11.12.90

July 1989
volume 161, *number* 1

CUK-H01344-31-P024-295

American Journal of OBSTETRICS AND GYNECOLOGY

Copyright © 1989 by The C.V. Mosby Company

31

Editor in Chief
JOHN I. BREWER

Editors
FREDERICK P. ZUSPAN • E. J. QUILLIGAN

Associate Editor
ALBERT B. GERBIE

Official Publication

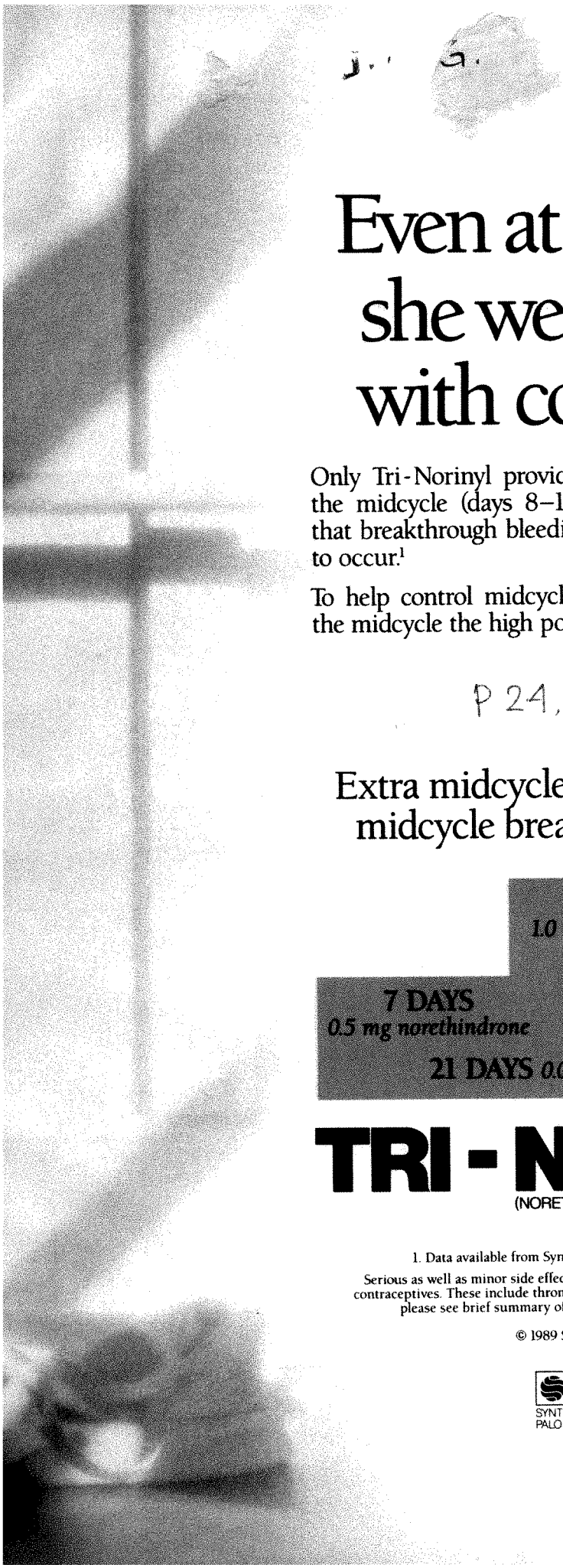
AMERICAN GYNECOLOGICAL AND OBSTETRICAL SOCIETY
CENTRAL ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS
SOUTH ATLANTIC ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS
PACIFIC COAST OBSTETRICAL AND GYNECOLOGICAL SOCIETY
AMERICAN BOARD OF OBSTETRICS AND GYNECOLOGY
SOCIETY FOR GYNECOLOGIC INVESTIGATION
SOCIETY OF PERINATAL OBSTETRICIANS

Published by

THE C.V. MOSBY COMPANY
St. Louis, Missouri 63146-3318

ISSN 0002-9378





Even at midcycle, she wears white with confidence

Only Tri-Norinyl provides a full 1 mg of progestin at the midcycle (days 8–16), when clinical data indicate that breakthrough bleeding and spotting are most likely to occur.[1]

To help control midcycle breakthrough bleeding: make the midcycle the high point of protection.

P 24,295

31

Extra midcycle progestin to reduce midcycle breakthrough bleeding

9 DAYS
1.0 mg norethindrone

7 DAYS
0.5 mg norethindrone

5 DAYS
0.5 mg norethindrone

21 DAYS *0.035 mg ethinyl estradiol*

TRI-NORINYL®

(NORETHINDRONE AND ETHINYL ESTRADIOL) TABLETS

1. Data available from Syntex Laboratories, Inc., document #92363.

Serious as well as minor side effects have been reported following the use of all oral contraceptives. These include thromboembolic disease. Before prescribing Tri-Norinyl, please see brief summary of prescribing information on following page.

© 1989 Syntex Laboratories, Inc.

SYNTEX

SYNTEX LABORATORIES, INC.
PALO ALTO, CA 94304

CALCUTTA UNIVERSITY CENTRAL LIBRARY

TRI-NORINYL®

(NORETHINDRONE AND ETHINYL ESTRADIOL) TABLETS

TRI-NORINYL® 21-Day Tablets (7 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg, 9 green tablets of norethindrone 1.0 mg with ethinyl estradiol 0.035 mg, 5 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg)

TRI-NORINYL® 28-Day Tablets (7 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg, 9 green tablets of norethindrone 1.0 mg with ethinyl estradiol 0.035 mg, 5 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg; 7 inert tablets)

BREVICON® 21-Day Tablets (norethindrone 0.5 mg with ethinyl estradiol 0.035 mg)

BREVICON® 28-Day Tablets (21 norethindrone 0.5 mg with ethinyl estradiol 0.035 mg tablets followed by 7 inert tablets)

NORINYL® 1 + 35 21-Day Tablets (norethindrone 1 mg with ethinyl estradiol 0.035 mg)

NORINYL® 1 + 35 28-Day Tablets (21 norethindrone 1 mg with ethinyl estradiol 0.035 mg tablets followed by 7 inert tablets)

NORINYL® 1 + 50 21-Day Tablets (norethindrone 1 mg with mestranol 0.05 mg)

NORINYL® 1 + 50 28-Day Tablets (21 norethindrone 1 mg with mestranol 0.05 mg tablets followed by 7 inert tablets)

NORINYL® 1 + 80 21-Day Tablets (norethindrone 1 mg with mestranol 0.08 mg)

NORINYL® 1 + 80 28-Day Tablets (21 norethindrone 1 mg with mestranol 0.08 mg tablets followed by 7 inert tablets)

NORINYL® 2 mg Tablets (norethindrone 2 mg with mestranol 0.1 mg)

NOR-Q.D.® (norethindrone) Tablets 0.35 mg

ORAL CONTRACEPTIVE (O.C.) AGENTS

Indications Prevention of pregnancy. *DOSE-RELATED RISK OF THROMBOEMBOLISM.* **Because** studies have shown a positive association between OC estrogen dose and risk of thromboembolism, it is prudent to minimize estrogen exposure. Prescribe an OC with the least amount of estrogen compatible with an acceptable pregnancy rate and patient acceptance. Start new users on OCs containing 0.05 mg or less of estrogen.

Contraindications 1. Known or suspected pregnancy (see Warning #5). 2. Thrombophlebitis or thromboembolic disorders. 3. Past history of deep vein thrombophlebitis or thromboembolic disorders. 4. Undiagnosed abnormal genital bleeding. 5. OCs should not be used by women who have or have had any of the following: a. cerebral vascular or coronary artery disease, including myocardial infarction. b. known or suspected carcinoma of the breast. c. known or suspected estrogen dependent neoplasia. d. benign or malignant liver tumor that developed during use of OCs or other estrogen containing products.

WARNINGS: Cigarette smoking increases the risk of serious cardiovascular side effects from OC use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use OCs should be strongly advised not to smoke.

The use of OCs is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, liver tumor, gall bladder disease, visual disturbances, fetal abnormalities, and hypertension. Practitioners prescribing OCs should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with OC use is established. One study demonstrated an increased relative risk for fatal venous thromboembolism and several studies demonstrated it for non-fatal venous thromboembolism. They estimate that OC users are 4-11 times more likely than nonusers to develop these diseases without evident cause. One British study reported an excess death rate of 40% in OC users, most of which resulted from cardiovascular disease. Another British study showed a lower death rate in OC users than controls; an increase in cardiovascular deaths was seen but was not statistically significant. A U.S. prospective study failed to disclose increased mortality rates from cardiovascular disorders, but a subset analyzed as a retrospective, case-control study showed significant increases in venous thromboembolism. CEREBROVASCULAR DISORDERS. Two American studies demonstrated an increased relative risk for stroke not shown in prior British studies. In an American study of cerebrovascular disorders in women with and without predisposing causes, relative risk of hemorrhagic stroke was estimated as 2.0 times greater and thrombotic stroke as 4-9.5 times greater in users than nonusers. A British long-term follow-up study reported in 1976 a highly significant association between OC use and stroke. Another study had suggested an association in 1974, but the number of cases was too small to estimate the risk. Subarachnoid hemorrhage has been shown to be increased by OC use in British and American studies. Smoking alone increases incidence of these accidents; smoking and pill use appear to increase risk more than either alone.

MYOCARDIAL INFARCTION (MI). Increased relative risk of MI associated with OC use has been reported. One British study found that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing MI, regardless of OC use. OCs were an additional risk factor. In terms of relative risk, it has been estimated that nonsmoking OC users (smoking is considered a major predisposing condition to MI) are twice as likely to have a fatal MI as nonsmoking nonusers. OC users who are smokers have a 5-fold increased risk of fatal infarction compared to nonsmoking users, and a 10-12-fold increased risk compared to nonsmoking nonusers. The number of cigarettes smoked is important. In determining the importance of these relative risks, baseline rates for various age groups must be considered. (Estimates are based on British vital statistics which show acute MI death rates 2-3 times less than in the U.S. so U.S. death rates could be higher.) Importance of other predisposing conditions in determining relative and absolute risks has not been quantified; other synergistic actions may exist. RISK OF DOSE. Using data from several national adverse reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to estrogen dose in OCs. OCs with 0.1 mg or more of estrogen were associated with a higher risk of thromboembolism than those containing 0.05-0.08 mg but quantity of estrogen may not be the sole factor. This was supported by a U.S. study. A British study found a positive association between dose of progestogen or estrogen and certain thromboembolic conditions. Swedish authorities noted decreased reporting of thromboembolic episodes when higher estrogen preparations were no longer prescribed. Careful epidemiological studies to determine degree of thromboembolic disease risk associated with progestogen-only OCs have not been done. Thromboembolic disease has been reported in women using these products, and they should not be considered free of excess risk. PERSISTENCE OF RISK. Two studies have suggested an increased risk may persist for 6 years after discontinuation of OC use for cerebrovascular disease and 9 years for MI; another study suggested the persistence of risk for subarachnoid hemorrhage. ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES. A large British prospective study estimated mortality rate per 100,000 women per year from circulatory system diseases for OC users and nonusers according to age, smoking habits, and duration of use. The overall annual excess death rate for OC users was estimated to be 20/100,000 (ages 15-34–5/100,000; ages 35-44–33/100,000; ages 45-49–140/100,000). Risk is concentrated in long-term users and in smokers, and may persist after OC discontinuation. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all occurred in women 35 or older. An update provided the following rates: ages 15-34–1/6700 for nonsmokers and 1/2000 for smokers; ages 45 and over–1/2500 for nonsmokers and 1/500 for smokers. Risk appeared to increase with parity, but not with duration of use. Until more women under 35 with continuous use for 5 or more years are available, it is not possible to assess relative risk for this age group. Data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. Estimates include combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease for OCs) plus risk attributable to pregnancy or abortion if the method fails (which varies with the effectiveness of the contraceptive method). The study concluded that mortality associated with all contraceptive methods is below that of childbirth, except for OCs in women over 40 who smoke. (Rates given for pill only/smokers for each age are for smokers as a class. For "heavy" smokers (more than 15 cigarettes a day), the rates would be about double; for "light" smokers (less than 15), about half.) The lowest mortality is with the condom or diaphragm backed up by early abortion. The study also concluded that OC users who smoke, especially over 30, have greater mortality risk than OC users who do not smoke.

Risk of thromboembolic and thrombotic disease associated with OCs increases with age after 30 and for MI is further increased by hypertension, hyperlipidemias, obesity, diabetes, or history of preeclamptic toxemia, and especially by smoking. The following chart gives a gross estimate of risk of death from circulatory disorders associated with OC use.

SMOKING HABITS AND OTHER PREDISPOSING CONDITIONS–RISK ASSOCIATED WITH USE OF OCs

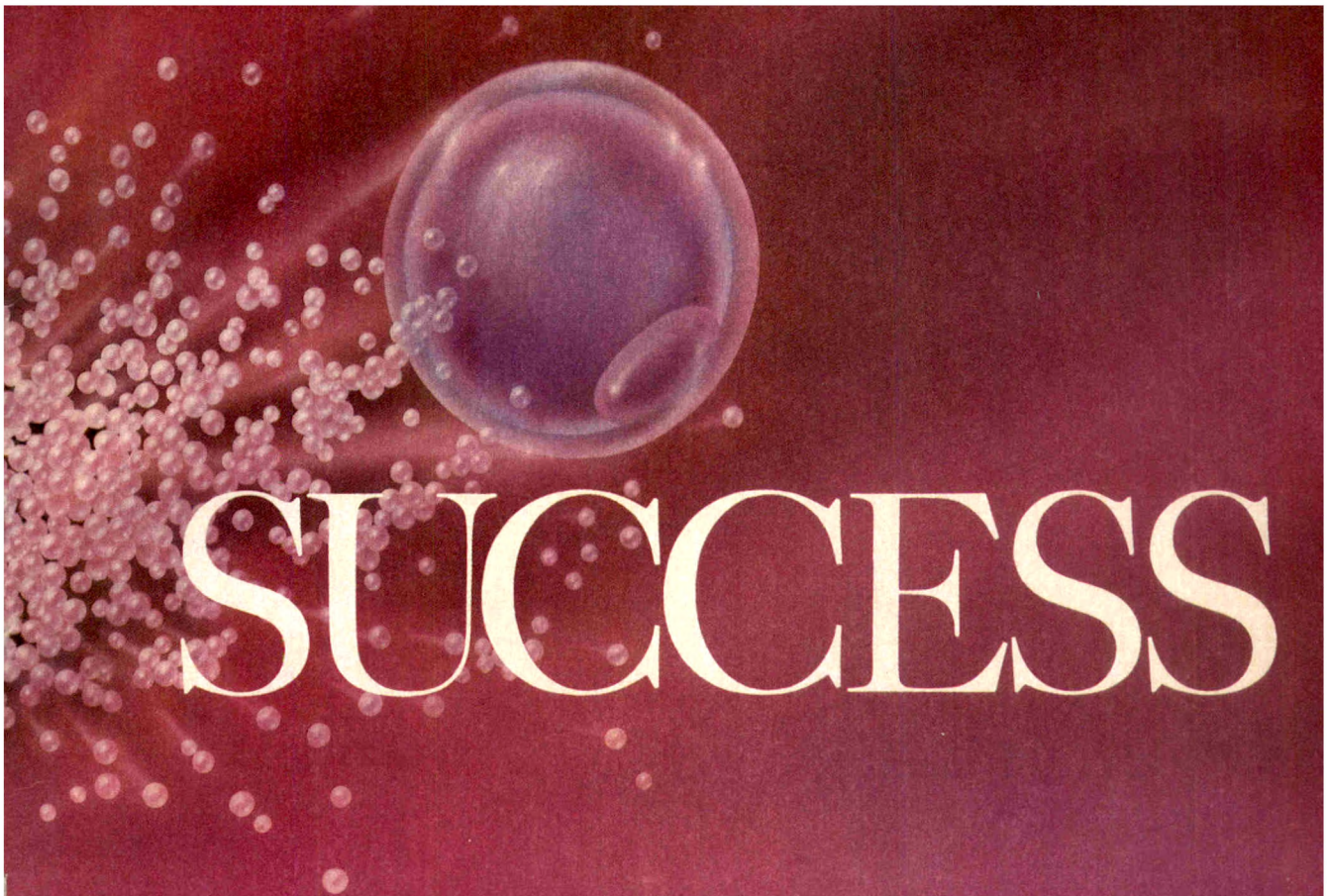
Age	Below 30	30-39	40 +
Heavy smokers	C	B	A
Light smokers	D	C	B
Nonsmokers (no predisposing conditions)	D	C,D	C
Nonsmokers (other predisposing conditions)	C	C,B	B,A

A–Use associated with very high risk
B–Use associated with high risk
C–Use associated with moderate risk
D–Use associated with low risk

Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, discontinue OC immediately. A 4-6 fold increased risk of post-surgery thromboembolic complications has been reported in OC users. If feasible, discontinue OCs at least 4 weeks before surgery associated with increased risk of thromboembolism or prolonged immobilization. Before resuming OC after major surgery or bedrest, balance risks of post-surgery thromboembolic complications with contraceptive needs. Data suggest varicose veins substantially increase risk of superficial venous thrombosis of the leg, the risk depending on the severity of the varicosities. 2. *Ocular Lesions:* Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OC use. Discontinue OC if there is unexplained, sudden, or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions; and institute appropriate diagnostic and therapeutic measures. 3. *Carcinoma:* Long-term continuous administration of natural or synthetic estrogen in certain animals increases certain tumors, benign or malignant, such as breast, cervix, vagina, uterus, ovary, pituitary and liver. Certain synthetic progestogens, none currently in OCs, increase the incidence of mammary nodules, benign and malignant, in dogs. Several retrospective case-control studies reported an increased relative risk (3.1-13.9 times) associating endometrial carcinoma with the prolonged use of estrogens in postmenopausal women. One publication reported the first 30 cases submitted to the registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the adenocarcinomas found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding when OCs were first given, polycystic ovaries), nearly all occurred in women who had used sequential OCs, which are no longer marketed. No statistical association has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs, although individual cases have been reported. Studies have shown no increased risk of breast cancer to OC or estrogen users. One study found no overall increased risk of breast cancer in OC users but a greater risk was suggested for OC users with documented benign breast disease and for long-term (2-4 years) users. Another study found a history of breast cancer among grandmothers or aunts was significantly more frequent among breast cancer patients who had used an OC continuously for one or more years than among nonusers with breast cancer. One other study indicated an increasing risk of breast cancer in women taking menopausal estrogens, which increased with duration of follow-up. One author suggests that extended (over 6 years) OC use prior to first full term pregnancy was associated with a significant relative risk of breast cancer, and other studies suggested an increased risk of breast cancer with long-term OC use before age 25. A reduced occurrence of benign breast tumors in OC users has been well documented. One study reported malignant melanoma more frequently in OC users than controls and suggests an increased incidence of urinary tract and thyroid cancers. A prospective study of women with cervical dysplasia found an increase in severity and conversion to cancer *in situ* in OC users compared to nonusers. This became statistically significant after 3-4 years of use. Nonreversal of dysplasia within the first 6 months of pill use was suggested to predict progression after prolonged exposure. One study disclosed an increased risk of cancer of the cervix (largely carcinoma *in situ*) in OC users under 40, particularly those who had used OCs over 4 years. Two other studies suggested an increasing incidence of cervical neoplasms with longer duration of OC use. There have been other reports of microglandular hyperplasia of the cervix in OC users. One study reported an association between OC use and endocervical adenocarcinoma. In summary, there is no confirmed evidence from human studies of increased risk of cancer associated with OCs. Close clinical surveillance of all OC users is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, take appropriate diagnostic measures to rule out malignancy. Monitor OC users with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms with particular care. (See also Warnings, 4. *Liver Tumors.*) 4. *Liver Tumors:* Sudden severe abdominal pain or shock may be due to rupture and hemorrhage of a liver tumor. There have been reports associating benign or malignant liver tumors with short-term and long-term OC use. One study reported use of OCs with high hormonal potency and age over 30 may further increase risk of hepatocellular adenoma. Two studies relate risk with duration of use, risk being much greater after 4 or more years of use. Long-term OC users have an estimated annual incidence of hepatocellular adenoma of 3-4/100,000. Although an uncommon lesion, it should be considered in women presenting with an "acute abdomen". The tumor may cause serious or fatal hemorrhage. Patients with liver tumors have demonstrated variable clinical features which may make preoperative diagnosis difficult. Some cases presented because of right upper quadrant masses, while most had signs and symptoms of acute intraperitoneal hemorrhage. Routine radiological and laboratory studies may not be helpful. Liver scans may show a focal defect. Hepatic arteriography may be useful in diagnosing primary liver neoplasm. Cases of hepatocellular carcinoma have been reported in long-term users of OCs.[105-107] An epidemiological study suggested a 4-fold increase in relative risk for hepatocellular carcinoma in OC users for 8 years or more, which increased to 7 in women with markers of hepatitis B. A second study suggested a risk of about 20 for use in excess of 8 years. 5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring:* Use of female sex hormones–estrogenic and progestational agents–during early pregnancy may seriously damage the offspring. Females exposed *in utero* to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1/1000 exposures or less. Although there is no evidence that OCs further enhance the risk of developing this type of malignancy, such OC users should be monitored with particular care. A high percentage of women exposed to diethylstilbestrol (30-90%) have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are a precursor of vaginal malignancy. Male children so exposed may develop urogenital tract abnormalities. Although similar data are not available on other estrogens, it cannot be presumed that they would not induce similar changes. Increased risk of congenital anomalies, including heart and limb defects, has been reported following use of sex hormones, including OCs, in pregnancy. One case-control study estimated a 4.7-fold increased relative risk of limb-reduction defects in infants exposed *in utero* to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some exposures involved only a few days of treatment. Data suggest risk of limb-reduction defects in exposed fetuses is somewhat less than 1/1000 live births. In a large prospective study, cardiovascular defects in children born to women who received female hormones, including OCs, during early pregnancy occurred at 18.2/1000 births, compared to 7.8/1000 for children not so exposed *in utero*. These results are statistically significant. A Welsh study found a statistically significant excess of neural tube defects among offspring of prior OC users (within 3 months) than among controls. The incidence of twin births may be increased for women who conceive shortly after discontinuing OC use. In the past, female sex hormones were used during pregnancy in an attempt to treat threatened or habitual abortion. There is evidence that estrogens are ineffective, and there is no evidence from well controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. If the patient has not adhered to the prescribed schedule, consider possible preg
nancy at the first missed period (or 45 days from the last menstrual period if
progestogen-only OCs are used) and discontinue OC use until pregnancy has
been ruled out. For any patient who has missed two consecutive periods, rule
out pregnancy before continuing the OC. If pregnancy is confirmed, tell th
patient about potential risks to the fetus and discuss advisability of continuit
the pregnancy. Women who discontinue OCs to become pregnant should use
alternate form of contraception for a period of time before attempting to c
ceive. A 3-month period is supported by a study suggesting increased frequen
of neural tube defects in women impregnated during the first 3 months aft
cessation of OC use. Do not use progestogen-only or progestogen-estroge
combinations to induce withdrawal bleeding as a pregnancy test. 6. *Gall Bladde
Disease:* Studies report increased risk of gall bladder disease in OC or estroge
users. In one study, an increased risk appeared after 2 years of use and double
after 4-5 years; in another study, an increased risk was apparent between 6 an
12 months. 7. *Carbohydrate and Lipid Metabolic Effects:* Because a decrease
glucose tolerance has been observed in a significant percentage of patients
OCs, prediabetic and diabetic OC users should be carefully observed. An increa
in triglycerides and total phospholipids has been observed in OC users but i
clinical significance is unknown. 8. *Elevated Blood Pressure:* An increase
blood pressure has been reported with OC use. Hypertension may occur within
few months of beginning OCs. In the first year of use, incidence of hypertensio
may be no higher in OC users than in nonusers. Incidence in users increases wit
exposure and in the fifth year of use is 2.5-3 times that in the first year. Age
strongly correlated with hypertension in OC users. Women with a history
elevated blood pressure (hypertension), preexisting renal disease, history
toxemia or elevated blood pressure during pregnancy, familial tendency to hype
tension or its consequences, or history of excessive weight gain or fluid retentio
during the menstrual cycle may be more likely to develop elevated blood pressur
when given OCs and should be monitored closely. Even though elevated blooc
pressure may remain within the "normal" range, closely watch elevations, partic
ularly for women with other risk factors for cardiovascular disease or stroke. High
blood pressure may or may not persist after OC discontinuation. 9. *Headache:*
Discontinue OC and evaluate the cause of onset or exacerbation of migraine or
development of a new pattern of headache which is recurrent, persistent, or
severe. 10. *Bleeding Irregularities:* Breakthrough bleeding, spotting, and missed
menses often make users discontinue OCs. In breakthrough bleeding, as in all
cases of irregular bleeding from the vagina, consider nonfunctional causes. In
undiagnosed persistent or recurrent abnormal bleeding from the vagina, use
adequate diagnostic measures to rule out pregnancy or malignancy. If pathology
has been excluded, time or another formulation may solve the problem. While
potentially useful in minimizing menstrual irregularity, change to an OC with
higher estrogen content only if necessary since this may increase the ris
thromboembolic disease. Women with a history of oligomenorrhea or secon
amenorrhea or young women without regular cycles may tend to remain a
latory or to become amenorrheic after OC discontinuation. Women with
preexisting problems should be advised of this and encouraged to use
contraceptive methods. Post-use anovulation, possibly prolonged, may occ
women without previous irregularities. A higher incidence of galactorrhea a
pituitary tumors (e.g., adenomas) has been associated with amenorrhe
former users compared with nonusers. One study reported a 16-fold incre
incidence of pituitary prolactin-secreting tumors among patients with po
amenorrhea when galactorrhea was present. 11. *Infertility:* There is evide
impairment of fertility in women discontinuing OCs in comparison with
contraceptive methods, which appears to be independent of duration
While impairment diminishes with time, there is an appreciable differenc
results in nulliparous women for OC and other groups 30 months after di
uation of birth control. For parous women the difference is not appar
months after cessation of contraception. One study suggests that the inc
of *Chlamydia trachomatis* infection, the leading cause of nongonococcal P
increased by OCs. 12. *Ectopic Pregnancy:* Ectopic as well as intrauterin
nancy may occur in OC failure. In progestogen-only OC failures, the
ectopic to intrauterine pregnancies is higher than in nonusers, since
are more effective in preventing intrauterine than ectopic pregnancies.
Feeding: OCs in the postpartum period may interfere with lactation by
ing quantity and quality of breast milk. A small fraction of OC hormona
has been identified in milk of mothers receiving OCs. Effects, if any,
breast-fed child have not been determined. If feasible, defer OC use
infant has been weaned. **Precautions** *GENERAL* 1. Take a complete medi
family history before starting OCs. Pretreatment and periodic physical
should include special reference to blood pressure, breasts, abdomen and
organs, including Papanicolaou smear and relevant lab tests. As a gener
OCs should not be prescribed for longer than 1 year without another physi
Under the influence of estrogen-progestogen preparations, preexisting
leiomyomata may enlarge. 3. Observe patients with a history of psychic d
sion and discontinue OCs if depression recurs to a serious degree. Pa
becoming significantly depressed while taking OCs should stop the OC ar
an alternate method of contraception to determine whether the symptom i
related. 4. OCs may cause some fluid retention. Prescribe with caution, an
with careful monitoring, in patients with conditions that might be aggrava
fluid retention, such as convulsive disorders, migraine syndrome, asth
cardiac, hepatic or renal insufficiency. 5. Patients with a past history of jau
during pregnancy have an increased risk of recurrence while using OCs. D
tinue OC if jaundice develops. 6. Steroid hormones may be poorly metaboliz
patients with impaired liver function and should be administered with cauti
such patients. 7. OC users may have disturbances in normal tryptophan me
lism that may result in a relative pyridoxine deficiency. The clinical significa
unknown. 8. Serum folate levels may be depressed by OC use. Since the pe
woman is predisposed to folate deficiency and incidence of folate defic
increases with increasing gestation, if a woman becomes pregnant shortly
stopping OCs, she may have a greater chance of developing folate deficienc
related complications. 9. Advise the pathologist of OC use when relevant
mens are submitted. 10. Certain endocrine and liver function tests and
components may be affected by estrogen-containing OCs. For exar
Increased: sulfobromophthalein retention. b. Increased prothrombin an
VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine
platelet aggregability. c. Increased thyroid binding globulin (TBG) lea
increased circulating total thyroid hormone, as measured by protein-
iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin upt
decreased, reflecting the elevated TBG; free T4 concentration is unalter
Decreased pregnanediol excretion. e. Reduced response to metyrapone t
Increased phospholipids and triglycerides. g. Temporarily decreased gl
tolerance. 11. Contact lens wearers who develop visual changes or chang
lens tolerance should be assessed by an ophthalmologist and remain
permanent cessation of wear considered. DRUG INTERACTIONS. OCs m
less effective and there may be increased breakthrough bleeding from in
tions with rifampin, isoniazid, ampicillin, tetracycline, neomycin, penicil
chloramphenicol, sulfonamides, nitrofurantoin, barbiturates, phenytoin,
done, analgesics, tranquilizers, antimigraine preparations, antihistamines,
griseofulvin. OCs may alter effectiveness of such other drugs as oral antico
lants, anticonvulsants, tricyclic antidepressants, antihypertensive agents
guanethidine), vitamins, hypoglycemic agents, tranquilizers, hypnotic prep
tions, theophylline, and beta blockers. Toxic liver effects have been reported
a combination of an OC and cyclosporin A. *CARCINOGENESIS* See Warni
PREGNANCY Pregnancy category X. See Contraindications and Warni
NURSING MOTHERS See Warnings. **Adverse Reactions** An increased risk of
following serious adverse reactions has been associated with OC use (see W
ings): thrombophlebitis, thrombosis, pulmonary embolism, coronary throm
sis, cerebral thrombosis, mesenteric thrombosis, liver tumors, cereb
hemorrhage, hypertension, gall bladder disease, congenital anomalies, ne
ocular lesions, e.g., retinal thrombosis and optic neuritis, Budd-Chiari syndro
Raynaud's disease, arterial thromboembolism. The following adverse reacti
have been reported in OC users and are believed to be drug related: bleed
irregularities (breakthrough bleeding, spotting, missed menses during treatm
amenorrhea after treatment), gastrointestinal symptoms (nausea, vomit
bloating, abdominal cramps, colitis), dysmenorrhea, infertility after discont
ance of treatment, edema, chloasma or melasma which may persist after drug
discontinued, breast changes (tenderness, enlargement, and secretion), intole
ance to contact lenses, changes in corneal curvature (steepening), change
weight (increase or decrease), change in cervical erosion and cervical secretion
possible diminution of lactation when given immediately postpartum, cholestati
jaundice, migraine, increase in size of uterine leiomyomata, rash (allergic), me
tal depression, reduced tolerance to carbohydrates, vaginal candidiasis, prola
tin-secreting pituitary tumors, chilblains. The following adverse reactions ha
been reported in OC users and the association has been neither confirmed
refuted: premenstrual-like syndrome, cataracts, changes in libido, chore
changes in appetite, cystitis-like syndrome, headache, nervousness, dizzine
hirsutism, loss of hair, erythema multiforme, erythema nodosum, hemorrha
eruptions, vaginitis, porphyria, impaired renal function, malignant nephrosc
rosis (hemolytic uremic syndrome), malignant hypertension, acute renal failu
premature supraventricular contractions. **Information for the Patient** (See Pati
Package Insert).

Rev. April 19

SYNTEX



SUCCESS

When pregnancy is the goal, successful ovulation is the first hurdle. Serophene has demonstrated its effectiveness for six years—with ovulation rates of 50% to 94% and pregnancy rates of 11% to 52%.

For effective first-line ovulation-induction therapy, success starts with Serophene.

Starts with SEROPHENE®

(clomiphene citrate tablets, USP) 50 mg

The first choice for ovulation induction

8, Serono Laboratories, Inc.

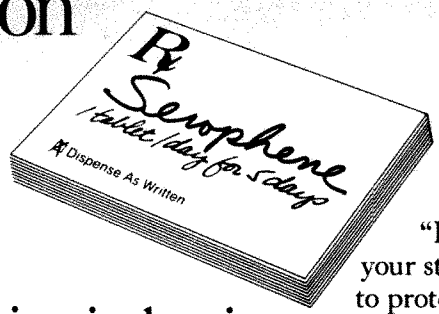
e see brief summary of prescribing information on adjacent page.

The World Leader in Infertility Therapy

Serono

Successful ovulation starts with SEROPHENE®

(clomiphene citrate tablets, USP) 50 mg



Be sure your patients receive the drug you prescribe. Specify "Dispense as Written" or your state's approved wording to protect the integrity of your Serophene prescription.

The first choice for ovulation induction

Serophene®
(clomiphene citrate tablets, USP)
Brief Summary:
Indications:
Clomiphene citrate is indicated for the treatment of ovulatory failure in patients desiring pregnancy, and whose husbands are fertile and potent. Impediments to this goal must be excluded or adequately treated before beginning therapy. Administration of clomiphene citrate is indicated only in patients with demonstrated ovulatory dysfunction and in whom the following conditions apply:
1. Normal liver function.
2. Physiologic indications of normal endogenous estrogen (as estimated from vaginal smears, endometrial biopsy, assay of urinary estrogen, or from bleeding in response to progesterone). Reduced estrogen levels, while less favorable, do not prevent successful therapy.
3. Clomiphene citrate therapy is not effective for those patients with primary pituitary or ovarian failure. It cannot substitute for appropriate therapy of other disturbances leading to ovulatory dysfunction, e.g., diseases of the thyroid or adrenals.
4. Particularly careful evaluation prior to clomiphene citrate therapy should be done in patients with abnormal uterine bleeding. It is most important that neoplastic lesions are detected.

Contraindications:
Pregnancy
Although no direct effect of clomiphene citrate therapy on the human fetus has been seen, clomiphene citrate should not be administered in cases of suspected pregnancy as such effects have been reported in animals. To prevent inadvertent clomiphene citrate administration during early pregnancy, the basal body temperature should be recorded throughout all treatment cycles, and therapy should be discontinued if pregnancy is suspected. If the basal body temperature following clomiphene citrate is biphasic and is not followed by menses, the possibility of an ovarian cyst and/or pregnancy should be excluded. Until the correct diagnosis has been determined, the next course of therapy should be delayed.
Liver Disease
Patients with liver disease or a history of liver dysfunction should not receive clomiphene citrate therapy.
Abnormal Uterine Bleeding
Clomiphene citrate is contraindicated in patients with abnormal uterine bleeding.

Warnings:
Visual Symptoms
Patients should be warned that blurring and/or other visual symptoms may occur occasionally with clomiphene citrate therapy. These may make activities such as driving or operating machinery more hazardous than usual, particularly under conditions of variable lighting. While their significance is not yet understood (see ADVERSE REACTIONS), patients having any visual symptoms, should discontinue treatment and have a complete ophthalmologic evaluation.

Precautions:
Diagnosis Prior to Clomiphene Citrate Therapy
Careful evaluation should be given to candidates for clomiphene citrate therapy. A complete pelvic examination should be performed prior to treatment and repeated before each subsequent course. Clomiphene citrate should not be given to patients with an ovarian cyst, as further ovarian enlargement may result.
Since the incidence of endometrial carcinoma and of ovulatory disorders increases with age, endometrial biopsy should always exclude the former as causative in such patients. If abnormal uterine bleeding is present, full diagnostic measures are necessary.
Ovarian Overstimulation During Treatment with Clomiphene Citrate
To minimize the hazard associated with the occasional abnormal ovarian enlargement during clomiphene citrate therapy (see ADVERSE REACTIONS), the lowest dose producing good results should be chosen. Some patients with polycystic ovary syndrome are unusually sensitive to gonadotropin and may have an exaggerated response to usual doses of clomiphene citrate. Maximal enlargement of the ovary, whether abnormal or physiologic, does not occur until several days after discontinuation of clomiphene citrate. The patient complaining of pelvic pains after receiving clomiphene citrate should be examined carefully. If enlargement of the ovary occurs, clomiphene citrate therapy should be withheld until the ovaries have returned to pretreatment size, and the dosage or duration of the next course should be reduced. The ovarian enlargement and cyst formation following clomiphene citrate therapy regress spontaneously within a few days or weeks after discontinuing treatment. Therefore, unless a strong indication for laparotomy exists, such cystic enlargement always should be managed conservatively.
Multiple Pregnancy
In the reviewed publications, the incidence of multiple pregnancies was increased during those cycles in which clomiphene citrate was given. Among the 1803 pregnancies on which the outcome was reported, 90% were single and 10% twins. Less than 1% of the reported deliveries resulted in triplets or more.
Of these multiple pregnancies, 96-99% resulted in the births of live infants. The patient and her husband should be advised of the frequency and potential hazards of multiple pregnancy before starting treatment.

Adverse Reactions:
Symptoms
Side effects are not prominent at the recommended dosage of clomiphene citrate, and infrequently interfere with treatment. Side effects tend to occur more frequently at higher doses and in the longer treatment courses used in some early studies. The more common side effects and the percent of patients experiencing them include vasomotor flushes (11%), abdominal discomfort (7.4%), abnormal uterine bleeding (0.5%), ovarian enlargement (14%), breast tenderness (2.1%), and visual symptoms (1.6%). The vasomotor symptoms resemble menopausal "hot flushes," and are not usually severe. They promptly disappear after treatment is discontinued. Abdominal discomfort may resemble ovulatory (mittelschmerz) or premenstrual phenomena, or that due to ovarian enlargement. In addition, nausea and vomiting (2.1%), nervousness and insomnia (1.9%), headache (1%), dizziness and lightheadedness (1%), increased urination (0.9%), depression and fatigue (0.8%), urticaria and allergic dermatitis (0.6%), weight gain (0.4%), and reversible hair loss (0.3%) have been reported.
When clomiphene citrate is administered at the recommended dose, abnormal ovarian enlargement (see PRECAUTIONS) is infrequent, although the usual cyclic variation in ovarian size may be exaggerated. Similarly, mid-cycle ovarian pain (mittelschmerz) may be accentuated. With prolonged or higher dosage, ovarian enlargement and cyst formation (usually luteal) may occur more often and the luteal phase of the cycle may be prolonged. Patients with polycystic ovary disease may be unusually sensitive to clomiphene therapy. Rare occurrences of massive ovarian enlargement have been reported, for example, in a patient with polycystic ovary syndrome whose clomiphene citrate therapy consisted of 100 mg daily for 14 days. Since abnormal ovarian enlargement usually regresses spontaneously, most of these patients should be treated conservatively.
The incidence of visual symptoms (see WARNINGS for further recommendations), usually described as "blurring" or spots or flashes (scintillating scotomata), correlates with increasing total dose. The symptoms disappear within a few days or weeks after clomiphene citrate is discontinued. This may be due to intensification and/or prolongation of afterimages. Symptoms often appear first, or are accentuated, upon exposure to a more brightly lit environment. While measured visual acuity has not generally been affected, in one patient taking 200 mg daily, visual blurring developed on the seventh day of treatment, and progressed to severe diminution of visual acuity by the tenth day. No other abnormality was coincident, and the visual acuity was normal by the third day after treatment was stopped. Ophthalmologically definable scotomata and electroretinographic retinal function changes have also been reported.
BSP Laboratory Studies
Greater than 5% retention of sulfobromophthalein (BSP) has been reported in approximately 10 to 20% of patients in whom it was measured. Retention was usually minimal but was elevated during prolonged clomiphene citrate administration or with apparently unrelated liver disease. In some patients, pre-existing BSP retention decreased even though clomiphene citrate therapy was continued. Other liver function tests were usually normal.
Other Laboratory Studies
Clomiphene citrate has not been reported to cause a significant abnormality in hematologic or renal tests, in protein bound iodine, or in serum cholesterol levels.
Birth Defects
Of 1803 births following clomiphene citrate administration, 45 infants with birth defects were reported for a cumulative rate of 2.5%.
Six cases of Down's Syndrome, one neonatal death with multiple malformations and one case each of the following were reported: extropia, club foot, tibial torsion, blocked tear duct and hemangioma. The other congenital abnormalities were not described. The investigators did not report that these were presumed to be due to therapy. The cumulative rate of congenital abnormalities does not exceed that reported in the general population.

Available only on prescription
Manufactured by Ikapharm Ltd, Kfar Sava, Israel.
Distributed by: Serono Laboratories, Inc., 280 Pond Street, Randolph, MA 02368.
© Serono Laboratories, Inc.

SLSJA 1/88

The World Leader in Infertility Therapy

Serono

Toll-free: 800-225-5185
In Mass: 617-963-8154

Serono Laboratories, Inc.
280 Pond Street
Randolph, MA 02368

Mosby

American Journal of Obstetrics and Gynecology

Founded in 1920

Copyright © 1989 by The C.V. Mosby Company

Contents

July
1989

CLINICAL SECTION

Clinical Opinion

Fetal and neonatal cardiovascular complications from β-sympathomimetic therapy for tocolysis 1
Vern L. Katz, MD, and John W. Seeds, MD
Chapel Hill, North Carolina

β-Sympathomimetic tocolytic agents have significant fetal cardiac side effects that may occasionally be life-threatening.

Understanding and eliminating errors in perinatal research 5
Barry S. Block, MS, MD
Chicago, Illinois

The valid interpretation of the results and conclusions of experimental studies is encouraged to avoid publication of misleading studies that adversely affect clinical practice.

A case-control study of epithelial ovarian cancer 10
Patricia Hartge, ScD, Mark H. Schiffman, MD, Robert Hoover, MD, Larry McGowan, MD, Linda Lesher, MHS, and Henry J. Norris, MD
Bethesda, Maryland, and Washington, D.C.

Study results confirm the importance of parity, infertility, family history, and previous breast cancer in the etiology of epithelial ovarian cancer.

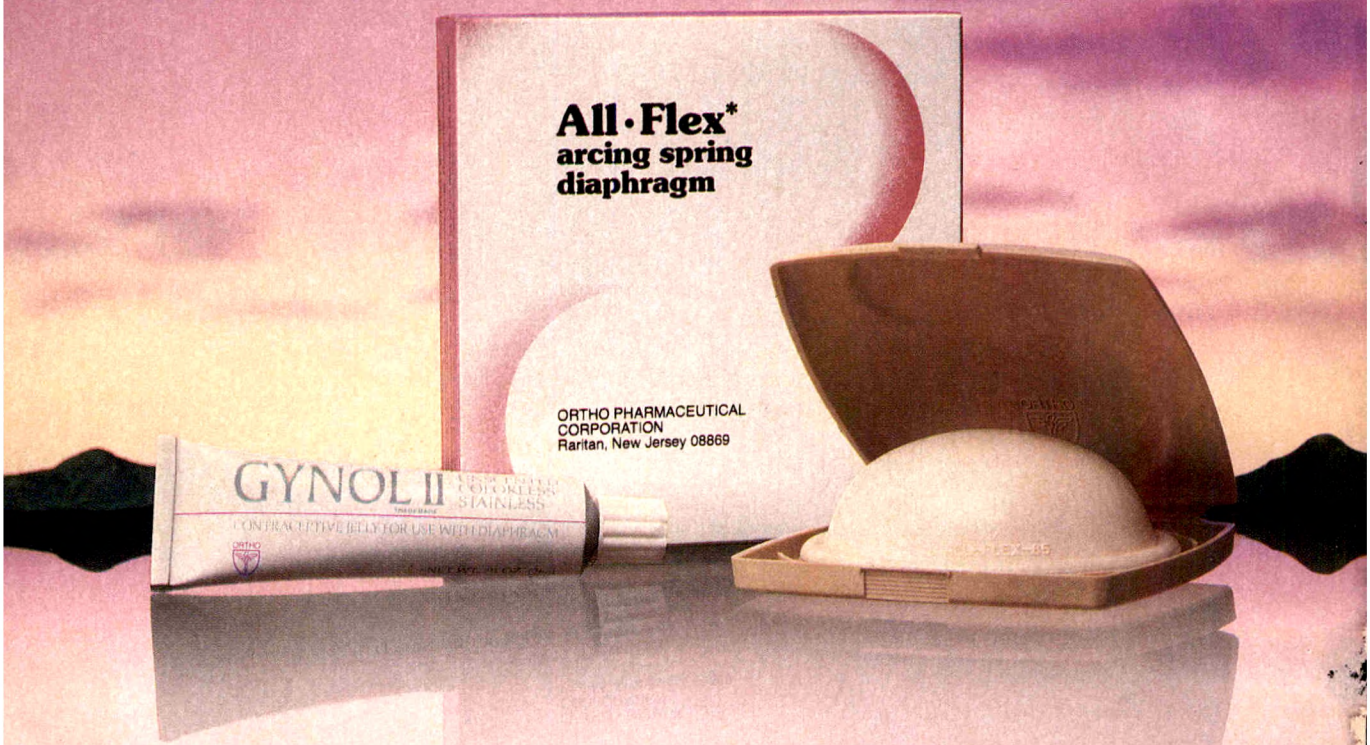
Heuristic determination of relevant diagnostic procedures in a medical expert system for gynecology 17
Steven L. Small, PhD, and Eberhard K. Muechler, MD
Pittsburgh, Pennsylvania, and Rochester, New York

We describe a medical expert system for problem solving in the gynecologic domain of infertility.

(Contents continued on page 7A)

Vol. 161, No. 1, July 1989. The American Journal of Obstetrics and Gynecology (ISSN 0002-9378) is published monthly (six issues per volume, two volumes per year) by The C.V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318. Second-class postage paid at St. Louis, Missouri, and additional mailing offices. POSTMASTER: Send address changes to The C.V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318, (314) 872-8370, ext. 351. Annual subscription rates for 1989: domestic, $65.50 for individuals and $118.00 for institutions. Printed in the U.S.A. Copyright © 1989 by The C.V. Mosby Company.

Satisfaction realized:



87% patient continuation rate† with Ortho ALL-FLEX* Arcing Spring Diaphragm.

Easy for patient to insert—
the Ortho ALL-FLEX* Diaphragm folds easily.

Easy for patient to correctly position in vagina—
the Ortho ALL-FLEX* Diaphragm can be compressed at any two opposing points around the rim.

Easy to use with **GYNOL II* (2% nonoxynol-9) Contraceptive Jelly**—
greaseless, odorless spermicide.

Easy to clean—one-step molding process eliminates gap between spring and dome so jelly or secretions can't accumulate.

Ortho ALL-FLEX*
DIAPHRAGM

†Percentage of patients continuing the method for 12 consecutive months.

Consult the revised patient brochure for new information on Toxic Shock Syndrome (TSS) and wearing time.

ORTHO PHARMACEUTICAL CORPORATION
Raritan, New Jersey 08869-0602

ORTHO

©OPC 1987 OJ-899 *Trademark

Contents continued from page 5A

Clinical Articles

Breech presentation after cesarean section: Always a section? 25
Ella Ophir, MD, Moshe Oettinger, MD, Arie Yagoda, MD, Yacov Markovits, MD, Nathan Rojansky, MD, and Harry Shapiro, MD
Nahariya, Israel

A trial of labor seems reasonable in carefully selected cases of breech presentation after a previous cesarean section.

Vaginal birth after cesarean delivery in the twin gestation 29
Thomas H. Strong, Jr., MD, Jeffrey P. Phelan, MD, Myoung Ock Ahn, MD, MPH, and Albert P. Sarno, Jr., MD, Maj., USA
Los Angeles, California

No significant increase in morbidity or mortality was noted among women with prior cesarean deliveries who attempted a trial of labor with twins.

Pharmacokinetics of orally administered ritodrine 32
Steven N. Caritis, MD, Raman Venkataramanan, PhD, Margaret Cotroneo, RN, and Jye-Ping Chiao, MS
Pittsburgh, Pennsylvania

Analysis of ritodrine concentration data after oral administration to pregnant subjects indicates that plasma concentrations are low and are unlikely to prevent recurrent preterm labor.

(Contents continued on page 8A)

1989 Annual subscription rates	U.S.A.	International (surface mail) All regions	International (airmail)° Canada	Mexico	Region 1	Region 2	Region 3
Institutions†	$118.00	$142.50	$170.00	$176.25	$188.50	$230.25	$272.00
Individuals‡	$ 65.50	$ 90.00	$117.50	$123.75	$136.00	$177.75	$219.50
Students, residents‡	$ 38.00	$ 62.50	$ 90.00	$ 96.25	$108.50	$150.25	$192.00

Subscription rates include supplements. Single copies are $7.00. Remittances should be made by check, draft, or post office or express money order, in U.S. funds drawn through a U.S. bank, payable to this JOURNAL. Claims for missing issues will be serviced only within six months of cover date. Single copy prices will be charged on missing issue claims older than six months from cover date. Back issues generally are available for the previous five years. Contact the Publisher to confirm availability of specific issues.

°*Airmail breakdown—Domestic:* First-class and Priority rates for the U.S. and possessions are available upon request. *Canada:* All provinces of Canada. *Mexico:* All localities within Mexico. *Region 1:* Colombia, Venezuela, Central America, Caribbean Islands, Bahamas, Bermuda, and St. Pierre and Miquelon (also from American Samoa to Western Samoa and from Guam to the Philippines). *Region 2:* South America (except Colombia and Venezuela), Europe (except Estonia, Latvia, Lithuania, and U.S.S.R), and North Africa (Morocco, Algeria, Tunisia, Libya, and Egypt). *Region 3:* Estonia, Latvia, Lithuania, U.S.S.R., Asia, Australia, New Zealand, Pacific Ocean Islands, Africa (other than North Africa), Indian Ocean Islands, and the Middle East.

†Institutional (multiple-reader) subscriptions are available to public and private libraries, schools, hospitals, and clinics; city, county, state, provincial, and national government bureaus and departments; and all commercial and private institutions and organizations.

‡Individual subscriptions and all student-rate subscriptions must be in the names of, billed to, and paid by individuals. All student-rate requests must indicate training status and name of institution.

Subscriptions may begin at any time.

Contents continued from page 7A

Neuromuscular blockade with magnesium sulfate and nifedipine 35
Scott W. Snyder, MD, and Michael S. Cardwell, MD
Columbia, Missouri

Neuromuscular blockade and respiratory distress occurred in a patient who received a small dose of magnesium after tocolysis with nifedipine.

Usefulness of continuous fetal heart rate monitoring during cesarean section 36
Boris M. Petrikovsky, MD, PhD, Mario Cohen, MD, and M. Leon Tancer, MD
Brooklyn, New York, and Farmington, Connecticut

Two cases in which continuous fetal heart rate monitoring during cesarean section modified the obstetric management are presented.

Risk factors for vulvar lichen sclerosus 38
Mario Sideri, MD, Fabio Parazzini, MD, Maria Teresa Rognoni, MD, Carlo La Vecchia, MD, Eva Negri, ScD, Salvatore Garsia, MD, Elena Arnoletti, MD, and Gabriela Cecchetti, MD
Milan, Italy, and Lausanne, Switzerland

The risk factors for vulvar lichen sclerosus have been assessed in a case-control study; the risk increased with increasing parity and decreasing intake of retinoid, but no relation emerged with menopausal status, socioeconomic indicators, smoking, and sexual habits.

Fetal liver length in normal and isoimmunized pregnancies 42
Alistair B. Roberts, MD, Jennifer M. Mitchell, and Neil S. Pattison, MD
Auckland, New Zealand

Ultrasonographic assessment of fetal liver length measurements correlates well with fetal hemoglobin levels in isoimmunized pregnancies.

Does midtrimester ΔOD_{450} of amniotic fluid reflect severity of Rh disease? 47
Uma Ananth, MD, And John T. Queenan, MD
Washington, D.C.

Second-trimester elevated amniotic fluid delta optical densities indicate severely affected fetuses.

Increased frequency of detection of human papillomavirus deoxyribonucleic acid in exfoliated cervical cells during pregnancy 50
Robert F. Rando, PhD, Steven Lindheim, MD, Lisa Hasty, MD, Thomas V. Sedlacek, MD, Mark Woodland, MD, and Catherine Eder, BA
Philadelphia, Pennsylvania

Detectable levels of HPV DNA in exfoliated cervical cells increased dramatically during pregnancy and a natural fluctuation in the detectable levels of HPV was observed over time.

Contents

Mechanism for human papillomavirus transmission at birth 55
Thomas V. Sedlacek, MD, Steven Lindheim, MD, Catherine Eder, BA, Lisa Hasty, MD, Mark Woodland, MD, Avi Ludomirsky, MD, and Robert F. Rando, PhD
Philadelphia, Pennsylvania

HPV DNA was detected in the nasopharyngeal cavity of neonates.

A ten-year experience treating cervical intraepithelial neoplasia with the CO_2 laser 60
Michael S. Baggish, MD, James H. Dorsey, MD, and Mark Adelson, MD
Syracuse, New York, and Baltimore, Maryland

Cure rates for cervical intraepithelial neoplasia between 94% and 97% were obtained in 4024 women treated with the CO_2 laser.

Anal submucosal injection: A new route for drug administration in pelvic malignancies. IV. Submucosal anal injection in the treatment of cancer of uterine cervix—Preliminary study 69
Ahmed Shafik, MD
Cairo, Egypt

Seven patients with advanced cancer of the uterine cervix, treated by anal submucosal injection of methotrexate, showed complete response with no side effects.

Aggressive angiomyxoma first diagnosed as levator hernia 73
Gregory P. Sutton, MD, Robert E. Rogers, MD, Lawrence M. Roth, MD, and Clarence E. Ehrlich, MD
Indianapolis, Indiana

Physical findings of an aggressive angiomyxoma first suggested a levator hernia; computed tomography aided in the diagnosis and excision.

Asymptomatic thrombocytopenia associated with chorioangioma of placenta 76
Nirmala S. Limaye, MD, and Jean-Gilles Tchabo, MD
Arlington, Virginia

A case of chorioangioma is presented.

First-trimester and early second-trimester diagnosis of nuchal cystic hygroma by transvaginal sonography: Diverse prognosis of the septated from the nonseptated lesion 78
M. Bronshtein, MD, S. Rottem, MD, N. Yoffe, MD, and Z. Blumenfeld, MD
Haifa, Israel

Half of eight cases of nuchal cystic hygroma were nonseptated and normal fetuses resulted; the other four cases had septated lesions and ended in fetal death or were associated with aneuploidy.

(Contents continued on page 10A)

Contents continued from page 9A

Enlarged cisterna magna in trisomy 18: Prenatal ultrasonographic diagnosis 83
Amy S. Thurmond, MD, David W. Nelson, MD, Richard I. Lowensohn, MD, William P. Young, MD, and Lowell Davis, MD
Portland, Oregon

Ultrasonographic visualization of an enlarged cisterna magna was the initial clue that led to the prenatal diagnosis of trisomy 18 in five patients.

Neonatal mortality in normal birth weight babies: Does the level of hospital care make a difference? 86
Cynthia J. Berg, MD, Charlotte M. Druschel, MD, MPH, Brian J. McCarthy, MD, Michael LaVoie, and R. Louise Floyd, RN, MSN
Atlanta, Georgia

Women with high prepartum risk scores who were delivered of normal birth weight infants had a lower neonatal mortality rate if delivery occurred in a level 2 or 3 hospital.

Postdate fetal surveillance: Is 41 weeks too early? 91
Denise A. Guidetti, MD, Michael Y. Divon, MD, and Oded Langer, MD
Bronx, New York

The abnormal fetal testing and adverse perinatal outcome associated with pregnancies >42 weeks were also found in pregnancies between 41 and 42 weeks' gestation.

Sarcoma associated with pregnancy 94
Yutaka Kyodo, MD, Kenji Inatomi, MD, and Tadayuki Abe, MD
Shizuoka, Japan

Sarcoma associated with pregnancy is reported; the patient underwent a cesarean section with myomectomy.

Primary stress urinary incontinence and pelvic relaxation: Prospective randomized comparison of three different operations 97
Arieh Bergman, MD, Paul P. Koonings, MD, and Charles A. Ballard, MD
Los Angeles, California

The Burch urethropexy resulted in a better cure rate in women with pelvic relaxation and stress urinary incontinence than did the Pereyra procedure or anterior colporrhaphy.

Saline solution amnioinfusion for oligohydramnios after premature rupture of the membranes 102
Motoharu Imanaka, MD, Sachio Ogita, MD, and Tadashi Sugawa, MD
Osaka, Japan

Saline solution that contained antibiotics was infused directly into the amniotic cavity by means of a new cervical catheter to successfully treat oligohydramnios after premature rupture of the membranes.

Contents

Prematurity among insulin-requiring diabetic gravid women 106
Michael F. Greene, MD, John W. Hare, MD, Martha Krache, RN, MPH, Mark Phillippe, MD, Vanessa A. Barss, MD, Daniel H. Saltzman, MD, Allan Nadel, MD, M. Donna Younger, MD, Linda Heffner, MD, PhD, and J. Elizabeth Scherl, MD
Boston, Massachusetts

Hypertension was the single most important immediate cause of and risk factor for premature delivery among 420 diabetic gravid women.

Management of the nonvertex second twin: Primary cesarean section, external version, or primary breech extraction 111
Stephen E. Gocke, MD, Michael P. Nageotte, MD, Thomas Garite, MD, Craig V. Towers, MD, and Wendy Dorcester, PhD
Fresno and Long Beach, California

Similar neonatal outcomes were seen when nonvertex second twins were delivered by cesarean section, external version, or breech extraction; however, intrapartum complications were more common when external version was attempted.

Magnesium supplementation during pregnancy: A double-blind randomized controlled clinical trial 115
Baha M. Sibai, MD, Marco A. Villar L., MD, and Eileen Bray, RNC
Memphis, Tennessee

Magnesium supplementation during pregnancy starting at 13 to 26 weeks' gestation did not reduce the incidence of either preeclampsia or fetal growth retardation.

Methicillin-resistant staphylococcal mastitis 120
Charles Kalstone, MD
Miami, Florida

Postpartum mastitis that does not respond to standard antibiotic therapy may require oral trimethoprim and sulfamethoxazole (Bactrim, Septra) or intravenous vancomycin for methicillin-resistant staphylococci.

Gestational trophoblastic disease: A case-control study from the People's Republic of China 121
Louise A. Brinton, PhD, Bao-Zhen Wu, MD, Wen Wang, MD, Abby G. Ershow, ScD, Hong-Zhao Song, MD, Jun-Yao Li, MD, Michael B. Bracken, PhD, and William J. Blot, PhD
Bethesda, Maryland, Beijing, People's Republic of China, and New Haven, Connecticut

A case-control study in the People's Republic of China of 331 patients with complete hydatidiform mole and 662 community controls showed reduced risks associated with a prior term birth or a history of infertility and increased risks with prior induced abortions, use of herbal medicines during pregnancy, and extended durations of oral contraceptive use.

(Contents continued on page 12A)

Contents continued from page 11A

Randomized treatment of mucopurulent cervicitis with doxycycline or amoxicillin 128
Jorma Paavonen, MD, Pacita L. Roberts, MS, Claire E. Stevens, MA, PA, Pål Wølner-Hanssen, MD, Robert C. Brunham, MD, Sharon Hillier, PhD, Walter E. Stamm, MD, Cho-Chou Kuo, MD, Timothy DeRouen, PhD, King K. Holmes, MD, PhD, and David A. Eschenbach, MD
Seattle, Washington

Doxycycline and amoxicillin effectively treated chlamydial and nonchlamydial cervicitis, as assessed by clinical and microbiologic criteria.

Right ventricular dysfunction in human fetal compromise 136
Juha Räsänen, MD, Pertti Kirkinen, MD, and Pentti Jouppila, MD
Oulu, Finland

Fetal M-mode echocardiography demonstrated decreased right ventricular fractional shortening and increased right/left ratio of ventricular inner end-diastolic diameters in fetuses with severe intrauterine distress.

Preterm and term fetal cardiac and movement responses to vibratory acoustic stimulation 141
Ronald L. Thomas, MD, Timothy R. B. Johnson, MD, Richard E. Besinger, MD, Deedra Rafkin, RN, Catharine Treanor, RN, and Donna Strobino, PhD
Baltimore, Maryland

High occurrence of increased baseline, tachycardia, and unusual fetal heart rate patterns accompanies use of vibratory acoustic stimulation.

Effect of physician characteristics on the cesarean birth rate 146
Gertrud S. Berkowitz, PhD, Gale S. Fiarman, MD, Michael A. Mojica, MD, Jay Bauman, MD, and Roberta Haynes de Regt, MD
New York and Elmhurst, New York

Physician characteristics may affect the indications for which cesarean sections are performed.

Court-ordered cesarean section: An analysis of ethical concerns in compelling cases 150
Thomas E. Elkins, MD, H. Frank Andersen, MD, Mel Barclay, MD, Tina Mason, MD, Noelle Bowdler, MD, and Garland Anderson, MD
Ann Arbor, Michigan, and Memphis, Tennessee

A discussion is presented of the ethical concerns that arise when a court order is sought to perform a cesarean section.

Removing a retained placenta by oxytocin—A controlled study 155
Charlotte Wilken-Jensen, MD, Verner Strøm, MD, Meta Damkjær Nielsen, MSc, and Bo Rosenkilde-Gram, MD
Gentofte, Denmark

Retained placenta can successfully and safely be treated with injection of 100 IU of oxytocin into an umbilical vein.

Contents

The use of transvaginal ultrasonography in the diagnosis of ectopic pregnancy 157
Ilan E. Timor-Tritsch, MD, Ming N. Yeh, MD, David B. Peisner, MD, Karen B. Lesser, MD, and Theresa A. Slavik, BA, RDMS
New York, New York

High-frequency transvaginal ultrasonography proved to be effective in the workup of the patient with suspected ectopic pregnancy.

Ileocecal vaginal construction 162
Rainer A. Bürger, MD, Hubertus Riedmiller, MD, Paul G. Knapstein, MD, Volker Friedberg, MD, and Rudolf Hohenfellner, MD
Mainz, West Germany

Indications for ileocecal and cecal vaginoplasty with detailed descriptions of operative and vaginal replacement techniques are reviewed and discussed.

Renal artery flow-velocity waveforms in normal and hypoxemic fetuses 168
S. Vyas, MD, K. H. Nicolaides, MD, and S. Campbell, MD
London, England

The fetal renal artery was identified by color flow mapping, and a reference range of the pulsatility index with gestation was established; subsequently, 18 SGA fetuses were examined and a significant correlation found between degree of fetal hypoxemia and increase in renal artery pulsatility index.

Twin pregnancy in premature ovarian failure after estrogen treatment: A case report 172
Lawrence Tang, MD, and Robert S. Sawers, MD
Birmingham, England

A patient with premature ovarian failure was given estrogen treatment that unexpectedly induced superovulation, resulting in a dizygotic twin pregnancy.

Uterine development and endocrine relationships after menarche 174
Eleonora Porcu, MD, Stefano Venturoli, MD, Raffaella Fabbri, BSc, Luigi F. Orsini, MD, Emilia Sganga, MD, Lorena Brondelli, MD, Roberto Paradisi, MD, and Carlo Flamigni, MD
Bologna, Italy

The uterus continues to grow after menarche.

Leiomyoma of the ovary with ascites and hydrothorax 177
Jonathan J. Nicoll, MA, and Peter J. Cox, MS
London, England

We describe a case in which leiomyoma of the ovary occurred with ascites and hydrothorax.

(Contents continued on page 14A)

Contents continued from page 13A

Individual growth curve standards in twins: Prediction of third-trimester growth and birth characteristics 179
Theodor Stefos, MD, Russell L. Deter, MD, Reba M. Hill, MD, and Nicolas V. Simon, MD
Houston, Texas, and York, Pennsylvania

Rossavik growth models for five anatomic parameters, derived from second-trimester growth curves, accurately predicted third-trimester growth and birth characteristics in twins, as they do in singletons.

Effect of birth weight, race, and sex on survival of low-birth-weight infants in neonatal intensive care 184
Michael B. Resnick, EdD, Randy L. Carter, PhD, Mario Ariet, PhD, Richard L. Bucciarelli, MD, Janet H. Evans, MSPA, Robert R. Furlough, PhD, William W. Ausbon, MD, and John S. Curran, MD
Gainesville, Tallahassee, and Tampa, Florida

Curves by race and sex illustrate the probability of survival of neonates at any specific birth weight.

Effects of maternal hyperoxia on the biophysical assessment of fetuses with suspected intrauterine growth retardation 188
David A. Ruedrich, MD, Lawrence D. Devoe, MD, and Nancy Searle, BA
Augusta, Georgia

The biophysical effects of maternal hyperoxia may improve the diagnostic accuracy of detecting intrauterine growth retardation.

Fetal radius length: A critical evaluation of race as a factor in gestational age assessment 193
Lyndon M. Hill, MD, David Guzick, MD, PhD, Marion L. Thomas, RT, RDMS, and Joanne K. Fries, RDMS
Pittsburgh, Pennsylvania

Assessment of gestational age from radius length incorporates racial factor.

Pregnancy after aortofemoral bypass grafting in a diabetic patient 200
P. J. Bradley-Watson, FRCS, FRCOG
Dyfed, Wales

A successful pregnancy after aortofemoral bypass grafting in a diabetic patient is described; this is believed to be the first case of its kind to be reported.

Fentanyl citrate analgesia during labor 202
William Rayburn, MD, Anne Rathke, RN, M. Patricia Leuschen, PhD, Jan Chleborad, MD, and William Weidner, MD
Omaha, Nebraska

Intravenous fentanyl citrate, a potent and short-acting narcotic, was helpful during labor without immediate or prolonged hazards to the mother or unborn infant.

Contents

Significance of D-mannose as a sperm receptor site on the zona pellucida in human fertilization 207
Kazumasa Mori, MD, Toshifumi Daitoh, MD, DMSc, Minoru Irahara, MD, DMSc, Masaharu Kamada, MD, DMSc, and Toshihiro Aono, MD, DMSc
Tokushima, Japan

Pretreatments of spermatozoa with D-mannose or of the zona pellucida with concanavalin A completely inhibited sperm penetration through the human zona pellucida.

The importance of ultrasonography in infertile women with "forgotten" intrauterine contraceptive devices 211
Raphael Ron-El, MD, Zvi Weinraub, MD, Rami Langer, MD, Ian Bukovsky, MD, and Eliahu Caspi, MD
Zerifin and Tel Aviv, Israel

Ultrasonography is a reliable method in diagnosis of an in situ "forgotten" intrauterine contraceptive device.

Acid-base status at birth and neurodevelopmental outcome at four and one-half years 213
Jennifer Dennis, DM, Ann Johnson, MD, Lesley Mutch, MD, Patricia Yudkin, MA, and Paul Johnson, MB, ChB
Oxford, England

A study of 230 term infants showed no statistically significant association between acid-base status at birth and any aspect of neurodevelopment at age 4½ years.

BASIC SCIENCE SECTION

The effect of elevated blood glucose on the electroencephalogram and cerebral metabolism during short-term brain ischemia in fetal sheep 221
Conrad R. Chao, MD, A. Roger Hohimer, PhD, and John M. Bissonnette, MD
Portland, Oregon

Elevation of blood glucose before cerebral ischemia prevents the attenuation of electroencephalographic amplitude seen during ischemia at normal blood glucose levels.

Uncoupling of excitation from contraction in uterine smooth muscle in near-term ewes 229
Deborah L. Reid, PhD, Mark C. Hollister, MD, Susan R. Davidson, MD, Terrance M. Phernetton, BS, and John H. G. Rankin, PhD
Madison, Wisconsin

A new method of assessing tocolytic efficacy is described; prostaglandin E_2–induced changes in intrauterine pressure were suppressed by forskolin but the uterine electromyogram was unaffected.

Gestational diabetes in the BHE rat: Influence of dietary fat 234
Juliana M. Bue, BS, Dorothy B. Hausman, PhD, and Carolyn D. Berdanier, PhD
Athens, Georgia

Long- and short-term feeding to BHE rats of a diet similar to that of human beings results in gestational diabetes.

(Contents continued on page 16A)

Contents continued from page 15A

Effect of inhibiting prostaglandin synthesis in pregnant sheep with 4-aminoantipyrine under normothermic and hyperthermic conditions 241
Peter Andrianakis, BSc(Hons.), David W. Walker, PhD, Meredith M. Ralph, PhD, and Geoffrey D. Thorburn, MD
Clayton, Victoria, Australia

The inhibition of prostaglandin synthesis by 4-aminoantipyrine severely compromises fetal well-being during episodes of hyperthermia.

Progesterone antagonist lilopristone: A potent abortifacient in the common marmoset 248
Chander P. Puri, PhD, Rohini K. Patil, BSc, Sanjiv D. Kholkute, PhD, Walter A. G. Elger, MD, and Xavier R. Swamy, BA
Bombay, India, and Berlin, Federal Republic of Germany

Administration of ZK 98.734 around the time of implantation or during early or mid gestation terminates pregnancy in the common marmoset.

CORRESPONDENCE

Fetal death: Diagnosis and management Paul Merlob, MD *Petah Tikva, Israel* 254

Data sought to link adverse pregnancy outcome with maternal serum α-fetoprotein levels in black gravid women Roger R. Lenke, MD *Toledo, Ohio* 254

Reply James N. Macri, PhD, Ramana V. Kasturi, BE, MS, Mannshya G. Hu, PhD, David A. Krantz, BS, Timothy J. Douros, Paul Sajda, and Edward J. Cook, PhD *Carle Place, New York* 254

Frequency and timing of intravascular intrauterine transfusions reconsidered Liliana S. Voto, MD, and Miguel Margulies, MD *Buenos Aires, Argentina* 255

Reply Michael L. Socol, MD, Scott N. MacGregor, DO, Bruce W. Pielet, MD, Ralph K. Tamura, MD, and Rudy E. Sabbagha, MD *Chicago, Illinois* 255

Basic reference gambles recommended for utility assessment J. G. Thornton, MD, and R. J. Lilford, PhD *Leeds, England* 256

Reply Richard Silver, MD, and Jack Minogue, DMin *Evanston, Illinois* 257

Detection of fetal blood Joseph R. Leiberman, MD, Moshe Mazor, MD, and Adolph Cohen, MD *Beer Sheva, Israel* 257

Reply Michael S. Cardwell, MD *Columbia, Missouri* 257

Role of ferritin supported in diagnosis of anemias of pregnancy Robert C. Goodlin, MD *Denver, Colorado* 258

Reply Ron B. Schifman, MD *Tucson, Arizona* 258

Does terbutaline cause uterine atony and increase intraoperative blood loss? Mark S. Brown, MD *Columbus, Ohio* 259

Reply Michael S. Patriarco, DO *Allentown, Pennsylvania* 259

Oxytocin may explain neonatal seizures Robert C. Goodlin, MD *Denver, Colorado* 259

Contents

ITEMS

Items 260

INFORMATION FOR AUTHORS

Information for authors 25A

Index to advertisers on page 60A

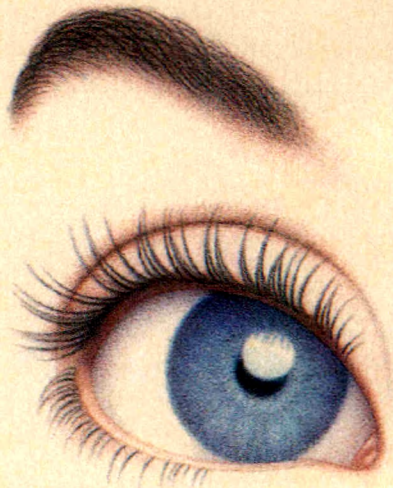
Make researching easier with the Ten-Year Cumulative Index (1978-1987) to the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY

The Ten-Year Cumulative Index is a complete guide to more than 30,000 pages of original articles, case reports, letters, and editorials published in the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY from 1978 through 1987 (volumes 130 to 157). Numbering more than 800 pages, the Cumulative Index includes both a subject and an author index. This hard-cover volume is $64.50 ($69.00 international). Prices include shipping. *Payment must accompany all orders and must be in U.S. funds, drawn on a U.S. bank.*

Contact The C.V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318 USA; phone (314) 872-8370, ext. 394. In the U.S., call toll-free: 1-800-325-4177, ext. 394. Please allow six weeks for delivery.

The Journal is abstracted and/or indexed in *Index Medicus, Science Citation Index, Current Contents/Clinical Medicine, Current Contents/Life Sciences, Excerpta Medica,* BRS Colleague, and MEDLINE.

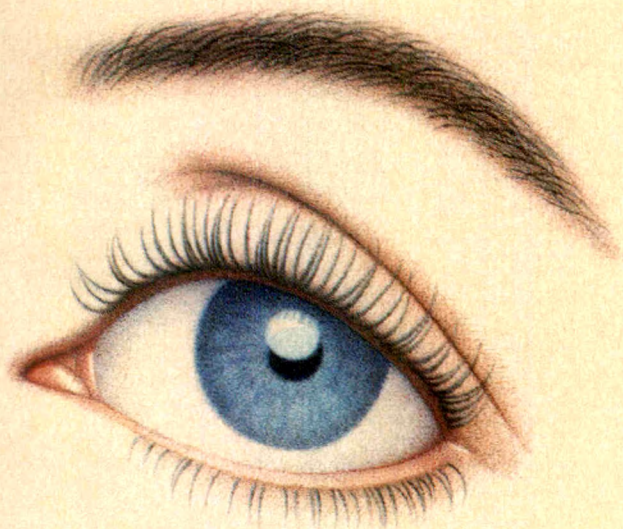
Authorization to photocopy items for internal or personal use, or the internal or personal use of specific clients, is granted by The C.V. Mosby Company for libraries and other users registered with the Copyright Clearance Center (CCC) Transactional Reporting Service, provided that 10¢ per page per copy is paid directly to CCC, 27 Congress St., Salem, MA 01970; phone (508)744-3350. The U.S. Government retains a nonexclusive, royalty-free license in and to any copyright on articles authored by government employees. 0002-9378/89 $00.00 + .10.



"When breakthrough bleeding happened to me I was scared, and that's why I called my doctor."

She deserves a





better start

TRI·LEVLEN®

Levonorgestrel and ethinyl estradiol tablets—Triphasic regimen

54%* less breakthrough bleeding† than Ortho-Novum® 7/7/7

Demonstrated in the largest parallel comparative trial of triphasic OCs to date[1]

*Serious as well as minor side effects have been reported with the use of all oral contraceptives. The physician should remain alert to the earliest symptoms of serious disease and discontinue oral contraceptive therapy when appropriate. Please see full prescribing information, a brief summary of which follows.

†100 women randomly assigned to Tri-Levlen® tablets or Ortho-Novum® 7/7/7; 50 women placed on nonhormonal contraception as a control. Evaluations were made at baseline and once a month for 6 months.

One cycle of Tri-Levlen® tablets therapy provides: ethinyl estradiol–30 mcg/day for 6 days, 40 mcg/day for 5 days and 30 mcg/day for 10 days; levonorgestrel–50 mcg/day for 6 days, 75 mcg/day for 5 days and 125 mcg/day for 10 days. One cycle of Ortho-Novum® 7/7/7 therapy provides: ethinyl estradiol–35 mcg/day for 21 days; norethindrone–500 mcg/day for 7 days, 750 mcg/day for 7 days and 1000 mcg/day for 7 days.

Ortho-Novum® is a registered trademark of Ortho Pharmaceutical Corp.

BERLEX™ Dedicated to innovation in OB/GYN medicine

TRI-LEVLEN® 28 Levonorgestrel and ethinyl estradiol
TRI-LEVLEN® 21 tablets–Triphasic regimen

BRIEF SUMMARY

Tri-Levlen®—6 brown tablets, each containing 0.050 mg of levonorgestrel (d(-)-13 beta-ethyl-17-alpha-ethinyl-17-beta-hydroxy-gon-4-en-3-one), a totally synthetic progestogen, and 0.030 mg of ethinyl estradiol (19-nor-17α-pregna-1,3,5(10)-trien-20-yne-3, 17-diol); 5 white tablets, each containing 0.075 mg levonorgestrel and 0.040 mg ethinyl estradiol; 10 light-yellow tablets, each containing 0.125 mg levonorgestrel and 0.030 mg ethinyl estradiol (7 light-green tablets containing inert ingredients are included in the 28-day triphasic regimen).

Indications and Usage—Tri-Levlen Tablets are indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OCs) as a method of contraception.

Contraindications—OCs should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5). 8. Benign or malignant liver tumor which developed during the use of OCs or other estrogen-containing products.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

Thromboembolic Disorders and Other Vascular Problems: An increased risk of thromboembolic and thrombotic disease associated with the use of OCs is well established. Three principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OCs are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION—An increased risk of myocardial infarction associated with the use of OCs has been reported, confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing myocardial infarction, regardless of whether the patient was an OC user or not. OCs, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE—In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism, including coronary thrombosis, is directly related to the dose of estrogen used in OCs. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the U.K. estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OCs according to age, smoking habits and duration of use. The overall excess death rate annually from circulatory diseases for OC users was estimated to be 1 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), the risk being concentrated in older women, in those with a long duration of use and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking and duration of use, or to compare the effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. The available data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. The estimates of risk of death for each method include the combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OCs) plus the risk attributable to pregnancy or abortion in the event of method failure. This latter risk varies with the effectiveness of the contraceptive method. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OCs in women over 40 who smoke. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with OCs increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes or history of preeclamptic toxemia and, especially, by cigarette smoking. The physician and the patient should be alert to the earliest manifestations of thrombophlebitis and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four-to-six-fold increased risk of postsurgery thromboembolic complications has been reported in OC users. If feasible, OCs should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Great Britain involving cerebrovascular disease and other study in the United States concerning myocardial infarction suggest that an increased risk of these conditions in users of OCs persists after discontinuation of the OC. In the British study, the risk of cerebrovascular disease remained elevated in former OC users for at least six years after discontinuation. In the U.S. study, an increased risk of myocardial infarction persisted for at least 9 years in women 40- to 49-years-old who had used OCs for five or more years. The findings in both these studies require confirmation since they are inconsistent with other published information.

Ocular Lesions: There have been reports of neuro-ocular lesions, such as optic neuritis or retinal thrombosis, associated with the use of OCs. Discontinue OC medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

Carcinoma: Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina and liver. Certain synthetic progestogens, none currently contained in OCs, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case-control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of endometrial carcinoma of the endometrium in women under 40 on OCs. Of the cases found in women without predisposing risk factors for endometrial carcinoma of the endometrium (e.g., irregular bleeding at the time OCs were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs. Several studies have found no increase in breast cancer in women taking OCs or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with OCs, found an excess risk in the subgroups of OC users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of OCs has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with OCs. Close clinical surveillance of all women taking OCs is nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use OCs.

Hepatic Tumors: Benign hepatic adenomas have been found to be associated with the use of OCs. One study showed that OC formulations with high hormonal potency were associated with a higher risk than lower potency formulations. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of OCs. Two studies relate risk with duration of use of OCs, the risk being much greater after 4 or more years of use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking OCs. The relationship of these drugs to this type of malignancy is not known at this time.

Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring and Malignancy in Female Offspring: The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been known that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a rare form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence at the present time that OCs further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OCs. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are biologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be assumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including OCs, in pregnancy. One case-control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses in women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. It is recommended that, for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period and further use of OCs should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue OCs with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease:* Studies report an increased risk of surgically confirmed gallbladder disease in users of OCs and estrogens. In one study, an increased risk appeared after 2 years of use and doubled after 4 or 5 years of use. In one of the other studies, increased risk was apparent between 6 and 12 months of use.

7. *Carbohydrate and Lipid Metabolic Effects:* A decrease in glucose tolerance has been observed in a significant percentage of patients on OCs. For this reason, prediabetic and diabetic patients should be carefully observed while receiving OCs. An increase in triglycerides and total phospholipids has been observed in patients receiving OCs. Three studies have been performed with the Tri-Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets Triphasic Regimen) formulation and no significant alterations in lipid metabolism were noted, with the exception of a slight increase in triglyceride levels in one study. The clinical significance of the findings remains to be defined.

8. *Elevated Blood Pressure:* An increase in blood pressure has been reported in patients receiving OCs. In some women, hypertension may occur within a few months of beginning OC use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure and in the fifth year of use is two-and-a-half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given OCs. Hypertension that develops as a result of taking OCs usually returns to normal after discontinuing the drug.

9. *Headache:* The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent or severe, requires discontinuation of OCs and evaluation of the cause.

10. *Bleeding Irregularities:* Breakthrough bleeding, spotting and amenorrhea are frequent reasons for patients discontinuing OCs. Breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. Undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea in young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of OCs. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. *Breast-feeding:* OCs given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in OCs has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of OCs should be deferred until the infant has been weaned.

Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OCs. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical examination and PAP smear being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. OCs may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving OC therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by OC therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of OC therapy when relevant specimens are submitted. 10. Certain endocrine and liver-function tests and blood components may be affected by estrogen-containing OCs: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX and X; decreased antithrombin 3, increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test.

Information for the Patient—See Patient Package Labeling

Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Carcinogenesis—See "Warnings" section for information on the carcinogenic potential of OCs.

Pregnancy—Pregnancy Category X. See "Contraindications" and "Warnings."

Nursing Mothers—See "Warnings."

Adverse Reactions—An increased risk of the following serious adverse reactions has been associated with the use of OCs (see "Warnings"): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies.

There is evidence of an association between the following conditions and the use of OCs, although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients receiving OCs and are believed to be drug-related: Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally: gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; dysmenorrhea; amenorrhea during and after treatment; temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement and secretion; change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses.

The following adverse reactions have been reported in users of OCs, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, hemolytic uremic syndrome.

Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OCs by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Dosage and Administration—To achieve maximum contraceptive effectiveness, Tri-Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets—Triphasic Regimen) must be taken exactly as directed and at intervals not exceeding 24 hours. (If Tri-Levlen is first taken later than the first day of the first menstrual cycle of medication or postpartum, contraceptive reliance should not be placed on it until after the first 7 consecutive days of administration. The possibility of ovulation and conception prior to initiation of medication should be considered.) Any time the patient misses 1 or 2 brown, white or light-yellow tablets, she should also use another method of contraception until she has taken a tablet daily for 7 consecutive days.

For full details on dosage and administration see prescribing information in package insert. 6061I-1 5/86

Reference: 1. Data on file, Berlex Laboratories, Inc.

TRI-LEVLEN is a registered trademark of Berlex Laboratories, Inc.

TRI-LEVLEN®
Levonorgestrel and ethinyl estradiol tablets–
Triphasic regimen

BERLEX™
Dedicated to innovation in OB/GYN medicine

BERLEX Laboratories, Inc., Wayne, New Jersey 07470

© 1989, Berlex Laboratories, Inc. All rights reserved. 430-118

P 24, 295

American Journal of Obstetrics and Gynecology

Editors

JOHN I. BREWER, *Editor in Chief*
Sandra Fernandez, *Senior Editorial Assistant*

FREDERICK P. ZUSPAN, *Editor*
Donna Peterson, *Editorial Assistant*

E. J. QUILLIGAN, *Editor*
Sherry A. Merryman, *Editorial Assistant*

ALBERT B. GERBIE, *Associate Editor*

Advisory committee on policy

Garland Anderson
William R. Anderson
Mason C. Andrews
E. Forrest Boyd
David C. Figge
A. Jerome Freeland
Edgar O. Horger III
W. Glenn Hurt
James M. Ingram
Walter S. Keifer
Emmet J. Lamb
Roger A. Lobo
Douglas J. Marchant
James H. Maxwell
Arthur M. McCausland
Allan G. W. McLeod
James A. Merrill
Daniel R. Mishell, Jr.
John C. Morrison
Carl J. Pauerstein
Richard Paul
Karl C. Podratz
John A. Read
Ruth W. Schwartz
Henry A. Thiede
Carol Trumbold

Board of corresponding editors

Stuart Campbell, *England*
Luigi Carenza, *Italy*
Eliahu Sadovsky, *Israel*
Nils-Otto Sjöberg, *Sweden*
Masakuni Suzuki, *Japan*

Publisher
THE C.V. MOSBY COMPANY, 11830 Westline Industrial Drive,
St. Louis, Missouri 63146-3318
Kay G. Goehler, *Senior Manuscript Editor*

Michael Wilson
BA: Iowa
MD: Iowa
Residency: Mayo Clinic
Specialty: Orthopedic Surgery
AFB: Lackland

For career and country: Why Dr Mike Wilson became an Air Force physician

"When I finished my residency at the Mayo Clinic, I headed home to Iowa to fulfill my dream of being a community-based orthopedic surgeon. But after several years in which it seemed I spent more time shuffling papers than seeing patients, I realized I was missing the things I wanted from my career in medicine. Helping. Teaching. Learning.

"That's when I investigated the Air Force. I was impressed by what it could offer: The opportunity to see, treat, and follow up on a diverse, interesting population; the academic stimulation of preparing and participating in teaching conferences; the chance to be part of a team that has a clearly defined, vital mission in the world.

"Sure, I don't gross as much as my peers in private practice, but I don't pay out as much, either. Health insurance is not necessary and I earn 30 days of vacation with pay each year; I don't pay a penny for malpractice insurance, staff salaries, or rent. I even receive an untaxed housing allowance, and an excellent, non-contributory pension plan.

"Plus, there's the intangible satisfaction of doing something meaningful for a country that, frankly, has been very good to me.

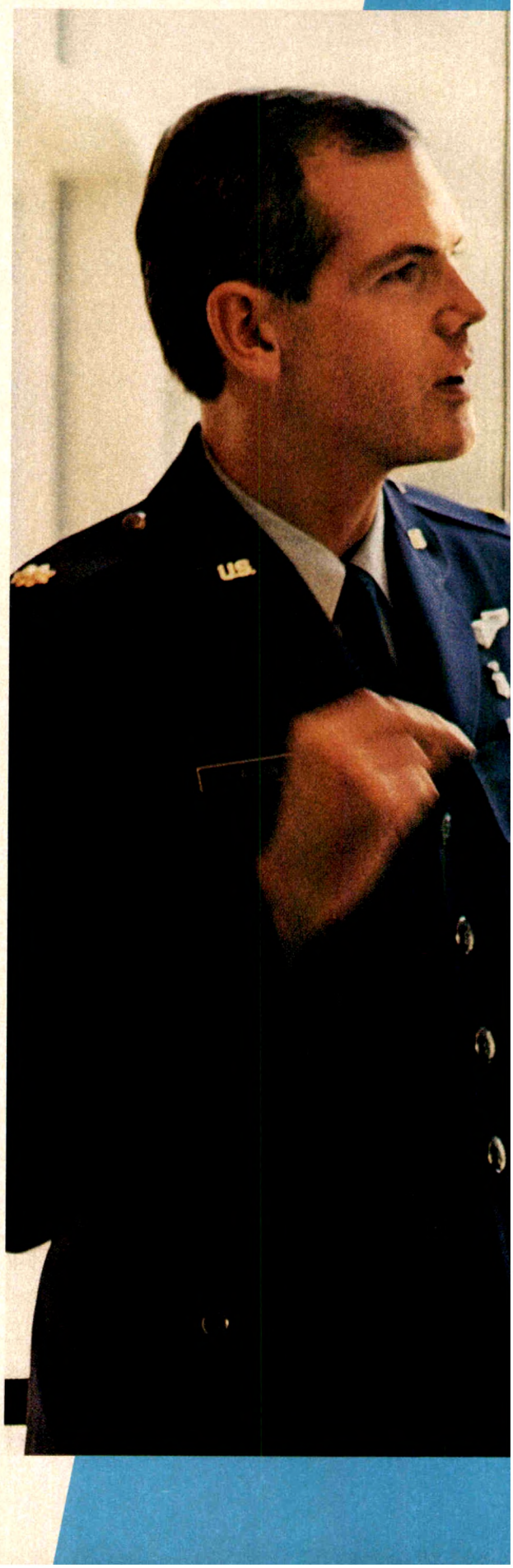
"For the right person, being an Air Force physician is the best practice in the world. It is for me."

Wilson MD

For career and country, find out more about becoming an Air Force physician. Call toll-free 1-800-423-USAF. Or send your curriculum vitae to Col William E. Patterson, HQ USAFRS/RSH, Randolph AFB, TX 78150.

Expand your horizons.
Become an Air Force physician.

AIR FORCE



Charles Johnson
BS: Air Force Academy
MD: Univ. of Southern California
Specialty: Ear, Nose, & Throat

James Spies
BS: Univ. of California (Davis)
MD: Georgetown
Specialty: Radiology



American Journal of Obstetrics and Gynecology

Societies

in addition to those listed on the front cover
the Journal is the official publication
of the following societies

NEW YORK OBSTETRICAL SOCIETY

OBSTETRICAL SOCIETY OF PHILADELPHIA

BROOKLYN GYNECOLOGICAL SOCIETY

ST. LOUIS GYNECOLOGICAL SOCIETY

NEW ORLEANS GYNECOLOGICAL AND OBSTETRICAL SOCIETY

THE OBSTETRICAL AND GYNECOLOGICAL SOCIETY OF MARYLAND

CHICAGO GYNECOLOGICAL SOCIETY

CINCINNATI OBSTETRICAL AND GYNECOLOGICAL SOCIETY

WASHINGTON GYNECOLOGICAL SOCIETY

PITTSBURGH OBSTETRICAL AND GYNECOLOGICAL SOCIETY

BOSTON OBSTETRICAL SOCIETY

LOUISVILLE OBSTETRICAL AND GYNECOLOGICAL SOCIETY

SEATTLE GYNECOLOGICAL SOCIETY

ALABAMA ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS

AKRON OBSTETRICAL AND GYNECOLOGICAL SOCIETY

KANSAS CITY GYNECOLOGICAL SOCIETY

CENTRAL NEW YORK ASSOCIATION OF GYNECOLOGISTS
AND OBSTETRICIANS

NEW JERSEY OBSTETRICAL AND GYNECOLOGICAL SOCIETY

IOWA OBSTETRIC AND GYNECOLOGIC SOCIETY

THE TEXAS ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS

OKLAHOMA CITY OBSTETRICAL AND GYNECOLOGICAL SOCIETY

MEMPHIS OBSTETRICAL AND GYNECOLOGICAL SOCIETY

UTAH OBSTETRICAL AND GYNECOLOGICAL SOCIETY

ROCHESTER OBSTETRICAL AND GYNECOLOGICAL SOCIETY

ARKANSAS OBSTETRICAL AND GYNECOLOGICAL SOCIETY

TENNESSEE STATE OBSTETRICAL AND GYNECOLOGICAL SOCIETY

NEW YORK GYNECOLOGICAL SOCIETY

PACIFIC NORTHWEST OBSTETRICAL AND GYNECOLOGICAL ASSOCIATION

BUFFALO OBSTETRICAL AND GYNECOLOGICAL SOCIETY

SAN FRANCISCO GYNECOLOGICAL SOCIETY

JACKSON GYNECIC SOCIETY

INDIANA OBSTETRICAL AND GYNECOLOGICAL SOCIETY

THE MINNESOTA OBSTETRICAL AND GYNECOLOGICAL SOCIETY

COLUMBUS OBSTETRIC AND GYNECOLOGIC SOCIETY

GREATER HARTFORD OBSTETRICAL AND GYNECOLOGICAL SOCIETY

American Journal of Obstetrics and Gynecology

Copyright © 1989 by The C.V. Mosby Company

Information for Authors

Editors

John I. Brewer, *Editor in Chief*
710 North Fairbanks Court, Suite 8300,
Chicago, IL 60611

Sandra Fernandez, *Senior Editorial Assistant*
Tel.: (312) 908-5266

Frederick P. Zuspan, *Editor*
The Ohio State University, Fifth Floor, Means Hall,
1654 Upham Dr., Columbus, OH 43210

Donna Peterson, *Editorial Assistant*
Tel.: (614) 293-8798

E. J. Quilligan, *Editor*
University of California, Irvine, California College of Medicine, Med Surge 1, Room 109H, Irvine, CA 92717

Sherry A. Merryman, *Editorial Assistant*
Tel.: (714) 856-5092

Albert B. Gerbie, *Associate Editor*
710 North Fairbanks Court, Suite 8300,
Chicago, IL 60611

Publisher
THE C.V. MOSBY COMPANY, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318

Kay G. Goehler, *Senior Manuscript Editor*
Tel.: (314) 872-8370 (ext. 591)

INFORMATION FOR AUTHORS

Editorial policies

The requirements for manuscripts submitted to the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY conform to the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" established by the International Committee of Medical Journal Editors and published in *Annals of Internal Medicine* 1988;108:258-65. Certain requirements unique to our JOURNAL are provided in Information for Authors, published in each issue of the JOURNAL, and in more detail in the *Guide to Writing for the American Journal of Obstetrics and Gynecology*. The latter may be obtained from The C.V. Mosby Company or the Editors on request.

Manuscript submission. Manuscripts should be submitted to one of the three Editors as follows:

1. **Dr. Brewer**—all manuscripts originating from the southeastern quadrant of the United States or Canada and those presented before one of the official sponsoring societies, except the Society for Gynecologic Investigation and The Society of Perinatal Obstetricians.

2. **Dr. Zuspan**—manuscripts from the northeastern quadrant of the United States and from Japan, Israel, Italy, and England, manuscripts written for Clinical Opinion and Current Development, and Letters to the Editors. Dr. Zuspan is responsible for manuscripts from The Society of Perinatal Obstetricians. Manuscripts from The Society of Perinatal Obstetricians should be submitted to Dr. John A. Read, 9102 Lake Steilacoom Point Road, S.W., Tacoma, WA 98498.

3. **Dr. Quilligan**—manuscripts from the north central states (including Ohio), states west of the Mississippi River, Hawaii, Alaska, and abroad (except Japan, Israel, Italy, and England). Dr. Quilligan is responsible for manuscripts from the Society for Gynecologic Investigation. Manuscripts from the Society for Gynecologic Investigation should be submitted to Dr. Roger A. Lobo, Women's Hospital, 1240 North Mission Road, Room 1M2, Los Angeles, CA 90033.

Author's designation of reviewers. When authors submit their manuscripts, they may provide the names and addresses of three reviewers for consideration by the Editors.

Copyright statement. Effective July 1, 1988, all manuscripts must be accompanied by the following written statement, signed by *all* authors: "The undersigned author(s) transfers all copyright ownership of the manuscript [title of article] to The C.V. Mosby Company in the event the work is published. The undersigned author(s) warrants that the article is original; is not under consideration by another publication; and its essential substance, tables, or figures have not been previously published. This restriction does not apply to abstracts or press reports published in connection with scientific meetings. The author(s) confirms the final manuscript has been read and each author's contribution has been approved by the appropriate author. The author(s) responsible for the manuscript must be identified."

Previous publication. If a report by the same author(s) has been previously published in any medium that deals in any respect whatever with the same patients, same animals, same laboratory experiments, or same data, in part or in full, as those reported in the manuscript being submitted, two reprints of the article or two copies of the manuscript, be it a full-length report or an abstract, must be submitted with the manuscript. The author(s) should inform the Editor of the circumstances of the two reports. This requirement also applies to the submission of a manuscript in which a few different patients, animals, laboratory experiments, or data were added to those reported in a previous publication or in a submitted or accepted manuscript. Articles previously published in another language will not be considered.

Human and animal experimentation. It is assumed by the Editors that manuscripts emanating from a particular

institution are submitted with the approval of the requisite authority. Human experimentation that requires local institutional approval must have this approval *before the experiment is started* and approval must be so indicated in the Methods section of the submitted manuscript. Reports of experiments on animals must *state in the Methods section of the manuscript* that the guidelines for the care and use of the animals *approved by the local institution* were followed.

Authorship. For manuscripts with two or more authors, each author must qualify by having participated actively and sufficiently in the study that is being performed and reported. The inclusion of each author in the authorship list of a report is based only (1) on substantial contributions to (a) concept and design, or analysis and interpretation of data and (b) drafting the manuscript or revising it critically for important intellectual content; and (2) on final approval by each author of the version of the manuscript. Conditions 1 (a and b) and 2 must both be met. Others contributing to the work should be recognized separately in an Acknowledgment. In the covering letter that accompanies the submitted manuscript, it must be confirmed that all authors fulfilled both conditions.

Conflict of interest. Authors are expected to inform the Editor, in a letter accompanying the submitted manuscript, of any commercial association that might pose a conflict of interest, such as ownership, stock holdings, equity interests and consultant activities, or patent-licensing situations. Such information is confidential, is not given to the consultants, and does not play a part in the decision of the quality or timeliness of the manuscript. If the manuscript is accepted, the author and the Editor will determine how best to release the information. The usual and customary listing of sources of support and institutional affiliations on the title page is proper and does not imply a conflict of interest; only where there is a possible conflict of interest is the author(s) expected to inform the Editor.

Disclaimer. Statements and opinions expressed in articles and communications herein are those of the author(s) and not necessarily those of the Editor(s) or publisher, and the Editor(s) and publisher disclaim any responsibility or liability for such material. Neither the Editor(s) nor the publisher guarantees, warrants, or endorses any product or service advertised in this publication, nor do they guarantee any claim made by the manufacturer of such product or service.

General requirements for preparation of manuscripts

The original and two good-quality photocopies of the manuscript and three sets of glossy prints of illustrations are required.

Manuscripts must be typed double-spaced on one side only of 22 × 28 cm (8½ × 11 inch) white bond paper with 1-inch margins at top, bottom, and sides. Number pages consecutively in the upper right-hand corner in the following order: title page, condensation, abstract, body of text, acknowledgments, references, legends, and tables.

Title page. The title page (page 1) should contain in sequence the title (concise and suitable for indexing purposes); author line with first name, middle initial, and last name of each author and each author's highest academic degree (both MD and PhD are acceptable); city(ies), state(s) in which the study was conducted; divisional, or departmental, and institutional affiliations at the time the study was performed; source(s) of financial support; presented line, if applicable; disclaimers, if any; name, address, and business and home telephone numbers of author to whom requests for reprints should be addressed (if reprints will not be available, it should be so stated); and name, address, and business and home telephone numbers of author responsible for correspondence concerning the manuscript if different from author to whom reprint requests are addressed. At the bottom of the title page supply a short title for the running head not exceeding 52 characters (including word spaces).

Condensation. On page 2 of the manuscript provide a brief, concise condensation, typed double-spaced, that will appear with the title in publication of the Contents pages of the JOURNAL. It should be a single sentence, limited to a maximum of 25 words, delineating the essential point(s) made in the manuscript.

Abstract page and key words/phrases. On manuscript page 3 type the abstract, double-spaced, with the required margins and headed by the title of the article and name(s) of author(s). Abstracts for regular articles, Current Investigation, Clinical Opinion, and Current Development may not exceed 150 words. Abstracts for case reports and brief communications may not exceed 50 words. Below the abstract list 3 to 5 *key words* or short phrases for indexing purposes.

Text. Do not hesitate to write your manuscript in the first-person, active voice if it is more appropriate to the information you wish to convey. The passive voice is generally more effective for describing techniques or observations, since the emphasis is on the "action" rather than on the person performing the action.

Only standard abbreviations are to be used. Consult the *Council of Biology Editors Style Manual* or the AMA's *Manual for Authors and Editors*. Abbreviations in the title are not acceptable. They should be avoided, if possible, in the abstract. In the text they should be kept to a practical minimum. The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement.

Either the generic, chemical, or proprietary names of drugs may be used. If the generic or chemical name is used, authors may, if they desire, insert the proprietary name in parentheses after the first mention in the text, with the name of the manufacturer and city and state.

Regular articles are customarily organized into the following sections: an introduction and headings that identify Material and Methods, Results, and Comment. Authors may wish to summarize their findings in a short paragraph at the end of the Comment section. This format may not be appropriate for some types of articles.

In the introduction, state concisely the purpose and rationale for the study and cite only the most pertinent references as background.

In the *Material and Methods* section describe briefly (but in sufficient detail to permit other workers to evaluate and reproduce the results) the plan, patients and/or experimental animals and controls, methods and procedures utilized, and statistical method(s) employed.

In the *Results* section present the detailed findings. Include mentions of all tables and/or figures. Avoid duplication of text and supporting material. Emphasize only your important observations; do not compare your

Estimating length of manuscripts

The length of text material (introduction through Comment section) in regular manuscripts accepted for publication normally ranges from 750 to 4200 words (an average of 2000 words). A 4200-word text can seldom be accepted, especially if tables and figures are included. The average manuscript of 2000 words of text with abstract, 3 tables with captions, 2 figures with legends, and references makes a 5.7-page article in the JOURNAL. The 2000 words of text alone make approximately 8 pages of manuscript typed double-spaced with the required 1-inch margins (approximately 250 words per page). A table or figure that occupies both columns of half a JOURNAL page is equivalent to approximately 500 words in manuscript. Thus, if a greater number of illustrations and tables are used, the length of the text should be adjusted accordingly.

observations with those of others. Such comparisons and comments are reserved for the Comment section.

In the *Comment* section state the importance and significance of your findings but do not repeat the details given in the Results section. Limit your opinions to those strictly indicated by the facts in your report. Compare your findings with those of others. No new data should be presented in this section.

Acknowledgments. Acknowledge only persons who have made substantive contributions to the study.

References. A reasonable number are allowed, except in case reports and brief communications (limited to 2) and in manuscripts for the Current Development section (for which there is no limit). Number references consecutively in the order in which they are mentioned in the text. Use the format of the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" (Vancouver style) (Ann Intern Med 1988;108:258-65). Journal titles should conform to abbreviations used in *Cumulated Index Medicus*.

Examples (if six or fewer authors, list all; if seven or more authors, list three then et al.):

JOURNAL: Flamm BL, Fischermann E, Quilligan EJ, et al. Vaginal delivery following cesarean section—use of oxytocin augmentation and epidural anesthesia. AM J OBSTET GYNECOL 1984;148:759-63.

BOOKS: James VHT, Folkerd EJ, Bonney RC, Beranek PA, Reed MJ. Factors influencing estrogen production and metabolism in postmenopausal women with endocrine cancer. In: van Herendael HB, Riphagen FE, Goessens L, van der Pas H, eds. The climacteric, an update. Lancaster, England: MTP Press, 1983:29.

Personal communications and unpublished data, if essential, may be used but not as numbered references. If they are used, they are to be referred to, within parentheses, at the appropriate location in the text. If used, the author(s) must obtain written and signed permission for their use from the individual being quoted. This signed permission must accompany the manuscript when it is submitted to the Editor. Abstracts are not acceptable as numbered references.

Illustrations and tables. Illustrations and tables should supplement, not duplicate, the text; presentation of data in either one or the other will suffice.

A reasonable number of halftone and line illustrations will be reproduced without charge, but special arrangements must be made with the Editors for *color illustrations* at a cost of $525 per page (one side).

For *color photographs* submit original transparencies and two sets of unmounted prints on glossy (smooth-surface) paper. Polaroid prints are not acceptable. Color transparencies must have a color balance (consistency in lighting and film speed) that is acceptable to the author and Editors before acceptance for publication. Please note that 35 mm transparencies are enlarged to twice their original size. If it is important to deviate from this standard, please so indicate when the material is submitted. The *top, first author's last name,* and *figure number* must be indicated on the front of each transparency and the back of each print. Consistency in size of illustrations within the article is strongly preferred.

For *black-and-white illustrations* submit three sets of 3 × 4 inch (minimum) to 5 × 7 inch (maximum) unmounted, glossy photographic prints. All lettering must be done with commercially available paste-on letters (or numbers) or by a professional; typed or freehand lettering is not acceptable. All lettering must be in proportion to the drawing, graph, or photograph. Original drawings, appropriately done in black India ink, roentgenograms, and other material must be submitted as glossy photographic prints with good black-and-white contrast. Consistency in size within the article is strongly preferred. Any special instructions regarding sizing should be clearly noted.

Do not use paper clips or mar the surface of prints in any way.

Figures must be cited consecutively in the text in Arabic numerals and identified thusly on the back of the print (gummed label with): author(s) name(s), title of article, number, and top marked clearly.

Figures will be returned only on request by the author.

Tables should be typed on separate sheets of paper, one table to a page, and included at the end of the text. They should be numbered in Roman numerals. Each table must be cited in sequence at an appropriate point in the text. Captions should be brief yet indicate clearly the purpose or content of each table, and each column should be precisely defined by headings. Abbreviations and special designations should be explained in a footnote to the table. *If a table or any part thereof has been taken from copyrighted material, a legend to the table must give full credit to the original source.* Special arrangements must be made with the Editors for elaborate tables because of space limitations.

Legends to illustrations. Legends for all figures must be typed double-spaced on paper separate from the text of the manuscript, and these pages must be numbered in sequence after the references. Titles should be included in

the legend, *not* on the print. Original magnifications should be provided. *If an illustration has been taken from copyrighted material, the legend must give full credit to the original source.*

Computer-generated illustrations. *Black-and-white illustrations* submitted must be legible and clearly printed in jet-black ink on heavy coated paper with either a glossy or dull finish. Any patterns or shadings must be dark enough for reproduction and must be distinguishable from each other. Lines, symbols, and letters should be both smooth and complete. The legend for the illustration should not appear on the print. On the back of each print the name of the first author and the figure number should be given and the top indicated. Original individual laser or plotter prints are to be submitted *unmounted* with the manuscript. Laser prints should be full size at 300 dots per inch (DPI) or greater full-page resolution; multiple illustrations on a page cannot be accepted. Dot matrix prints and photographic halftones are not acceptable. *Color illustrations* are acceptable, but special arrangements must be made with the Editors. The colors used must be dark enough and of sufficient contrast for reproduction. With the exception of fluorescent colors, all colors can be reproduced in four-color illustrations. The preparation and submitting of color prints should follow the preceding guidelines for black-and-white computer-generated illustrations.

Permissions. Direct quotations, tables, or illustrations that have appeared in copyrighted material must be accompanied by *written permission* for their use from the copyright owner and original author along with complete information as to source. Photographs of identifiable persons must be accompanied by signed releases or else all recognizable features masked.

Requirements for special sections

Case reports and brief clinical and basic science communications. Limit of 700 words, 2 references. Include abstract of 50 words maximum, 3 to 5 key words/phrases for indexing purposes, and short title. If tables and/or figures are used, an equivalent number of words must be deducted from the total (see "Estimating Length of Manuscript").

Current Investigation. Same requirements as for regular article.

Clinical Opinion. Limit of 3000 words. Include abstract of 50 to 150 words, 3 to 5 key words/phrases, and short title. Submit to Dr. Zuspan.

Current Development. Limit of 6000 words. Include abstract of 50 to 150 words, 3 to 5 key words/phrases, and short title. Submit to Dr. Zuspan.

Correspondence. Two types of correspondence will be considered for publication. (1) A Letter to the Editors commenting on an article that has appeared in the JOURNAL should be brief and directly related to the published article. The editorial staff reserves the right to shorten letters if necessary and to make minor editorial alterations without reference to the writer. Letters may be published together with a reply from the original author. If the original author does not respond, a notation indicating "Response declined" will be published. As space for letters is limited, only a selection of letters submitted may be published. (2) A brief case presentation or a short report of a pertinent observation in the form of a Letter to the Editors will be considered for publication. All letters should be typed double-spaced. The original and a good photocopy must be submitted. Letters should be sent to Dr. Zuspan.

Announcements. Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60 U.S., and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, The C.V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, MO 63146-3318.

Books. Books received will be listed in the JOURNAL. They should be sent to Dr. Gerbie. No books will be returned.

Reprints

Reprints of articles must be obtained from the author. The corresponding author will receive a price schedule and order form at the time of publication. Reprints in quantities must be ordered from the publisher with the author's consent.

Business communications

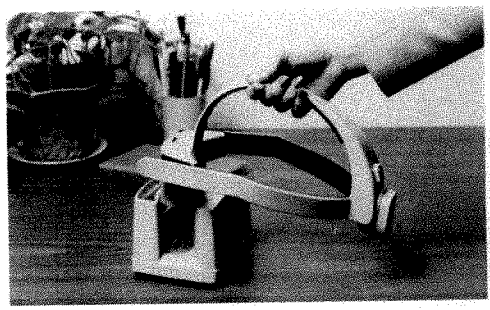
Communications of a business nature and all advertising communications should be addressed to: Journal Publisher, The C.V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, MO 63146-3318, or call Journal Advertising Production Manager (314) 872-8370.

Checklist

—Letter of submission
—Copyright transfer letter
—Copies of other manuscripts containing duplicated data (see paragraph "Previous Publication")
—Original and two photocopies of manuscript
—Title page
  —Title of article
  —Full name(s), highest academic degree(s), and affiliations of author(s)
  —Line citing financial support
  —Author to whom correspondence is to be sent, including address and business and home telephone numbers
  —Reprint requests line or line stating reprints not available
  —Short title
—Condensation (double-spaced)
—Abstract (double-spaced), 3 to 5 key words/phrases
—Article proper (double-spaced)
—References (double-spaced), on a separate sheet
—Legends (double-spaced), on a separate sheet
—Tables (double-spaced), on a separate sheet
— Illustrations, properly labeled (three sets of glossy prints)
—Permission to reproduce published material
—Informed consent for patient photographs

TIRED OF BEING TIED DOWN?
Introducing
THE RIGHT LIGHT™





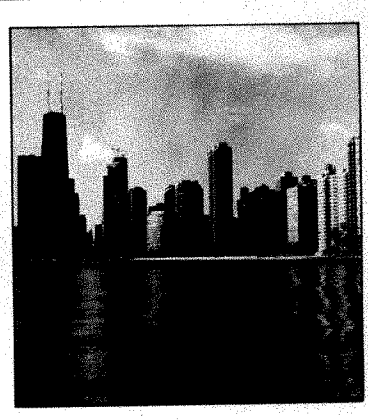
FINALLY, an examination light that allows you the freedom you wanted. There are no restrictive cords tying you to an electrical outlet. Both hands are free. Developed through the efforts of a physician team and an electrical engineer, THE RIGHT LIGHT™ answers the need for a reliable, cordless, lightweight head-mounted illuminator.

- Portable (No umbilical cord)
- Rechargeable
- High intensity halogen beam
- High capacity powerpack
- Easily adjusted headband
- Light lowers from headband for optimum line-of-sight illumination
- Swivel directed light beam
- Desk or wall-mounted charger
- Charger UL listed
- Recharges overnight
- Charging indicator
- Lasts 2 hours per charge
- ON-OFF switch to conserve energy

IdenTechs

To order your RIGHT LIGHT™ call 1-800-545-6775. In Illinois 312-690-0177. The price of $220.00 includes tax, shipping and handling. Visa and MasterCard charge accepted. If you prefer to order by mail send a check or money order to IdenTechs, P.O. Box 524, Wheaton, Illinois 60187.

Specialty Review in Obstetrics and Gynecology: Practical Aspects



October 15 – 21, 1989

• Chicago •

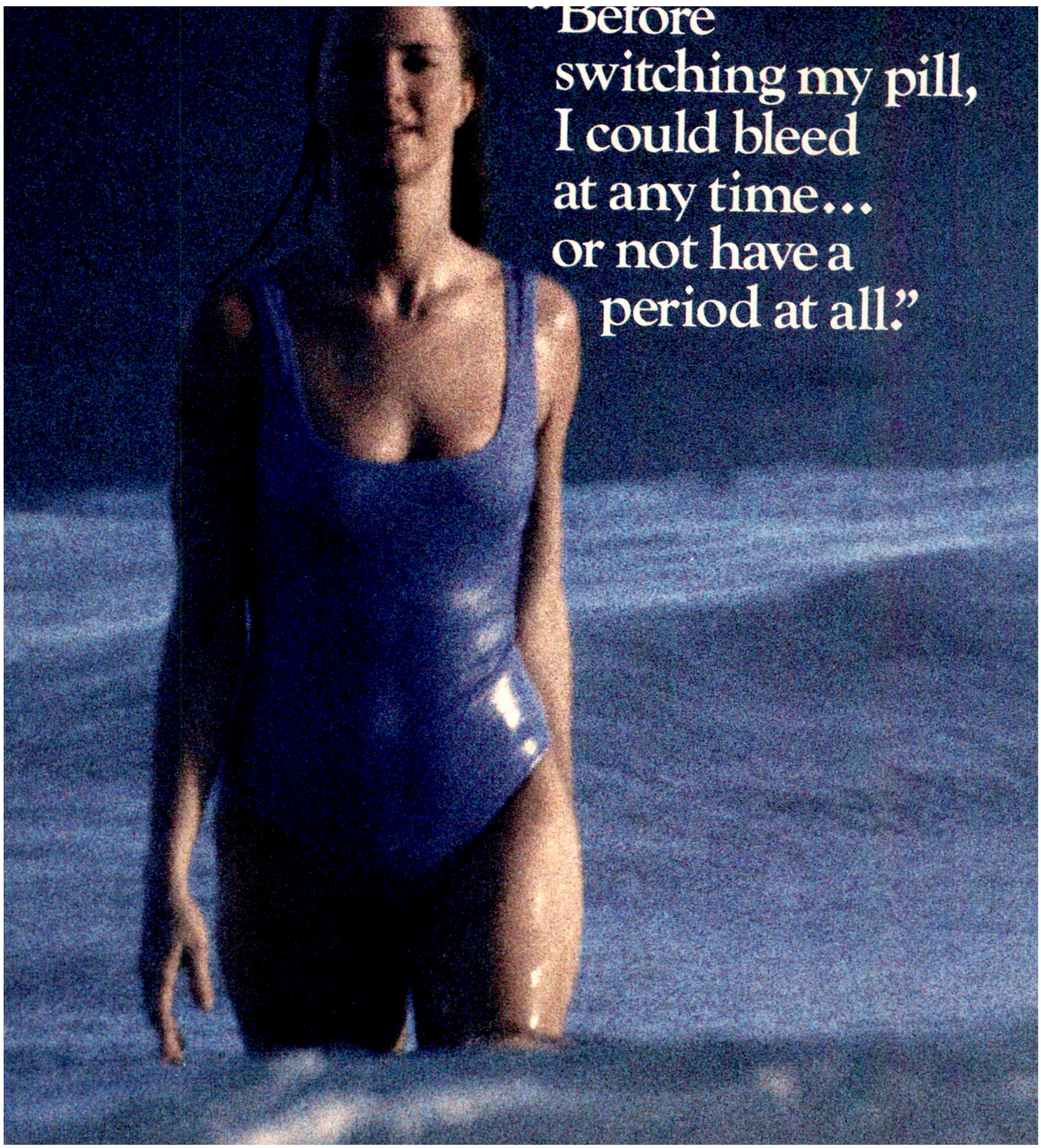
Course Coordinator: M. LeRoy Sprang, M.D., Assistant Professor of Clinical Obstetrics and Gynecology, Northwestern University Medical School, Chicago

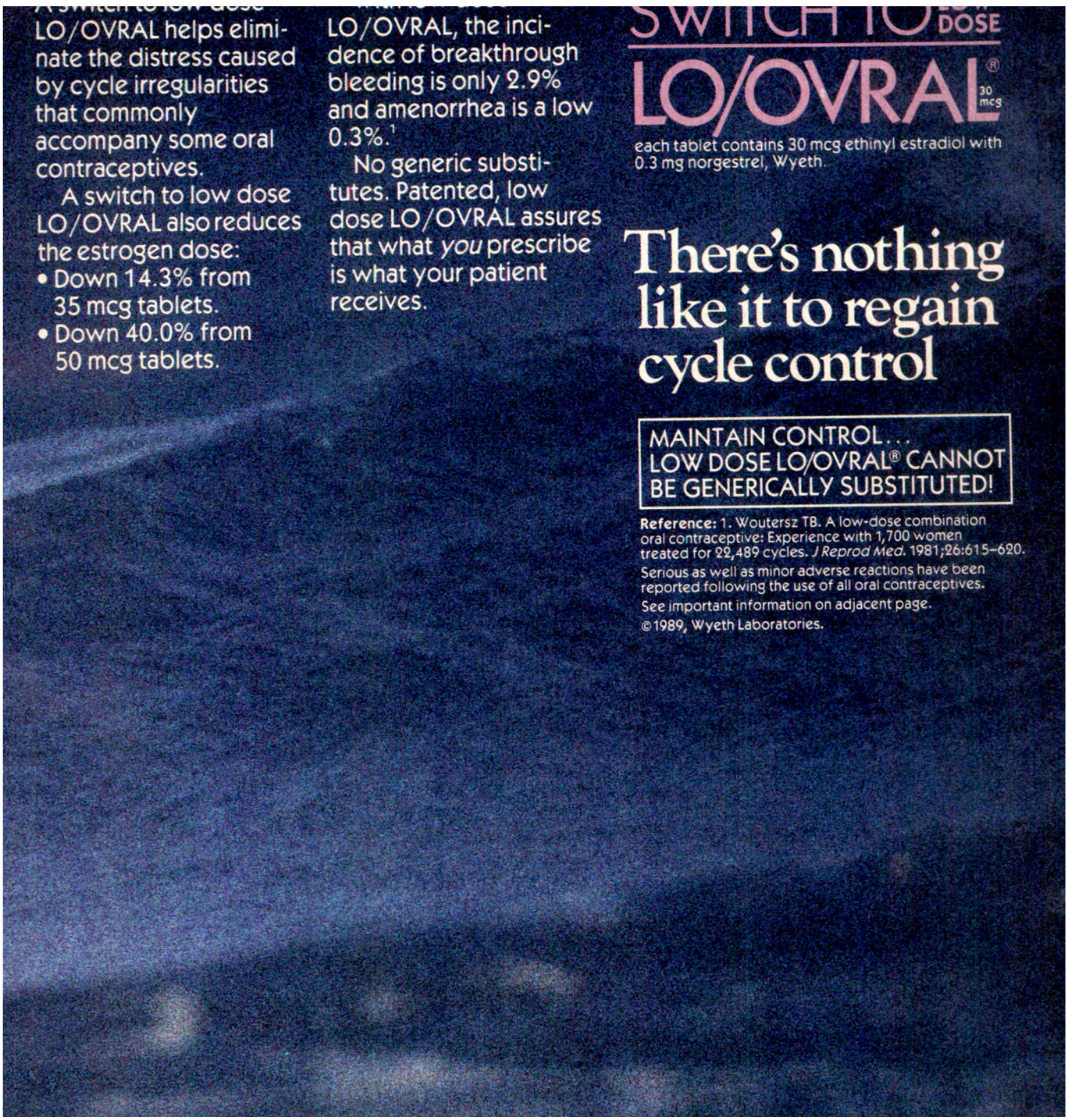
For those who seek a review of all aspects of Obstetric and Gynecologic practice. Particular emphasis is placed on the state of the art in diagnosis and management and contemporary solutions to common dilemmas. Lectures are presented by leading clinicians who are also known for their effective teaching skills. Two special features are an **Optional Microscope Slide Review,** offering you a unique opportunity for tutelage in pathologic identification; and a **Review of Case Lists,** affording the chance to review your cases with a panel of experts.

Participation earns **53 AMA Category 1 credit hours.** Application has been made for cognates from the American College of Obstetricians and Gynecologists.

THE COOK COUNTY GRADUATE SCHOOL OF MEDICINE
CME Since 1932

For more information, call toll-free anytime
1-800-621-4651
In Illinois, call **1-800-621-4649**
Or write to us at
707 South Wood Street
Chicago, Illinois 60612


"Before
switching my pill,
I could bleed
at any time...
or not have a
period at all."


LO/OVRAL helps eliminate the distress caused by cycle irregularities that commonly accompany some oral contraceptives.
A switch to low dose LO/OVRAL also reduces the estrogen dose:
• Down 14.3% from 35 mcg tablets.
• Down 40.0% from 50 mcg tablets.
LO/OVRAL, the incidence of breakthrough bleeding is only 2.9% and amenorrhea is a low 0.3%.[1]
No generic substitutes. Patented, low dose LO/OVRAL assures that what *you* prescribe is what your patient receives.
SWITCH TO DOSE
LO/OVRAL® 30 mcg
each tablet contains 30 mcg ethinyl estradiol with 0.3 mg norgestrel, Wyeth.
There's nothing like it to regain cycle control
MAINTAIN CONTROL . . .
LOW DOSE LO/OVRAL® CANNOT BE GENERICALLY SUBSTITUTED!
Reference: 1. Woutersz TB. A low-dose combination oral contraceptive: Experience with 1,700 women treated for 22,489 cycles. *J Reprod Med.* 1981;26:615–620.
Serious as well as minor adverse reactions have been reported following the use of all oral contraceptives.
See important information on adjacent page.
©1989, Wyeth Laboratories.

IN BRIEF:

Indications and Usage—LO/OVRAL® is indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OC's) as a method of contraception.

Contraindications—OC's should not be used in women with any of the following conditions:

1. Thrombophlebitis or thromboembolic disorders.
2. A past history of deep-vein thrombophlebitis or thromboembolic disorders.
3. Cerebral-vascular or coronary-artery disease.
4. Known or suspected carcinoma of the breast.
5. Known or suspected estrogen-dependent neoplasia.
6. Undiagnosed abnormal genital bleeding.
7. Known or suspected pregnancy (see Warning No. 5).
8. Benign or malignant liver tumor which developed during use of OC's or other estrogen-containing products.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems*—An increased risk of thromboembolic and thrombotic disease associated with use of OC's is well established. Three principal studies in Great Britain and 3 in the U.S. have demonstrated increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OC's are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION (MI)—An increased risk of MI associated with use of OC's has been reported, confirming a previously suspected association. These studies, conducted in the UK, found, as expected, that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclamptic toxemia) the higher the risk of developing MI, regardless of whether the patient was an OC user or not. OC's, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to MI) are about twice as likely to have a fatal MI as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, amount of smoking is also an important factor. In determining importance of these relative risks, however, baseline rates for various age groups must be given serious consideration. Importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; quite likely the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE—In an analysis of data derived from several national adverse-reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to dose of estrogen in OC's. Preparations containing 100 mcg or more of estrogen were associated with higher risk of thromboembolism than those containing 50-80 mcg. Their analysis did suggest, however, that quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the U.S.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the UK estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OC's according to age, smoking habits, and duration of use. Overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), risk being concentrated in older women, in those with long duration of use, and in cigarette smokers. It was not possible, however, to examine interrelationships of age, smoking, and duration of use, nor to compare effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess magnitude of relative risk for this younger group. Available data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates of risk of death for each method include combined risk of contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OC's) plus risk attributable to pregnancy or abortion in event of method failure. This latter risk varies with effectiveness of method. The study concluded that mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OC's in women over 40 who smoke. Lowest mortality is associated with condom or diaphragm backed up by early abortion. Risk of thromboembolic and thrombotic disease associated with OC's increases with age after about 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of pre-eclamptic toxemia, and especially cigarette smoking. Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A 4- to 6-fold increased risk of postsurgery thromboembolic complications has been reported in OC users.

If feasible, OC's should be discontinued at least 4 weeks before surgery of a type associated with increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Britain involving cerebrovascular disease and another in the U.S. concerning MI suggest an increased risk of these conditions in users of OC's persists after discontinuation of the OC's. In the British study, risk of cerebrovascular disease remained elevated in former OC users for at least 6 years after discontinuation. In the U.S. study, increased risk of MI persisted for at least 9 years in women 40- to 49-years-old who had used OC's for 5 or more years. Findings in both studies require confirmation since they are inconsistent with other published information.

2. *Ocular Lesions*—There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with use of OC's. Discontinue OC's if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. *Carcinoma*—Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in OC's, have been noted to increase incidence of mammary nodules, benign and malignant, in dogs. In humans, 3 case-control studies have reported an increased risk of endometrial carcinoma associated with prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OC's. Of cases found in women without predisposing risk factors (e.g., irregular bleeding at the time OC's were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These are no longer marketed. No evidence has been reported suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only OC's. Several studies have found no increase in breast cancer in women taking OC's or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women on OC's, found an excess risk in subgroups of OC users with documented benign breast disease. Reduced occurrence of benign breast tumors in users of OC's has been well documented. In summary, there is at present no confirmed evidence from human studies of increased risk of cancer associated with OC's. Close clinical surveillance of all women on OC's is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or abnormal mammograms should be monitored with particular care if they elect to use OC's.

4. *Hepatic Tumors*—Benign hepatic adenomas have been found to be associated with use of OC's. One study showed that OC's with high hormonal potency were associated with higher risk than lower potency OC's. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users. Two studies relate risk with duration of use of OC's, the risk being much greater after 4 or more years' use. While hepatic adenoma is rare, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women on OC's. Relationship of these drugs to this type of malignancy is not known.

5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring*—Use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have increased risk of developing in later life a form of vaginal or cervical cancer ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence now that OC's further enhance risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OC's. Furthermore, 30 to 90% of such exposed women have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with use of other estrogens, it cannot be presumed they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with use of sex hormones, including OC's, in pregnancy. One case control study estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OC's, hormonal withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some exposures involved only a few days. Data suggest that risk of limb-reduction defects in exposed fetuses is somewhat less than 1 in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OC's. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OC's is unknown. It is recommended that, for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing OC's. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at time of first missed period, and further use of OC's should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and advisability of continuation of the pregnancy should be discussed. It is also recommended that women who discontinue OC's with intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this. The administration of progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease*—Studies report increased risk of surgically confirmed gallbladder disease in users of OC's and estrogens. In one study, increased risk appeared after 2 years' use and doubled after 4 or 5 years' use. In one of the other studies, increased risk was apparent between 6 and 12 months' use.

7. *Carbohydrate and Lipid Metabolic Effects*—Decrease in glucose tolerance has been observed in a significant percentage of patients on OC's. For this reason, prediabetic and diabetic patients should be carefully observed while on OC's. Increase in triglycerides and total phospholipids has been observed in patients on OC's; clinical significance of this finding remains to be defined.

8. *Elevated Blood Pressure*—Increase in blood pressure has been reported in patients on OC's. In some women, hypertension may occur within a few months of beginning OC's. In the 1st year of use, prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. Prevalence in users increases, however, with longer exposure, and in the 5th year of use is 2½ to 3 times the reported prevalence in the 1st year. Age is also strongly correlated with development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure on OC's. Hypertension that develops as a result of taking OC's usually returns to normal after discontinuing the drug.

9. *Headache*—Onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of OC's and evaluation of the cause.

10. *Bleeding Irregularities*—Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing OC's. In breakthrough bleeding, as in all cases of irregular vaginal bleeding, nonfunctional causes should be borne in mind.

In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another OC may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase risk of thromboembolic disease. Women with past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuing OC's. Women with these pre-existing problems should be advised of this possibility and encouraged to use other methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. *Ectopic Pregnancy*—Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. *Breast-feeding*—OC's given in the postpartum period may interfere with lactation and decrease quantity and quality of breast milk. Furthermore, a small fraction of the hormones in OC's has been identified in the milk of mothers on OC's; effects, if any, on the breast-fed child have not been determined. If feasible, defer OC's until infant has been weaned.

Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OC's. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Pap smear and relevant laboratory tests.

As a general rule OC's should not be prescribed for longer than 1 year without another physical examination.

2. Under influence of estrogen-progestogen preparations, pre-existing uterine leiomyomata may increase in size.

3. Patients with history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while on OC's should stop OC's and use an alternate method to try to determine whether the symptom is drug-related.

4. OC's may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency.

5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while on OC's. If jaundice develops, OC's should be discontinued.

6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution.

7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. Clinical significance is undetermined.

8. Serum folate levels may be depressed by OC's. Since the pregnant woman is predisposed to development of folate deficiency and incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OC's, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency.

9. The pathologist should be advised of OC therapy when revelant specimens are submitted.

10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OC's: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test.

Information for the Patient—See Patient Package Labeling.

Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Carcinogenesis—See Warnings on carcinogenic potential of OC's.

Pregnancy—Category X. See Contraindications, Warnings.

Nursing Mothers—See Warnings.

Adverse Reactions—An increased risk of these serious adverse reactions has been associated with use of OC's (see Warnings): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies.

There is evidence of an association between the following conditions and use of OC's although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients on OC's and are believed to be drug-related: nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea, amenorrhea during and after treatment, temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement, and secretion; change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening), intolerance to contact lenses.

The following adverse reactions have been reported in users of OC's, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, hemolytic uremic syndrome.

Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OC's by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

2/27/85 3209-3

54541

LO/OVRAL® each tablet contains 0.3 mg norgestrel with 0.03 mg ethinyl estradiol.

WYETH AYERST™

American Journal of Obstetrics and Gynecology

Founded in 1920

volume 161 *number* 1 JULY 1989

CLINICAL SECTION

Clinical Opinion

Fetal and neonatal cardiovascular complications from β-sympathomimetic therapy for tocolysis

Vern L. Katz, MD, and John W. Seeds, MD

Chapel Hill, North Carolina

β-Sympathomimetic drugs cross the placenta freely. Just as these agents cause serious cardiovascular changes in the mother, they may cause severe cardiovascular complications in the fetus. β-Sympathomimetic agents for tocolysis have been associated with fetal heart rate and rhythm disturbances, hydrops, stillbirth, neonatal cardiac failure, myocardial ischemia and infarction, and neonatal death. Prospective studies have documented changes in interventricular septa of babies exposed to these drugs. Histologic changes have been reproduced in animal models and in vitro similar to those seen in infants with myocardial disease caused by β-mimetic therapy. The mechanism of β-mimetic toxicity appears to be increased myocardial intracellular calcium leading to overexcitation and cell necrosis. Since serious fetal cardiovascular effects may occur with β-mimetic use, benefits should clearly outweigh risks before these drugs are administered. (AM J OBSTET GYNECOL 1989;161:1-4.)

Key words: β-Sympathomimetics, fetal cardiac, tocolysis introduction

The use of β-sympathomimetics for tocolysis is now a mainstay in the prevention of premature birth. Unfortunately, these agents have potent cardiovascular effects. Attention to the adverse cardiovascular effects has focused primarily on the mother. However, serious cardiovascular complications may also occur in the fetus.

In 1924 Pierce Rucker[1] presented a report on the "Action of Adrenalin on the pregnant uterus" to the Southern Medical Association. Using 5 minims of 1 to 1000 solution of adrenalin "hypodermically," he caused a "cessation or diminution" in uterine contractions in 16 of 20 gravid women. This investigation followed his earlier observation of decreased contractions with the administration of epinephrine HCl (Adrenalin) and procaine HCl (Novocain) in caudal anesthetics. His report appears to be the first investigation of the use of adrenergic agents for tocolysis. He did not describe at that time any effects from his injections on the fetus.

Sixty years later in 1983, Thomas Benedetti[2] reviewed maternal complications of β-sympathomimetic therapy and cautioned: "There is a long and sobering list of potentially life threatening complications . . . we must ask ourselves by whom and under what circumstances should these drugs be used."[2] Careful attention and rapid response to maternal cardiac signs and symptoms, electrocardiographic changes, and fluid and electrolyte balance have improved the maternal risk/benefit ratio. Evaluations of the fetal cardiac status during exposure to the same potentially toxic medications have been rare.[3] Fortunately, most infants appear to tolerate

From the Division of Maternal and Fetal Medicine, Department of Obstetrics and Gynecology, University of North Carolina School of Medicine.

Reprint requests: Vern Katz, MD, Department of Obstetrics and Gynecology, Division of Maternal and Fetal Medicine, CB# 7570, MacNider Building, University of North Carolina, Chapel Hill, NC 27514.

Table I. Reports of cardiovascular complications

Author	*β-Sympathomimetic exposure*	*Complications*	*Treatment and outcome*
Brosset et al.[4]	Intravenous ritodrine 12 hr	Extrauterine superventricular tachycardia, cardiac failure	Digitalis, resolution of complications
Hermanse and Johnson[5]	Intravenous and oral ritodrine, 5 wk	In utero superventricular tachycardia	Delivery spontaneous, resolution of
Lenke and Trupin[6]	Intravenous terbutaline, 5 hr	Stillborn	—
Beitzke et al.[7]	Oral and inravenous ritodrine, 7 wk	In utero atrial flutter hydrops	Delivery, DC shock, digitalis, resolution of complication
Vogt et al.[8]	Intravenous fenoterol variable duration, series of 30 infants	Tachycardia, ECG changes, cardiomegaly, cardiac failure, perinatal death, myocardial infarction	Range from spontaneous resolution to neonatal death
Katz and Seeds	Intravenous ritodrine, oral terbutaline, 12 days	Hydrops, neonatal congestive failure, myocardial infarction	Delivery; digitalization; neonatal death

DC, Direct current; *ECG*, electrocardiographic.

β-mimetic exposure without problems, but a growing number of reports document serious fetal cardiac complications from β-mimetic therapy.[3-12, 14-16] The purpose of this article is to summarize and illustrate fetal cardiovascular complications of exposure to β-sympathomimetics and review the relevant literature regarding fetal cardiotoxicity from these drugs.

Prospective evaluation of fetal cardiovascular complications caused by β-mimetic therapy

Nuchpuckdee et al.[11] used M-mode echocardiography to assess newborn cardiac status in 41 infants whose mothers had been treated with ritodrine from 1 day to 7 weeks (mean, 16 days). Compared with control infants matched for gestational age, infants of ritodrine-treated mothers had disproportional septal hypertrophy. Interventricular septal thickening correlated ($r = 0.96$) with the duration of ritodrine use by the mother. All echocardiographic changes were gone within 3 months of age. The clinical outcomes were not different between the two groups.

Crawford et al.[12] performed echocardiograms on 34 infants exposed to β-mimetics in utero for tocolysis and compared them with controls. They also demonstrated a significant increase in interventricular septal thickness.[12]

Bohm and Adler[10] examined autopsy specimens of fetuses and newborns dying of noncardiac causes whose mothers were exposed to β-mimetic therapy (from 1 day to 12 weeks' duration). Autopsy specimens from gestational age–matched control subjects not receiving tocolysis were also reviewed. In 20 of 25 autopsy specimens from the β-mimetic exposed group, pathologic changes of myocardial ischemia and infarction were found. None of these changes were seen in the control group. These changes were classified as focal necrosis, fatty degeneration, and myocardial cell nuclear enlargement, primarily of the right ventricle.[10] The histopathologic findings at autopsy of the case presented below are entirely analogous to these changes.

Mechanism of β-mimetic toxicity to the fetal cardiovascular system

The cardiovascular complications described in case reports and prospective studies have a natural model. Catecholamine overload leading to cardiomyopathy, myocarditis, and necrosis is a well-understood phenomenon. Pheochromocytoma provides a pathologic model of this toxicity.[13-15] Elevated and prolonged exposure of the heart to catecholamines leads to cardiomyopathy, myocarditis, and infarction similar to that seen with β-mimetic therapy. In the case of tocolysis, the fetus is exposed to β-sympathomimetic drugs that cause prolonged and elevated activation of the adrenergic receptors.

Animal models have helped clarify the mechanism of myocardial damage.[13-16] The catecholamines or sympathomimetics affect the myocardial cells by increasing the flow of calcium through the cellular and intercellular membranes. Increased calcium leads to increased cellular metabolism and increased cardiac activity. The next step is a growing imbalance between oxygen supply and consumption. Structural integrity of the cell is lost and necrosis occurs.[14-16] Isoproternol, norepinephrine, and fenoterol have been used to create these lesions in vitro and in vivo in rats and dogs.[13-16]

An in vitro study used fetal myocardial tissue from abortuses, exposing the fetal myocardium to fenoterol. Focal parenchymal necrosis developed, and histologic changes similar to those seen in infants exposed in utero to β-sympathomimetics for tocolysis were documented.[10, 15] A calcium channel blocker (verapamil)

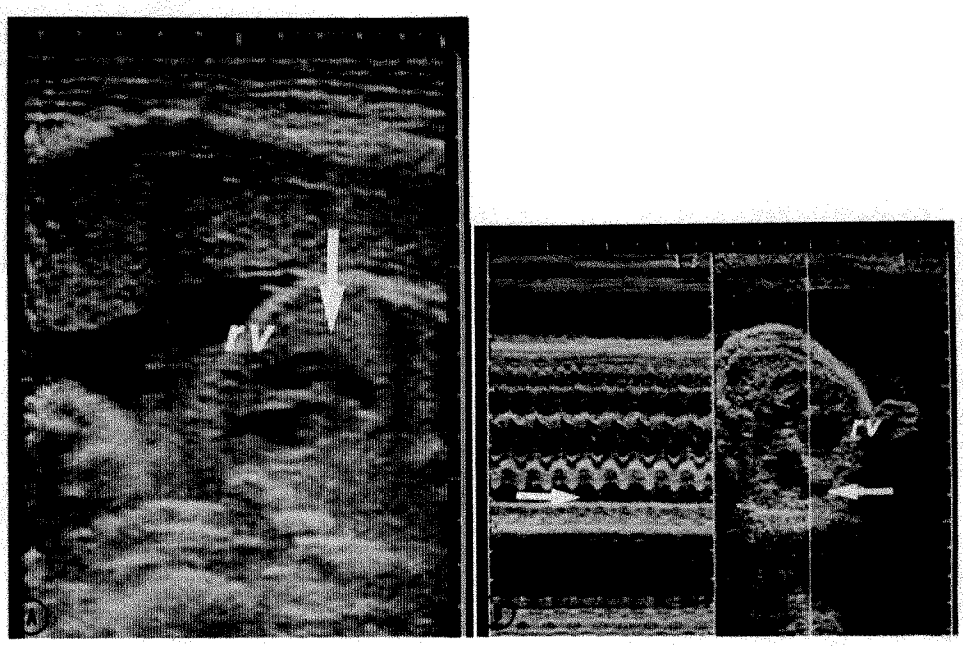


Fig. 1. A, Ultrasound scan of a transverse section of fetal chest, four-chamber view, before tocolytic therapy. *Arrow* points to fetal heart. **B,** M-mode echocardiogram of fetus 12 days after initiation of β-sympathomimetic therapy for tocolysis. Heart is now enlarged from **A,** and ventricles are dilated. Polyhydramnios is also present. *Arrows* point to pericardial effusion. *rv,* Right ventricles.

was added to the media and myocardial necrosis was prevented.[15] In fact, the combined use of β-mimetics and calcium channel blockers, usually verapamil, is now widely accepted in Europe. This practice was originally introduced to prevent unwanted maternal cardiovascular side effects.[16]

Most of the cardiovascular effects are initially transient and reversible.[14] However, the most severe changes of widespread cellular necrosis may lead to heart failure and death. The right ventricle, the area of greatest cardiac work in the fetus, appears most seriously affected by tocolytic medications. Myocardial damage is dose related, and the severity of changes is also related to the duration of exposure. The model of catecholamine or β-sympathomimetic overload leading to increased intracellular calcium and cellular necrosis matches the clinical picture of fetal myocardial toxicity caused by tocolytic therapy with β-mimetics.

Reports of fetal cardiac complications associated with β-sympathomimetic tocolysis

β-Sympathomimetic agents freely cross the human placenta and may be found in concentrations in umbilical cord serum equal to maternal serum.[3] Thus it is not surprising that fetuses and newborns have cardiac complications similar to adults exposed to these drugs. Myocardial ischemia, injury, and infarctions are well documented in mothers exposed to these agents.[2]

Elevations in baseline fetal heart rate from maternal β-sympathomimetic administration is a widely accepted side effect and not necessarily a complication. Complications are viewed as pathologic effects leading to potential compromise. A wide spectrum of fetal cardiovascular complications have been reported in association with β-sympathomimetic use for tocolysis. Fetuses and newborns have been reported to have rate and rhythm disturbances, including paroxysmal superventricular tachycardia and atrial flutter.[4, 5, 8] These lesions have typically resolved spontaneously within a few days to 2 weeks. Electrocardiographic abnormalities reported include transient nonspecific T wave changes and ischemic T wave changes.[4, 5, 7, 8] Vogt et al.[8] reported on a series of 30 infants admitted with cardiac disease after treatment with fenoterol. The spectrum of neonatal disease ranged from T wave changes to myocardial infarction. More serious fetal complications, including hydrops fetalis, pulmonary edema, and extrauterine cardiac failure, have also been seen.[4, 7, 8] Severe complications have included stillbirth, neonatal death, and histologic findings of myocardial infarction.[6, 8-10]

Table I lists cases of cardiovascular complications directly related to β-sympathomimetic therapy for tocolysis. In addition to the 34 cases listed in Table I, we recently cared for an infant who developed hydrops from a myocardial infarction in utero presumably caused by β-sympathomimetic therapy. The infant's mother, a 31-year-old gravida 2, para 0 white, was treated at 28 weeks' gestation for preterm labor. She

was given intravenous ritodrine, 15 μg/hr, for 2 hours and then 2.5 and 3.75 mg of oral terbutaline every 4 hours for 12 days. Fig. 1, *A*, shows the normal cardiac ultrasound study of the fetus before therapy. Fig. 1, *B*, shows the dilated heart 12 days after therapy was initiated. Pericardial effusion, cardiomegaly, and paradoxical interventricular septal motion were also noted on ultrasound examination. The infant was delivered and died on day 3. Results of viral cultures, inoculates, and titers were negative. The placenta was normal. The autopsy showed large areas of myocardial necrosis with calcification, primarily subendocardial and in the right ventricle. These findings were identical to those of Bohm and Adler[10] and Vogt et al.[8]

The cardiac complications reported in Table I mirror the spectrum of cardiovascular complications seen in mothers given β-sympathomimetics for tocolysis.[2] However, fetal cardiovascular complications may be underreported, since premature infants (exposed and unexposed) to β-sympathomimetics agents suffer from multiple problems that may overshadow β-sympathomimetic effects.[9, 10]

Comment

β-Sympathomimetics have been used for tocolysis for well over a decade. An estimation of the incidence of serious complications is difficult to make. Severe fetal cardiovascular complications are rare, but subclinical pathophysiologic changes seem to occur more commonly. Direct myocardial effects, including tachycardia, are usually transient. Interventricular septal hypertrophy in infants exposed to longer durations of tocolytic therapy seems to be reversible. Reports of severe and permanent myocardial damage leading to cardiac failure and perinatal mortality as described in our review are rare. Studies from multiple institutions would be needed to arrive at an absolute risk. However, in view of current knowledge, it is prudent to recommend careful consideration and evaluation of the risk/benefit ratio of the use of β-sympathomimetics in every case and consider alternatives with less cardiotoxic potential.

It appears appropriate to use β-sympathomimetics for limited duration and only when there is a very clear benefit to be gained. Use of these drugs beyond 33 completed weeks of gestation should only be considered after careful evaluation of alternatives and rarely prescribed solely for contractions without cervical change. When β-sympathomimetics are used, regular ultrasound examinations during and after the time of drug exposure to evaluate for early fetal cardiac changes should be considered. Ultrasound examinations should include measurements of biventricular end-diastolic diameter and interventricular septal thickness. This article emphasizes that serious potential effects may occur from the use of β-sympathomimetics. Benefits should outweigh risks before these potent agents are prescribed.

REFERENCES

1. Rucker MP. The action of Adrenalin on the pregnant human uterus. South Med J 1925;18:412-8.
2. Benedetti TJ. Maternal complications of parenteral β-sympathomimetic therapy for premature labor. Am J Obstet Gynecol 1983;145:1-6.
3. Gross TJ, Kuhnert BR, Kuhnert PM, et al. Maternal and fetal plasma concentration of ritodrine, Obstet Gynecol 1985;65:793-7.
4. Brosset P, Ronayette D, Pierce MC, et al. Cardiac complications of ritodrine in mother and baby. Lancet 1982; 1:1461.
5. Hermansen MC, Johnson GL. Neonatal supraventricular tachycardia following prolonged maternal ritodrine administration. Am J Obstet Gynecol 1984;149:798-9.
6. Lenke R, Trupin S. Sudden unforeseen fetal death in a woman being treated for premature labor. J Reprod Med 1984;29:872-4.
7. Beitzke A, Winter R, Zach M, Grubbauer HM. Kongenitales vorhofflaterm mit hydrops fetales durch mutterlichetokolitika medikation. Klin Padiatr 1979;191:410-27.
8. Vogt J, Schmidt-Redemann B, Urbanek R. Neugeborenon kardiotoxizitat mach tokolyse mit fenoteral hydrobromid. Padiat Padol 1979;14:355-62.
9. Brazy JE, Pupkin MJ. Effects of maternal isoxsuprine administration on perterm infants. J Pediatr 1979;94: 444-8.
10. Bohm N, Adler CP. Focal necrosis, fatty degeneration and subendocardial nuclear polyploidization in the myocardium in newborns. Eur J Pediatr 1986;136:149-57.
11. Nuchpuckdee P, Brodskyr, Porat T, et al. Ventricular septal thickness and cardiac function in neonates after in utero ritodrine exposure. J Pediatr 1986;109:687-91.
12. Crawford CS, Hall ML, Otis C. Echocardiographic effects of intrauterine ritodrine exposure. Pediatr Res 1982;16: 123A.
13. Imperato-McGinley J, Gautier T, Ehlers K, et al. Reversibility of catecholamine induced dilated cardiomyopathy in a child with pheochromocytoma. N Engl J Med 1987;316:793-6.
14. Fleckenstein A, Janke V, Doring HJ. Myocardial fiber necrosis due to intracellular Ca^{++} overload, a new perception in cardiac pathophysiology. In: Dhalla NS, ed. Myocardial biology. Baltimore: Baltimore University Press, 1974; 563-80.
15. Hofmann W, Schleich A, Schroeter D, et al. Herzmuskelnekrosen durch β-sympathomimetika. Virchows Arch 1977;373:85-95.
16. Strige R, Pfeiffer U, Erhardt W. Does the administration of the calcium antagonist verapamil in tocolysis with β-sympathomimetics still make sense? J Perinat Med 1981;9:235-47.

Understanding and eliminating errors in perinatal research

Barry S. Block, MS, MD
Chicago, Illinois

Research studies published in the scientific literature have had a rapid impact on medical practice, especially in clinical obstetrics. Recently, incentives to control medical costs without sacrificing the quality of care have placed increased importance on studies that truly demonstrate clinical effectiveness. In addition, there are ethical concerns that improperly designed or conducted studies adversely affect medical practice and place patients at unnecessary risk. This article summarizes potential sources of error and encourages the valid interpretation of the results and conclusions of experimental studies by making four suggestions: (1) the errors and limitations that may be present in the methodology should be described and explanations given how the errors might have affected the results and why the limitations are acceptable; (2) the methods selected should allow an answer to the question proposed; (3) the investigator should verify that the appropriate statistical test is used; and (4) when unexpected or hard to explain results are found, verification before publication should be undertaken. (Am J Obstet Gynecol 1989;161:5-9.)

Key words: Quality control, research design, statistics

Avoiding errors in the performance of experimental studies and the interpretation of the results and conclusions of such studies is especially important in clinical obstetrics. Bryce and Enkin[1] have described the vital role that perinatal clinicians have ". . . in evaluating the treatments we are now using, as well as new therapies in the future." Similarly, Tyson et al.[2] have stated that "adequate evaluation of (treatment) methods prior to their recommendation is needed to avoid widespread use of ineffective or hazardous treatment methods especially if high-risk (perinatal) patients are involved." Recent emphasis on the need to control medical costs without sacrificing the quality of care places increased importance on studies that truly demonstrate clinical effectiveness. Improperly conducted studies cost money, patients may be placed at risk by an inferior treatment, and animals may be killed to collect data that are incorrectly interpreted. Finally, as stated by Altman,[3] ". . . it is unethical to publish results that are incorrect or misleading." Several authors have addressed the issue of proper statistical design and analysis in previously published reports[2, 4, 5]; however, other issues remain. As stated by Feinstein,[4] ". . . the most important issues in biostatistics are not expressed with statistical procedures. The issues are inherently scientific, rather than purely statistical. . . ." Two examples of such scientific issues are (1) the theory of error and (2) the consideration of alternative conclusions after the statistical analysis is completed.

From the Section of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, The University of Chicago.
Reprint requests.: Barry S. Block, MD, Department of Obstetrics and Gynecology, Box 446, The University of Chicago, Chicago, IL 60637.

The ultimate responsibility for avoiding errors rests with the individual researcher, but reviewers and readers of scientific articles also have an important responsibility. After recent reports of academic fraud,[6] the reliability and integrity of scientific publications are under review. All investigators, including perinatal researchers, are influenced by the sophistication of our technical procedures and the intense competition for academic achievement. All of us must make a commitment to eliminate fraudulent or incorrect publications from the literature. The purpose of this article is to assist investigators, reviewers, and readers by reinforcing existing knowledge of the principles of error theory and by encouraging the valid interpretation of the results of experimental studies. Examples presented are chosen from publications in the perinatal literature.

Error theory[7]—definition of terms

The term "error" generally has two usages. First, it may refer to the difference between a measurement and the "true" value, and second, it may refer to the estimated uncertainty in an experiment. The term is frequently used improperly to denote a "discrepancy," that is, the difference between two measurements. There are two principal types of errors: determinant errors, or errors that can be evaluated by a theoretic or experimental procedure, and indeterminate errors that can not be so evaluated.

Errors that are differences between a measurement and a "true" value are classified as systematic, random, or illegitimate and are summarized in Table I. Systematic errors in measurement depend on the skill of the observer and include calibration errors, human errors, errors from experimental conditions, dynamic re-

Table I. The classification of errors that represent differences between a measurement and a true value

Type of error	*Cause*	*Example*
Systematic errors		
Calibration error	Incorrect calibration or "zero" drift with time	Thermometer that reads 101° F in boiling water
Human error	Observer bias; also called personal errors	Head position when reading a pH meter scale
Experimental conditions	Change in conditions from those during calibration	Temperature or pressure is different than during a calibration
Dynamic response error	Exceeding the design of the equipment	Nonlinear response to a fast changing signal
Imperfect technique	Loss of sample during collection	Splashing during 24 hr urine collection
Random errors		
Error of judgment	Observer estimates that vary from time to time	Reading fetal heart rate tracings at 3:00 AM when overtired
Fluctuating conditions	Temperature, pressure or line voltage variations	Power "brown out"
Small disturbances	Mechanical disturbances from nearby equipment	Vibrations from adjacent centrifuge
Error of definition	What is being measured is not an "exact" quantity	Measuring one's height because of irregular surfaces
Illegitimate errors		
Method error	Wrong or insufficient experimental method	Self-explanatory
Instrument error	Faulty instrument or misoperation	Use of outdated equipment
Blunder	Outright mistakes in reading instrument	Inadequate training of assistants
Error of computation	Arithmetic error during analysis	Error ("bug") in computer program or in data entry
Chaotic error	Disturbances that exceed natural random errors	Discontinue experiment until source is removed

sponse errors, and imperfect technique. Random errors include errors of judgment, fluctuating conditions, small disturbances, and errors of definition. They are caused by chance, and their elimination requires improving and refining experimental technique. Illegitimate errors, such as method errors, instrument errors, blunders, arithmetic errors, and chaotic errors, are avoidable errors that have no place in research publications.

Statistical terminology

The common uses of the statistical term "error" are summarized in Table II. Interestingly, the standard error is not an error and has nothing to do with standards. Similarly, the term "error variance" is also included in the statistical vocabulary but is a quantity that refers to observer variation not mistakes. The use of the term "error" is confusing, improper, and untrue but entrenched by time. Clearly, a better choice is dispersion or deviation rather than error.

Other uses of the term "error" include experimental error, observer error, and type I and II errors. Experimental error is introduced because a sample is taken from a large population. If the entire population were studied, the "true" value would be known. Experimental error is minimized by appropriate sample selection, and observation errors can be diminished by repeated observations by the observer. Experimental error can be biased or unbiased. If the ratio of observed error to sample size tends to zero as the sample size increases, the error is said to be unbiased. Also, the statistic calculated from the sample may be biased or unbiased and overestimate or underestimate the population parameter. If an experiment were repeated a large number of times and the statistic has the property that the estimations average out, then the statistic is said to be unbiased.

There are two ways that an investigator can make a mistake in hypothesis testing—rejecting a true hypothesis or accepting a false one. A type I error occurs if the hypothesis under test is rejected when in fact it is true. A type II error occurs if the hypothesis under test is accepted when in fact it is false. The p value is frequently, although incorrectly, thought to be either the probability of making a mistake or the importance of the difference observed. The p value is, by definition, the probability of obtaining an equally unlikely result if the hypothesis under test were actually true.

Statistical power is the probability of rejecting a null hypothesis when in fact it is false and should be rejected. In hypothesis testing the magnitude of difference that would lead to rejection of the hypothesis (D), the probability of committing a type I error (α), the probability of committing a type II error (β), and the sample size (n) are directly related. If any three (D, α, β, or n) are known, then the fourth is fixed mathematically. In a properly designed study the magnitude of the difference one desires to detect, α and the power

Table II. A description of some statistical uses of the term "error"

Statistical term	*Definition*
Absolute deviation	Absolute value of a deviation from the mean
Average deviation	Sum of absolute deviations divided by sample size
Fractional error	Ratio of absolute deviation to expected value
Percentage error	Fractional error expressed as a percentage
Probable error	That magnitude of deviation whose probability of being exceeded is one half
Standard deviation	Positive square root of sum of square deviations from mean divided by sample size less 1
Standard error	Standard deviation divided by square root of sample size
Error variance	Sum of square deviations from group mean divided by number of groups less 1
Error tolerance	Region with a given probability of containing "true" value

(1-β), are preselected, and the size of the sample required is then determined in advance. Failure to follow these guidelines may lead to a negative conclusion with low statistical power in which no effect was found but in which there was little if any chance of detecting an effect.

The terms "precision" and "accuracy" are used to describe how well measurements relate to each other and to the "true" value. Precision is a measure of reliability or how reproducible the results are. An experiment with small random errors has high precision, and the more precise of two measurements is the one with the smaller probable error. Accuracy is a measure of how close the experimental result comes to the "true" value. An experiment with small systematic errors has high accuracy. For a diagnostic test, the accuracy is the total number of correct diagnoses of both disease and nondisease divided by the number of tests performed.

Error tolerance is described in Table II. In layman terms, the confidence limits are an interval with a given likelihood of including a population parameter, such as the population mean or population variance. For example, if all possible samples of size n were taken from a population and a 90% confidence limit of the mean calculated from each sample, the population mean would be contained in 90% of the intervals. These limits apply only to samples of size n but are frequently used incorrectly and also applied to an individual value. The correct region for including a specified proportion of the individual values from a population is called the tolerance limits. For example, considering gestational age and biparietal diameter measurements obtained by ultrasound study, one may claim with 95% confidence that at least 90% of individual values will be between given limits. In summary, tolerance limits pertain to individual values, whereas confidence limits pertain to parameter estimates obtained from a sample of size n.

The expression "statistical significance" is generally both misunderstood and misused. Unfortunately, there is a tendency to equate significance in a statistical sense with clinical or medical importance. Actually, a small difference of no clinical importance may be statistically significant and an important clinical difference may not be statistically significant depending on the sample size selected. A statistically significant result should be thought of as representing evidence of rather than proof of a particular hypothesis, just as a positive test result does not prove that a patient has a disease. Similarly, a nonsignificant result does not imply nonimportance or nonexistence and would better be thought of as inconclusive or not proved. The choice of the statistical term "significance" was unfortunate. The misconception arises from a misunderstanding of the precise meaning of the term "p value." A significant p value, usually $p < 0.05$, is only a guide to interpretation and is not meant to be a strict rule. In fact, to accept as real a statistically significant result at the $p < 0.05$ level is both unwise and unfounded because one in 20 comparisons of equally effective treatments will be significant by definition.

When the p value is significant, an explanation other than that there is a "true" difference may be present. The difference may be explained by an artifact or the hidden association with a third factor. Artifacts can be caused by chance or bias. The statistical analysis quantifies the probability of obtaining a particular result by chance alone if the hypothesis is true. If the probability is low, the investigator rejects the hypothesis, accepting that there is a real risk of being wrong. Bias, if present, may explain a significant finding between equally effective treatments. Bias is particularly likely to occur in retrospective studies in which data are frequently analyzed in the search for significant results. Bias may occur in the selection of the treatment group, the control group, interpretation of the findings by chart review, or patient recall. Another explanation for an apparent correlation between two factors is that a hidden third factor is present, also called confounding effects. That is, two factors appear to be correlated; however, the explanation is their relationship with a third factor.

For example, a study of income and height would demonstrate a significant correlation. However, it is the relationship of sex with income (males earn more) and the relationship of height with sex that explains the finding.

Violations of underlying assumptions

Statistical tests are based on underlying assumptions, and each individual test may have a different set of underlying assumptions. For example, for the Student *t* test, the underlying assumptions are that the data are independent random samples from normal distributions with equal variances. With little reason to expect otherwise, one often assumes the deviations from these assumptions, if they exist, are small. The robustness of a statistical test is its property of maintaining the same proportion of type I errors when the underlying assumptions are violated as when they are true. Care is required because knowingly or unknowingly violating, these assumptions may lead to the wrong interpretation of the results and incorrect conclusions. Independence implies that the samples are not related. There can be no more than n independent measurements and n-1 degrees of freedom with n subjects. Randomization is the process used to obtain a sample—not the sample itself. In most clinical studies, patient selection is strongly biased. Patients with a disease choose the physician rather then the other way around. Data may be contaminated because of differences in diagnostic criteria or the presence of coexisting disorders. Also, a control group selected from hospitalized patients without the disease may also be biased. A normal or Gaussian distribution is usually assumed. However, obvious signs of nonnormality or skewness should be investigated. For example, if the mean and standard deviation of a measurement that can take only nonnegative values are of the same magnitude, the distribution cannot be Gaussian. Furthermore, if A and B are continuous normal distributions, the ratio A/B will not be normally distributed. The problems related to violations of the underlying assumptions can be avoided by selection of a nonparametric or other appropriate technique. If the variance increases with increases in the independent variable or if the distribution is nonnormal, transformation of the data may be helpful.

Anticipating and avoiding errors

Investigators can aid readers by providing a clear concise accounting of what they did, what they found, and what they conclude. They should anticipate reader concerns by describing errors or limitations that may be present in the methodology and explaining how the errors might have affected the results and why the limitations are acceptable. Before publication, the investigators should assess the validity of any assumptions they made and consider alternative hypotheses to explain their findings. In addition, the investigators should verify that the data and methods support their conclusion and that an appropriate statistical test was used. In the event that unexpected or hard to explain observations occur, verification before publication should be undertaken. To illustrate that improper patient selection may lead to a wrong conclusion and relate actual publications to the principles described herein, several examples are presented.

As previously stated, the methods selected must allow an answer to the question proposed. For example, Crombleholme et al.[8] found an increase in term births from 15.5% to 68.5% in patients whom they treated with cervical cerclage who had previous obstetric losses without a typical history of an incompetent cervix. They concluded that cervical cerclage improves outcome in patients with previous obstetric loss. However, Harger[9] noted the fallacy of using patients who were selected because of poor reproductive performance as their own controls. He presented data that demonstrated a successful outcome of 79% in a similar group of patients who were not treated by a cervical cerclage procedure. Harger was concerned because Crombleholme et al. did not follow a comparable group of patients who were untreated, and it is unclear whether the cervical cerclage procedure might even have decreased successful outcomes, perhaps from 79% to 69%. At the least, even in the event no difference in outcomes were found, the expense, morbidity, and risk of unnecessary surgery should be cause for concern.

The investigator should verify that the appropriate statistical test was used to avoid publishing misleading results. For example, Kennedy and Greenwald[10] investigated the relationship between cesarean section for cephalopelvic disproportion and shoe size and found that women less than 5 feet tall who wore a shoe size less than 5½ had a twofold increased risk of cesarean section with a $p < 0.05$ with the use of χ^2 test. Muncie[11] correctly noted that the proper statistical test is Fisher's exact test and that the probability calculated is $p = 0.498$, a nonsignificant result.

When unexpected or hard to explain results are found, caution is advised and verification before publication should be undertaken. This point is demonstrated by the following two examples. In the first example, Edem et al.[12] investigated the urinary protein profile in hypertensive pregnancies with nephelometry. They noted that "it was very surprising to find that the concentration of albumin and transferrin became nondetectable by nephelometric measurement with increasing severity of pregnancy-induced hypertension." van Kamp et al.[13] observed similar findings that were

at variance with the results of protein electrophoresis performed on the same urine samples. Before publication of their data, they investigated further and found that the concentration of albumin was so high that the antigen excess caused soluble complexes to form, resulting in low scatter and an "out of range, low" message on the nephelometer. They strongly suggested that preliminary protein electrophoresis be performed to determine the proper dilution so that ". . . false conclusions about the absence of 'hidden' proteins are avoided."

In the second example, Lueck et al.[14] presented an ". . . initial descriptive report of observations of multiple forms of an organism found in patients with gestational trophoblastic disease and in patients with preeclampsia-eclampsia," but noted that ". . . The taxonomy of these forms has not yet been determined." Long et al.[15] repeated the study and found ". . . that the objects described by Lueck et al. are artifacts created by cotton or other cellulose fibers and are unrelated to disease."

Comment

There are several ethical concerns about research that is substandard, including the misallocation of resources, as well as the investigator's time, the inconvenience and unjustified risks to which patients are exposed, and the likelihood that publication of misleading results might falsely influence medical practice and lead to unnecessary further work. There are many reasons why erroneous publications may have been intenally or unintentionally submitted. To avoid misrepresenting scientific data, individual researchers and research institutions and universities must meet their obligation to submit only truthful publications. To avoid unintentional errors, researchers must ensure that experiments are well designed and properly evaluated.

REFERENCES

1. Bryce RI, Enkin MW. Six myths about controlled trials in perinatal medicine. AM J OBSTET GYNECOL 1985;151:707-10.
2. Tyson JE, Furzan JA, Reisch JS, Mize SG. An evaluation of the quality of therapeutic studies in perinatal medicine. Obstet Gynecol 1983;62:99-104.
3. Altman DG. Statistics and ethics in medical research. Br Med J 1980;281:1182-4.
4. Feinstein AR. A survey of the statistical procedures in general medical journals. Clin Pharmacol Ther 1974; 15:97-107.
5. Schor S, Karten I. Statistical evaluation of medical journal manuscripts. JAMA 1966;195:1123-8.
6. Petersdorf RG. The pathogenesis of fraud in medical science. Ann Intern Med 1986;104:252-4.
7. Beers Y. Introduction to the theory of error. Reading, Massachusetts: Addison-Wesley, 1957:3-36.
8. Crombleholme WR, Minkoff HL, Delke I, Schwartz RH. Cervical cerclage: An aggressive approach to threatened or recurrent pregnancy wastage. AM J OBSTET GYNECOL 1983;146:168-74.
9. Harger JH. Effectiveness of cervical cerclage. AM J OBSTET GYNECOL 1984;148:834.
10. Kennedy JL, Greenwald E. Correlation of shoe size and obstetric outcome: an anthropometric study. AM J OBSTET GYNECOL 1981;140:466-7.
11. Muncie HL. Relationship of shoe size and cephalopelvic disproportion. AM J OBSTET GYNECOL 1982;142:931-2.
12. Eden RD, Wahbeh CJ, Williams AY, Gall SA. Nephelometric urinary protein profile as an index of renal involvement in hypertensive disorders of pregnancy. AM J OBSTET GYNECOL 1983;147:645-51.
13. van Kamp GJ, Koh-Verspuy A, Mast-Schat T. Urinary protein profiles in hypertensive pregnancies. AM J OBSTET GYNECOL 1984;149:697-8.
14. Lueck J, Brewer JI, Aladjem S, Novotny M. Observation of an organism found in patients with gestational trophoblastic disease and in patients with toxemia of pregnancy. AM J OBSTET GYNECOL 1983;145:15-26.
15. Long EG, Tsin T, Reinarz JA, Schnadig VJ, MsLucas E, Kely RT. "Hydatoxi lualba" identified. AM J OBSTET GYNECOL 1984;149:462-3.

A case-control study of epithelial ovarian cancer

Patricia Hartge, ScD,[a] Mark H. Schiffman, MD,[a] Robert Hoover, MD,[a] Larry McGowan, MD,[b] Linda Lesher, MHS,[b] and Henry J. Norris, MD[c]

Bethesda, Maryland, and Washington, D.C.

With data from a study of 296 patients with primary epithelial ovarian cancer and 343 patients hospitalized because of other conditions, we estimated ovarian cancer risk in accordance with reproductive and other factors. Risk was greatest among women of lower parity, especially among women who said they planned to have children but could not. The protective effect of oral contraceptives seen in other studies was observed only in subgroups of our study population. Women who had breastfed their children had decreased risk, but the number of months of breastfeeding was not related to risk. Incomplete pregnancies did not provide the protection seen for live births. A family history of ovarian cancer and a medical history of breast cancer were both strong risk factors. None of the nonreproductive factors that we examined, including childhood illnesses, tobacco and alcohol consumption, obesity, and selected adult diseases, was convincingly associated with risk. (Am J Obstet Gynecol 1989;161:10-6.)

Key words: Ovarian cancer, endogenous estrogens, infertility, oral contraceptives, epidemiology

The causes of ovarian cancer are mainly unknown.[1, 2, 3] Known risk factors are age (incidence rises until about age 60 years), race (white women are at higher risk than nonwhite women), low parity, infertility, a history of endometrial or breast cancer, a family history of ovarian cancer, and exposure to radiation. Probable protective factors are oral contraceptives and surgical menopause. Although the importance of reproductive and contraceptive history has been firmly established, questions remain. How do parity, pregnancy, and fetal loss jointly affect risk? Does the timing of the first birth affect risk? Is infertility an independent risk factor, separate from the effects of low parity? In addition, many nonreproductive factors have been suggested. With interview and medical records data from a case-control study, we have estimated ovarian cancer risk according to various reproductive and other suggested risk factors to add to our knowledge with regard to these persistent issues.

Methods

Study methods are described in detail elsewhere.[4] All eligible cases involved women aged 20 to 79 years who resided in the Washington, D.C., metropolitan area and who were first diagnosed by operation with microscopically confirmed primary epithelial ovarian cancer during the period August 1978 to June 1981. Cases included tumors of low malignant potential as well as frankly malignant tumors because risk factors for the two groups were similar.[5] For all participants with ovarian cancer, we obtained the diagnostic microscopic slides and pertinent medical records. After review we excluded 30 women found not to have definite primary ovarian cancer of the epithelial type by microscopic (H.J.N.) or clinical (L.M.) evaluation. Several of these cases had been classified as mucinous ovarian cancer but actually were of colorectal origin.

We identified 400 cases and interviewed 296 women (74%). Losses were a result of death (n = 44), disability (n = 12), physician's refusal (n = 8), patient's refusal (n = 33), or other reasons (n = 7). As shown in Table I, serous and endometrioid types predominated.

Control participants were identified from hospital discharge lists and were matched with women with epithelial ovarian cancer according to hospital, age, and race (Table II). A woman was not eligible to be a control if her discharge diagnosis was psychiatric or was potentially related to the major exposures of interest. For all potential control participants, we confirmed in the physician and hospital records that the women had at least one ovary intact and excluded those who did not because they were not at risk of ovarian cancer. We identified 439 controls of whom we interviewed 343 (78%). Losses were a result of death (n = 13), disability (n = 8), physician's refusal (n = 11), patient's refusal (n = 50), or other reasons (n = 14).

Trained, experienced medical interviewers obtained informed consent and administered a standardized questionnaire in the study participant's home shortly after diagnosis. (Questionnaire is available on request.)

Effects of ovarian cancer risk were measured by the

From the Environmental Epidemiology Branch, National Cancer Institute,[a] the Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, George Washington University Medical Center,[b] and the Armed Forces Institute of Pathology.[c]

Supported by the National Cancer Institute, Contract No. NC1-CP-81051.

Reprint requests: Mark H. Schiffman, NCI, Executive Plaza North, Room 443, National Institutes of Health, Bethesda, MD 20892.

Table I. Ovarian cancer cases according to histologic type

Histologic type	*Number*	*Percentage*
Serous	84	28
Serous, low malignant potential	35	12
Mucinous	17	6
Mucinous, low malignant potential	9	3
Endometrioid	76	26
Endometrioid, low malignant potential	8	3
Clear cell	12	4
Mixed epithelial	25	8
Undifferentiated	30	10
TOTAL	296	100

Table II. Discharge diagnoses among control group

Disease category	*Number*	*Percentage*
Infectious disease	6	2
Neoplasm	22	5
Endocrine-metabolic diseases	19	5
Disease of blood or blood-forming organs	4	1
Disease of nervous system	20	8
Disease of eye, ear, or mastoid	26	8
Varicose veins, hemorrhoids	5	1
Respiratory diseases	27	8
Digestive system diseases	55	16
Urinary diseases	18	5
Skin disorders	6	2
Musculoskeletal diseases	75	22
Congenital anomalies	4	1
Ill-defined conditions	18	5
Fractures and other injuries	38	11
TOTAL	343	100

estimated rate ratio—the ratio of ovarian cancer incidence in an exposed group to that in the corresponding unexposed group. A rate ratio of 2.0 for nulliparity, for example, indicates that the incidence rate is twice as great among the nulliparous women as among the parous women; conversely, a rate ratio of 0.5 indicates that the incidence is half as great. The rate ratio estimates were adjusted for the effects of confounding variables by stratified contingency table analysis (presented in the tables) and by logistic regression models, which gave similar results.[6] All trend tests used scored categorized data and were two-tailed.

For each estimate presented we adjusted for age and race. We assessed whether there were confounding effects of parity, difficulty conceiving, recent administration of oral contraceptives, surgical menopause, menopausal estrogen use, or family history of ovarian cancer, and we adjusted the estimates accordingly. If the number of confounders held constant exceeded three, we used a logistic regression model to produce smoothed estimates.

To derive unbiased estimates of the effects of tobacco, alcohol, and adult medical conditions, it was necessary to include in the control group only women who were admitted for conditions not caused by (or protected by) the exposure under assessment. Without such exclusions, a hospital-based control series would produce spurious results because the population of hospitalized women generally includes, for example, more smokers than the whole population of women at risk for ovarian cancer.

Results

A total of 12% of the patients with ovarian cancer and 14% of the controls were black, and the remainder were white. The mean age at diagnosis was 54.4 years among women with cancer and 54.7 years among controls. Because the control participants were chosen from the same hospitals as were the women with cancer, they were similar in social class and religion.

Greater parity was related to reduced risk in our study (Table III), and pregnancies that ended in spontaneous or induced abortion or stillbirth did not, as a group, confer any protection. When the effects of the different pregnancy outcomes were further separated, stillbirths appeared to increase risk, induced abortions appeared to slightly decrease risk, and spontaneous abortions appeared not to affect risk, but the estimates for the specific outcomes were rather unstable. There was also no clear pattern of risk when women were categorized according to the number of months pregnant with the different outcomes, adjusted for the effects of parity. It was necessary to adjust for the effects of parity in the examination of fetal loss because the two were correlated: about one fifth of the nulliparous women had some fetal loss, whereas about one half of the women with five or more live births had fetal loss. The strong protective effect of parity was seen in almost all subgroups and was strongest among women aged 20 to 39 years and weakest among those aged 60 to 79 years.

The protection afforded by greater parity was not explained by an effect of earlier first birth. On the other hand, as shown in Table IV, the apparent protection associated with earlier first birth, when this variable was considered alone, was eliminated by adjusting for parity. Also shown in Table IV is reduced risk among women who had breastfed their children, but an erratic relationship is shown between lifetime months of breastfeeding and risk.

The total group of women who had taken oral contraceptives showed no altered risk (Table V), but women who had taken them ≥3 years were at slightly reduced risk, as were women who had taken them recently. Women who took compounds with high levels of progestogen showed no excess ovarian cancer risk (data not shown). Those who quit after only 1 or 2 months, usually because of side effects, were not at

Table III. Estimated rate ratios according to pregnancies, births, and fetal losses

	Group with cancer	*Control group*	*Estimated rate ratio**	*95% Confidence interval*
Total pregnancies				
0	70	71	1.0	—
1	57	45	1.4	0.8-2.4
2	49	71	0.7	0.4-1.2
3	49	65	0.8	0.5-1.4
4	35	37	1.0	0.4-1.2
5+	35	54	0.7	0.4-1.2
Trend test		p (trend) = 0.10		
Live births				
0	89	84	1.0	—
1	54	53	1.0	0.6-1.7
2	71	85	0.8	0.5-1.3
3	43	60	0.7	0.4-1.2
4+	39	61	0.6	0.4-1.1
Trend test		p (trend) = 0.03		
Losses, adjusted for live births				
0	206	238	1.0	—
1	53	62	1.0	0.6-1.6
2	24	29	1.0	0.5-1.8
3+	13	14	1.2	0.5-2.7
Trend test		p (trend) = 0.70		

*Adjusted for age and race.

Table IV. Estimated rate ratios among parous women according to age at first birth and months of breastfeeding

	Group with cancer	*Control group*	*Estimated rate ratio**	*95% Confidence interval*
Age at first birth, adjusted for parity				
<20	42	52	1.0	—
20-24	72	108	0.7	0.4-1.2
25-29	58	62	1.0	0.5-1.7
≥30	34	33	1.0	0.5-2.0
Trend test		p (trend) = 0.22		
Months of breastfeeding, adjusted for parity				
0	112	121	1.0	—
1-9	62	84	0.8	0.5-1.2
10-18	16	37	0.5	0.2-0.9
19-110	13	15	1.1	0.5-2.6
Trend test		p (trend) = 0.14		

*Adjusted for age and race by logistic regression.

increased risk. The effect of oral contraceptives was similar for parous and nulliparous women, but it varied somewhat with age at diagnosis. Among women younger than 40 years old, oral contraceptives were weakly protective (rate ratio = 0.7), but among women aged 40 to 59 years, they were not (rate ratio = 1.1). Only one woman older than 59 years of age had taken oral contraceptives.

The effects of infertility were assessed in several ways. Married nulliparous women had a 70% higher risk than unmarried nulliparous women, an excess relative risk that was limited to those who reported difficulty conceiving (Table VI). Among the married nulliparous participants there was little difference in risk between those who had been pregnant and those who had not. In a separate confirmatory question, we asked married women who had never conceived why they had never been pregnant. Women who planned not to have children showed no increased risk compared with nonmarried nulliparous women (rate ratio = 1.0, 95% confidence interval = 0.4 to 2.4) but women who were unable to become pregnant had a corresponding rate ratio of 2.8 (95% confidence interval = 1.1 to 7.3). So few women reported each specific cause of infertility with confirmation by a physician that cause-specific estimates were quite unstable. Among parous women, a history of trouble conceiving was unrelated to risk (Table VI).

Participants were asked about a variety of medical factors found in previous studies to be related to risk of ovarian cancer. Our data on menopausal factors,

Table V. Estimated rate ratios according to oral contraceptive use for women younger than age 60 years

	Group with cancer	*Control group*	*Estimated rate ratio**	*95% Confidence interval*
Never took oral contraceptives	115	131	1.0	—
Ever took oral contraceptives	74	78	1.0	0.7-1.7
Duration (months)				
1-11	23	16	1.6	0.7-3.4
12-35	16	19	1.0	0.4-2.2
36-59	10	12	0.8	0.3-2.3
60+	25	31	0.8	0.4-1.5
Trend test		p (trend) = 0.76		
Latency (years since first taken)				
0-5	2	3	0.4	0.0-4.3
5-9	12	15	0.7	0.2-2.2
10-14	29	31	1.0	0.3-2.3
15+	30	29	1.1	0.6-2.1
Trend test		p (trend) = 0.72		
Recency (years since last taken)				
10+	28	24	1.4	0.7-2.6
1-9	38	41	0.9	0.5-1.6
<1	7	13	0.5	0.1-1.6
Trend test		p (trend) = 0.75		

*Adjusted for age and race; referent group for all comparisons is women who never took oral contraceptives.

Table VI. Estimated rate ratios according to reported difficulty conceiving*

	Group with cancer	*Control group*	*Estimated rate ratio†*	*95% Confidence interval*
Nulliparous women				
Never married	30	37	1.0	—
Ever married	59	47	1.7	0.9-3.4
No trouble conceiving or never tried	32	32	1.3	0.6-3.4
Had trouble conceiving	26	13	2.8	1.1-7.3
Parous women				
No trouble conceiving	164	209	1.0	—
Had trouble conceiving	42	50	1.0	0.6-1.7

*Women with incomplete responses excluded.

†Adjusted for age and race.

including protective effects of hysterectomy and menopausal estrogen, are detailed elsewhere. The results for selected childhood and adult diseases are given in Table VII. None of the childhood diseases influenced risk, regardless of whether it occurred before, after, or around the time of menarche. An extensive analysis of mumps revealed only that the protective effect of parity was absent among women who did not recall having had mumps.

Among the adult diseases, shingles was significantly protective but chickenpox was not. High blood pressure was weakly associated with increased risk. Thyroid disease was apparently protective, and hyperthyroidism was more protective than hypothyroidism (rate ratio = 0.5 and 0.9, respectively), but patients could not reliably make this distinction. Adrenal disease was reported by five women in the control group but no patients with cancer reported it. Diabetes was weakly protective. A history of asthma was also protective, but the effects of the illness and the asthma medication could not be distinguished because nearly all women with asthma had taken multiple medicines for their condition. Previous breast cancer was strongly related to risk but nulliparity, a strong risk factor for breast cancer and ovarian cancer, was present in only one of the 17 cases with previous breast cancer. There were only two histories of endometrial cancer among patients with cancer compared with four in the control group, a difference that was not statistically significant.

A family history of ovarian cancer in a sister, daughter or mother was reported by 13 patients with cancer and by only five women in the control group, which equals an elevated rate ratio of 3.3 (95% confidence interval = 1.1 to 9.4). No excess risks were seen for first-degree family histories of breast or endometrial cancer.

Obesity was not related to risk. We considered usual adult weight adjusted for height using Quetelet's index (weight ÷ height^2) divided into quartiles. The rate ra-

Table VII. Estimated rate ratios according to history of selected childhood and adult diseases

Disease	Number of participants who recalled disease*			
	Group with cancer	Control group	Estimated rate ratio†	95% Confidence interval
Mumps				
No	87	92	1.0	—
Yes	192	220	0.9	0.6-1.4
German measles				
No	99	124	1.0	—
Yes	112	136	1.0	0.7-1.5
Measles				
No	46	47	1.0	—
Yes	181	222	0.8	0.5-1.3
Chickenpox				
No	49	59	1.0	—
Yes	228	257	1.0	0.7-1.6
Shingles				
No	273	284	1.0	—
Yes	18	38	0.5	0.3-0.9
High blood pressure				
No	189	213	1.0	—
Yes	96	97	1.2	0.8-1.8
Thyroid disease				
No	237	236	1.0	—
Yes	56	78	0.7	0.5-1.1
Diabetes				
No	271	274	1.0	—
Yes	21	27	0.8	0.4-1.5
Asthma				
No	279	260	1.0	—
Yes	13	26	0.4	0.2-0.9
Breast cancer				
No	278	337	1.0	—
Yes	17	5	4.2	1.4-13.2

*Participants with uncertain responses excluded.
†Adjusted for age and race.

tios in increasing quartiles were 1.0, 1.4, 1.0, and 1.1, respectively.

As shown in Table VIII, ovarian cancer risk was related to drinking but was not related to cigarette smoking. Alcohol use was measured as the typical weekly number of shot glasses of alcohol, bottles of beer, and glasses of wine consumed over the past 10 years, with total alcohol consumption computed as the sum of the three subtotals. The rate ratio rose slightly with increasing total alcohol consumption, and with each of the three kinds of alcohol considered separately. The rate ratios were highest for whiskey (rate ratio = 1.6, 95% confidence interval = 1.1 to 2.3), followed by wine (rate ratio = 1.3, 95% confidence interval = 0.9 to 1.9) and beer (rate ratio = 1.1, 95% confidence interval = 0.7 to 1.6).

Comment

The results of this investigation confirm the importance of parity, infertility, family history, and previous breast cancer in the cause of epithelial ovarian cancer. The results also suggest the relative unimportance of some other factors associated with risk in previous epidemiologic investigations.

Several explanations of the protective effect of childbearing have been proffered, among them that pregnancy prevents ovulation, that it inhibits pituitary secretion of gonadotropins, or that an underlying problem leads both to low parity and increased risk. Estimation of the effects of different pregnancy outcomes may help reveal how childbearing reduces risk of ovarian cancer. If parity protected against ovarian cancer simply by the inhibition of ovulation, then a pregnancy would reduce risk approximately in proportion to its length, regardless of outcome. Our data suggest that pregnancies that lead to live births offer protection but those that lead to losses may not. Inasmuch as women with more live births tend to have had more losses, adjustment for parity is critical. One previous estimate of the rate ratio adjusted for parity was 0.8 per miscarriage.[7] Other investigations of fetal loss are not directly comparable, either because they were not adjusted for the effect of parity or because they measured the fraction of pregnancies that ended in

Table VIII. Estimated rate ratios according to cigarette and alcohol use*

	Group with cancer	*Control group*	*Estimated rate ratio†*	*95% Confidence interval*
Cigarette smoking				
Never smoked	128	130	1.0	—
Quit	91	69	1.3	0.9-2.0
Current	70	83	0.8	0.6-1.3
Cigarette smoking (duration)				
Never smoked	128	130	1.0	—
10 years or less	28	23	1.1	0.6-2.2
11-30 years	65	66	0.9	0.6-1.5
31+ years	66	64	1.1	0.7-1.8
Trend test		p (trend) = 0.87		
Alcohol (average weekly consumption)				
0	109	122	1.0	—
Occasional drink	49	49	1.1	0.7-1.9
1-6 drinks	63	54	1.4	0.8-2.3
7-13 drinks	36	32	1.2	0.7-2.2
14+ drinks	34	26	1.5	0.8-2.8
Trend test		p (trend) = 0.14		

*Three participants without data on smoking duration or currency were deleted.
†Adjusted for age and race.

miscarriage rather than the number or months of various pregnancy outcomes.

Apart from the reduced risk associated with higher parity, there clearly seems to be a separate link between infertility and risk evident among the nulliparous women in our investigation. Previous studies have shown that married nulliparous women have a higher risk than single nulliparous women.[1,2] We clarified this observation by showing that married nulliparous women who chose not to become pregnant were not at increased risk, but women who tried and failed definitely were at increased risk. Case-control studies of ovarian cancer cannot easily assess infertility as a risk factor, because older women must recall often ill-defined diagnoses from decades earlier. One follow-up study of infertile women found increased ovarian cancer risk among subjects with nonhormonal infertility (rate ratio = 3.2, 95% confidence interval = 0.3 to 32.9),[8] but another did not.[9] Both studies observed very few cancers and both derived the expected rates of ovarian cancer from the general population of parous and nulliparous women, so the effects of parity and infertility could not be separated. A long-term follow-up study of a large cohort of infertile women could discriminate between the effects of different kinds of infertility and could provide useful etiologic clues. For example, anovulation that leads to infertility should protect against ovarian cancer if incessant ovulation is a key mechanism.

Several reproductive factors probably are not strongly related to risk. A majority of studies indicate that the number of live births and not their timing is the key factor. Our finding of reduced risk to women who breastfed their children confirms a previous observation[10] and supports the hypothesis that anovulation reduces risk. The absence of dose response may reflect the poor correlation between the number of months a woman breastfeeds and the months of ovulation suppression because of other factors such as body mass and variability in breastfeeding regimens. Menstrual factors such as age at menarche, menstrual symptoms, and age at menopause do not appear to be strongly related to risk of ovarian cancer in these or other data, although the plateau in age-specific incidence around menopause strongly suggests a change in risk broadly related to cessation of ovarian function. It is not clear whether menopausal estrogen use affects risk. Hysterectomy does appear to decrease risk, but the association may reflect increased clinical surveillance rather than decreased risk.[11]

An anomalous finding in this study was that women who take oral contraceptives had only a very slight reduction in ovarian cancer risk. The estimated rate ratios from many studies have been in the protective range of 0.5. to 0.7. We know of no biases in the current study that would distort our estimate of the effects of oral contraceptives. Other known risk factors showed the same patterns in this study as in other studies, and women with diagnoses potentially related to oral contraceptives were not selected as control participants. From all of the available data we infer that oral contraceptives protect some women from ovarian cancer.

A history of mumps infection has been found to be a weak protective factor in several previous investigations.[1,2] This effect, if real, is certainly not strong. The biologic meaning is in doubt because recall of mumps

history has been shown to be a poor indicator of antibody titer.[13] A careful examination of mumps exposure, assessed serologically, would be worthwhile. Most of the other childhood and adult diseases that we considered probably do not influence the development of ovarian cancer. The apparent protection conferred by a history of shingles, in the absence of an effect of chickenpox, has no ready explanation. Nor does the effect of asthma. The lack of effect of hypertension, obesity, or diabetes, accords with the inconsistent findings of previous studies. Two medical conditions that warrant further study are thyroid disease and adrenal disease. The examination of adult diseases as risk factors in a hospital-based case-control study is difficult because the control group should include only patients whose admission diagnosis is not related (positively or negatively) to the adult disease risk factor. Data from population-based studies with well-documented medical histories may clarify whether these two associations are real.

Women with a previous history of breast cancer were at substantially increased risk of ovarian cancer. This observation is not new[14] and appears to represent a true association. One goal of our painstaking pathologic and clinical review was to exclude metastatic breast and colon cancer; we believe misdiagnosis does not explain the finding. A family history of ovarian cancer seems to pose at least a three- to fourfold increased risk, as indicated by our results and the results of others.[2]

Smoking was not related to ovarian cancer in this investigation, as in most previous work, but the effects of alcohol remain unclear. Our study and others (e.g., Fasoli and Franceschi)[15] estimate slightly increased incidence of ovarian cancer among drinkers, but other studies of ovarian cancer found reduced incidence among drinkers (e.g., Gwinn et al. and Byers et al.).[16, 17] The available data suggest that if there is an association, it is weak.

During the past decade, case-control interview studies with 200 to 500 women with ovarian cancer have offered numerous leads. Comparisons among existing studies suggest that some of the leads warrant further pursuit. Despite their cost, larger studies are needed to give the statistical power to disentangle the effects of specific causes of infertility, varying paterns of oral contraceptives, and specific pregnancy outcomes.

REFERENCES

1. Weiss N. Ovary. In: Schottenfeld D, Fraumeni JF Jr, eds. Cancer epidemiology and prevention. Philadelphia: WB Saunders, 1982:871-80.
2. Greene MH, Clark JW, Blayney DW. The epidemiology of ovarian cancer. Seminars in oncology 1984:11:209-26.
3. Richardson GS, Scully RE, Nikrui N, Nelson JH Jr. Common epithelial cancer of the ovary. N Engl J Med 1983; 312:415-24.
4. Hartge P, Hoover R, McGowan L, Lesher L, Norris HJ. Menopause and ovarian cancer. Am J Epidemiol 1988; 127:990-8.
5. McGowan L, Norris HJ, Hartge P, Hoover R, Lesher L. Risk factors for ovarian cancer. Eur J Gynecol Oncol 1988;9:195-9.
6. Rothman K. Modern epidemiology. Boston: Little, Brown, and Co., 1986.
7. Risch HA, Weiss NS, Lyon JL, Daling JR, Liff JM. Events of reproductive life and the incidence of epithelial ovarian cancer. Am J Epidemiol 1983;117:128-39.
8. Ron E, Lunenfeld B, Menczer J, et al. Cancer incidence in a cohort of infertile women. Am J Epidemiol 1987; 125:780-90.
9. Brinton LA, Melton LJ, Malkasian GD, Bond A, Hoover R. Cancer risk following evaluation for infertility. Am J Epidemiol 1989;129:712-22.
10. Schneider AP. Risk factor for ovarian cancer. N Engl J Med 1987;317:508-9.
11. Weiss NS, Harlow BL. Why does hysterectomy without bilateral oophorectomy influence the subsequent incidence of ovarian cancer? Am J Epidemiol 1986;124: 856-8.
12. Cramer DW, Welch WR, Cassells S, Scully RE. Mumps, menarche, menopause, and ovarian cancer. Am J Obstet Gynecol 1983;147:1-6.
13. Menczer J, Modan M, Ranon L, Golan A. Possible role of mumps virus in the etiology of ovarian cancer. Cancer 1979;43:1375-9.
14. Multiple primary cancers in Connecticut and Denmark. Bethesda, Maryland: National Cancer Institute, 1985; DHHS publication no. 68.
15. Fasoli M, Franceschi S. Dietary factors and the risk of epithelial ovarian cancer. JNCI 1987;79:663-9.
16. Gwinn ML, Webster LA, Lee NC, Layde PM, Rubin GL. Alcohol consumption and ovarian cancer risk. Am J Epidemiol 1986;123:759-66.
17. Byers T, Marshall J, Graham S, Mettlin C, Swanson M. A case-control study of dietary and nondietary factors in ovarian cancer. JNCI 1983;71:681-6.

Take the OC preference test.

1. In the United States last year, the OC group with the largest number of prescriptions was:

triphasic | low-dose monophasic | 1/50 formulations

2. In the last 18 months, more *new* prescriptions were dispensed for:

triphasics | low-dose monophasics

3. Which monophasic had an increase in new prescriptions 13 times the average of all low-dose monophasics in the last 18 months?

Demulen® 1/35* | Ortho-Novum® 1/35† | Lo/Ovral®‡

Scratch silver squares with a coin to reveal the answers.

*Demulen® 1/35 (ethynodiol diacetate 1 mg, ethinyl estradiol 35 mcg) is a registered trademark of G.D. Searle & Co.

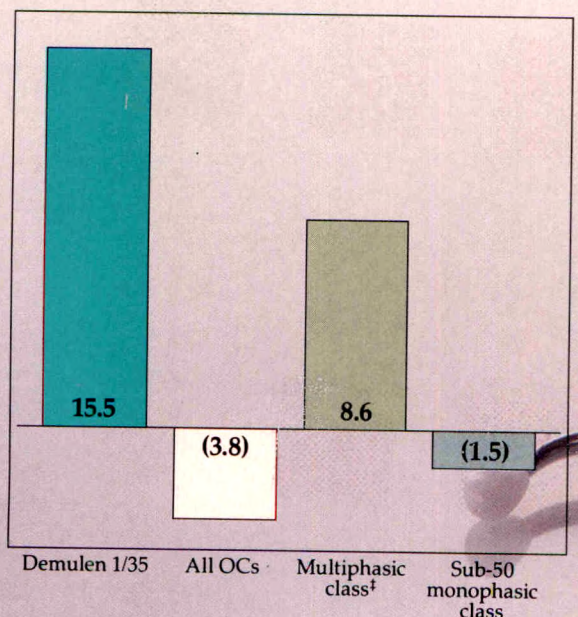
†Ortho-Novum® 1/35 (norethindrone 1 mg, .035 mg ethinyl estradiol) is a registered trademark of Ortho Pharmaceutical Corporation.

‡Lo/Ovral® (norgestrel and ethinyl estradiol tablets) is a registered trademark of Wyeth-Ayerst.

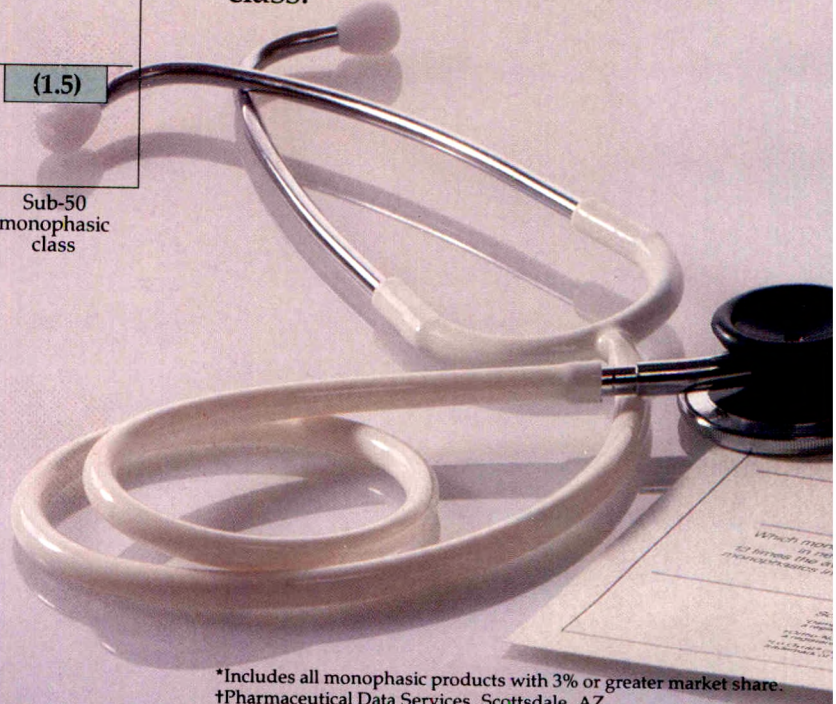
There's an OC in
and it's not

Now Demulen® 1/35 is the fastest growing monophasic*

New OC Rx's: *Percent change, October 1987 to March 1989†*



Today more and more physicians are taking an increased interest in Demulen 1/35 and prescribing it for their patients on oral contraceptive (OC) therapy. In fact, recent statistics show that new prescriptions for Demulen 1/35 rose by 16% during the last 18 months (October 1987 to March 1989).† That's a significantly greater increase than any other monophasic OC,* and nearly twice the growth rate of the multiphasic class.‡



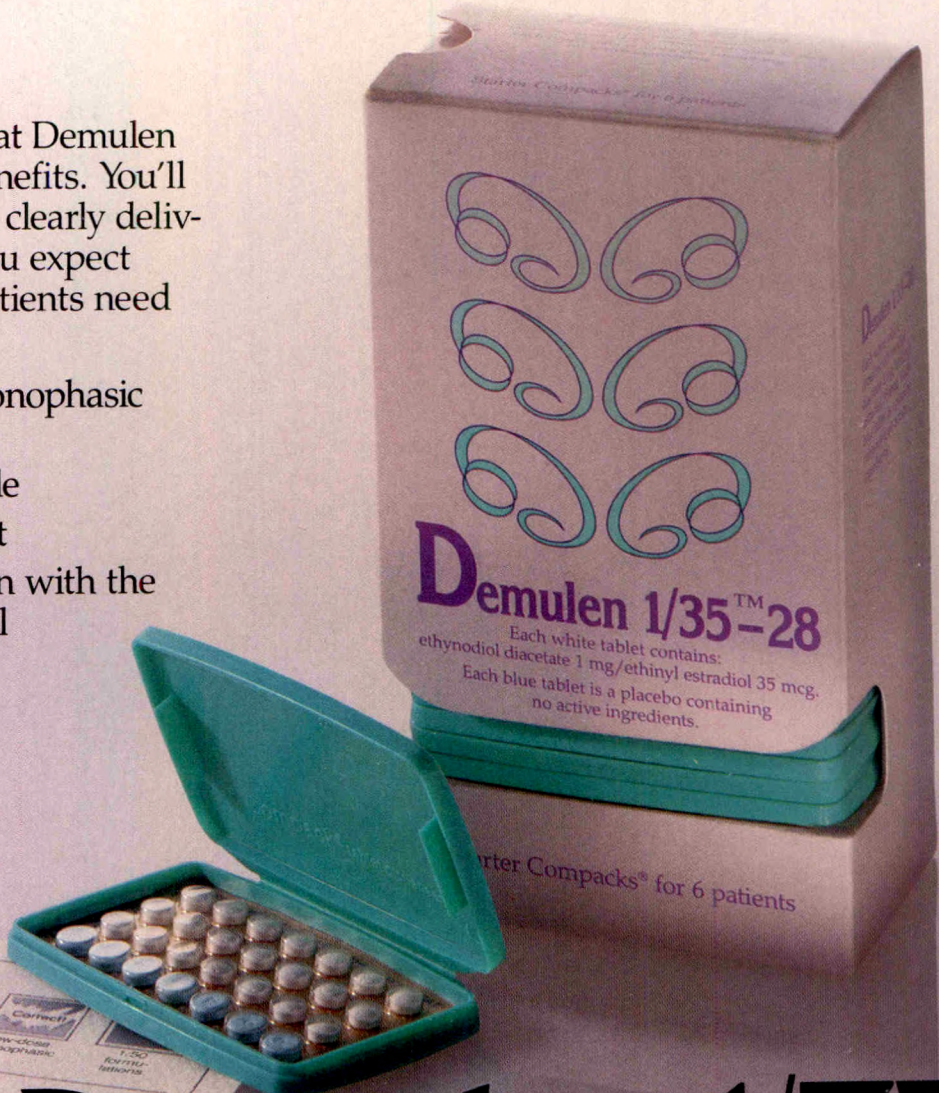
*Includes all monophasic products with 3% or greater market share.
†Pharmaceutical Data Services, Scottsdale, AZ.
‡Data derived from the change (by percentage) in the number of new prescriptions for all multiphasic products combined, from October 1987 to March 1989.

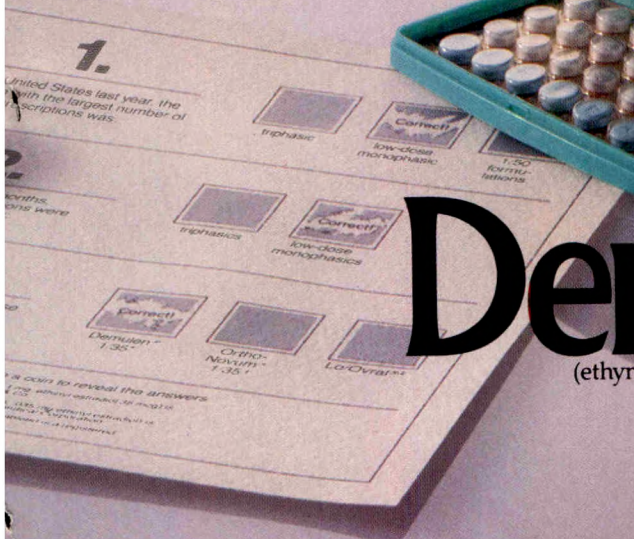
The physician should become familiar with the full prescribing information (labeling) for this product, which discusses reported risks, such as thromboembolic disorders (including strokes and heart attacks) and hepatic lesions, as well as the need to monitor patients for early symptoms of any diseases or medical conditions, so use can be discontinued where appropriate. A brief summary of the prescribing information accompanies this advertisement.

a growth phase—
a triphasic.

Take a closer look at Demulen 1/35 and add up the benefits. You'll find that Demulen 1/35 clearly delivers the performance you expect and the results your patients need from an OC.

- ☐ Reliable low-dose monophasic therapy
- ☐ Low side-effect profile
- ☐ Minimal lipid impact
- ☐ Exclusive formulation with the progestogen ethynodiol diacetate





Demulen® 1/35

(ethynodiol diacetate 1 mg, ethinyl estradiol 35 mcg)

Take a closer look

SEARLE

G. D. Searle & Co.
Box 5110, Chicago, IL 60680

© 1989, G.D. Searle & Co.

Please see brief summary of prescribing information on following page.

A89-A7450T

Demulen® 1/35

(ethynodiol diacetate 1 mg, ethinyl estradiol 35 mcg)

Demulen 1/35™ Each white tablet contains ethynodiol diacetate 1 mg and ethinyl estradiol 35 mcg. Each blue tablet in Demulen 1/35-28 is a placebo containing inert ingredients.

Demulen 1/50™ Each white tablet contains ethynodiol diacetate 1 mg and ethinyl estradiol 50 mcg. Each pink tablet in Demulen 1/50-28 is a placebo containing inert ingredients. **Indication and usage:** For prevention of pregnancy. Pregnancy rate for products with ≥35 mcg ethinyl estradiol or ≥50 mcg mestranol is ≤1/100 woman-years. With ≤35 mcg ethinyl estradiol, rate is slightly higher; with progestogen-only oral contraceptives (OCs), rate is about 3. Precise comparisons cannot be made. Rates for other methods have been published. *Dose-related Thromboembolic Risk*: Studies showed positive association between OC estrogen dose and thromboembolic risk. It is in keeping with good therapeutics to minimize estrogen exposure. Prescribe product containing least amount of estrogen compatible with acceptable pregnancy rate and patient acceptance. Start new users on ≤50 mcg estrogen. **Contraindications:** Thrombophlebitis, thromboembolic disorders, past history of deep vein thrombophlebitis or thromboembolic disorders; cerebrovascular disease, myocardial infarction or coronary artery disease, or past history of these; known or suspected breast carcinoma, estrogen-dependent neoplasia, or pregnancy; undiagnosed abnormal genital bleeding; past or present, benign or malignant liver tumors developing during use of estrogenic products. **Warnings:**

Cigarette-smoking increases risk of serious cardiovascular side effects from OC use. Risk increases with age and heavy smoking (15 or more cigarettes/day) and is quite marked in women over 35. OC users should be strongly advised not to smoke. OCs are associated with increased risk of several serious conditions including venous and arterial thromboembolism, thrombotic and hemorrhagic stroke, myocardial infarction, visual disorders, hepatic tumors, gallbladder disease, hypertension, fetal abnormalities. Practitioners should be familiar with the available information relating to these and other risks.

1. *Thromboembolic disorders and other vascular problems:* Increased risk of thromboembolic and thrombotic disease associated with OCs is well established. A British study showed increased risk for fatal venous thromboembolism (TE). British and U.S. studies showed increased risk for nonfatal disease. U.S. studies showed increased risk for stroke. OC users were 2-11 times more likely to manifest these diseases without evident cause. In a study of idiopathic deep vein thrombosis and pulmonary embolism (PE), projected hospitalization rates for ages 16-40 were 47/year/100,000 users and 5 for nonusers. Overall excess mortality from PE or stroke was 1.3-3.4 deaths/year/100,000 users and increased with age. *Cerebrovascular Disorders:* In a study of stroke with and without predispositions, risk of hemorrhagic stroke was 2 times greater and thrombotic stroke 4.0-9.5 times greater in users. Mortality trends in 21 countries indicate that changes in cardiovascular mortality were associated with changes in OC prevalence. The Royal College of GPs (RCGP) reported on its prospective study: "A statistically significant higher rate of reporting of cerebrovascular accidents in Takers is evident, but the numbers are too small to justify an estimation of the degree of risk." A 1981 analysis showed a 4-fold increased mortality in users from circulatory diseases, mainly myocardial infarction (MI) and hemorrhagic stroke. Excess mortality was associated with age and smoking. A 1983 analysis showed elevated cerebrovascular disease, increasing with use to 8 yrs, including cerebral thrombosis or embolism and TIA. Peripheral arterial disease and MI were increased. Women over 30 had more arterial disease, being greatest in smokers. Arterial case-fatality was 2-3 times greater for users who smoked. Family Planning Assoc. (FPA) found significant association between OC use and stroke; increased risk for venous thrombosis and PE was 4-fold and statistically significant. Mortality from nonrheumatic heart disease was greater in ever-users. In the Walnut Creek Study, subarachnoid hemorrhage risk was associated with smoking, age, and OC use. *Myocardial Infarction:* Increased risk of MI associated with OCs was reported in Britain, as previously suspected. The morbidity study found the greater the number of risk factors for coronary artery disease (smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk, regardless of OC use. OCs were an additional risk factor. Excess rate of fatal MI was 3.5/year/100,000 users aged 30-39 and 20 for ages 40-44. (These are based on British statistics with acute MI death rates 2-3 times less than the U.S. Similar estimates for the U.S. are 25.7 for users aged 30-39 with predispositions vs 1.5 without; for ages 40-44, 86.2 vs 5.1.) Excess hospitalization rate for nonfatal MI in Britain was 3.5/year/100,000 users aged 30-39 and 47 for ages 40-44. Smoking major predisposing condition to MI. In terms of relative risk, nonsmoking OC users are twice as likely to have fatal MI as nonsmoking nonusers. Smoking users have a 5-fold greater risk than nonsmoking users, but a 10-12-fold greater risk than nonsmoking nonusers. Amount of smoking is an important factor. In determining importance of these risks, give serious consideration to rates in table below. Importance of other predispositions cited above in determining relative and absolute risks has not been quantified; it is likely the same synergism exists, but perhaps to a lesser extent. U.S. studies similarly relate MI, OCs, smoking, and age.

Estimated Mortality Rate from Myocardial Infarction per 100,000 Women/Year

	Aged 30-39:	*Users*	*Nonusers*	*Aged 40-44:*	*Users*	*Nonusers*
All smokers		10.2	2.6		62.0	15.9
≥15 cigarettes daily		13.0	5.1		78.7	31.3
<15 cigarettes daily		4.7	0.9		28.6	5.7
Nonsmokers		1.8	1.2		10.7	7.4
Smokers and nonsmokers		5.4	1.9		32.8	11.7

Risk of Dose: Reports of TE after use of OCs with ≥50 mcg estrogen received by drug safety committees in Britain, Sweden, and Denmark were compared with distribution expected from market research sales estimates. A positive correlation was found between estrogen dose and reporting of TE, including coronary thrombosis, in excess of that predicted by sales. OCs with ≥100 mcg estrogen were associated with higher TE risk than those with 50-80 mcg. Estrogen quantity may not be the sole factor; influence of progestogens was not considered, which may be responsible for certain discrepancies in the data. No significant differences were noted between OCs containing same estrogen dose nor between ethinyl estradiol and mestranol. In Britain certain TE conditions were associated with progestogen or estrogen dose. In Sweden reports of TE decreased when higher estrogen doses were no longer prescribed. Studies on TE risk with progestogen-only OCs have not been done. Cases have been reported for these; they are not presumed free of risk. In a U.S. case-controlled study, relative risk of OC use 1 month before hospitalization for various types of TE was calculated. If relative estrogenic potency and possible influence of different progestogens are ignored, OCs may be divided into those with <100 mcg and those with ≥100 mcg estrogen. For all cases combined, larger doses were associated with only slightly higher risk; for idiopathic cases risk was doubled with larger doses, but confidence limits overlapped and differences were not significant. Apparently there was less increased risk for cases with predispositions. In FPA study no nonhemorrhagic strokes occurred with <50 mcg estrogen, vs 13 with higher doses. TE risk in users and nonusers increases with age. OCs have been considered an independent risk factor. *Persistence of Risk* after OC discontinuation was reported for circulatory disease, nonrheumatic heart disease, and cerebrovascular disease, including subarachnoid hemorrhage, cerebral thrombosis, and TIA. *Excess Mortality from Circulatory Diseases:* A U.K. prospective study estimated annual mortality from circulatory diseases for OC users and nonusers by age, smoking, and duration of use. Excess mortality was 23/100,000 users (2 and 10 for ages 25-34, 15 and 48 for ages 35-44, 41 and 179 for ages >44, for nonsmokers and smokers, respectively). Risk was significant only for smokers over 34. Most deaths were due to subarachnoid hemorrhage or ischemic heart disease. Risk for ever-users rose with parity. One study estimated risk of death with various contraceptive methods, each estimate including risk of method (eg, TE for OCs) plus risk due to pregnancy or abortion in cases of method failure. This latter risk varies with effectiveness. It concluded mortality with all methods is low and below that of childbirth, except for OC users over 40 who smoke. Heavy smokers have double the risk of smokers, light smokers about half. Mortality with OC use in nonsmokers over 40 is higher than with any other method in that age group. Lowest mortality is with condom or diaphragm backed up by early abortion. Risk of TE and thrombotic disease with OCs increases with age after 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia, and especially cigarette smoking. Based on current data, the chart gives gross estimates of risk of death from circulatory disorders associated with OCs:

Risk is: A—very high; B—high; C—moderate; D—low.	Age:	<30	30-39	≥40
Heavy smokers (≥15/day)		C	B	A
Light smokers (<15/day)		D	C	B
Nonsmokers, no predispositions		D	C,D	C
Nonsmokers, with predispositions		C	C,B	B,A

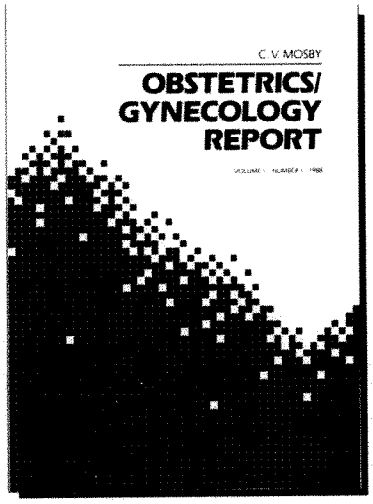
Be alert to earliest manifestations of TE and thrombotic disorders (eg, thrombophlebitis, PE, cerebrovascular insufficiency, coronary artery disease, MI, retinal thrombosis, mesenteric thrombosis). Should any be suspected, discontinue OCs immediately. A 4-7-fold increased risk of postsurgery TE was reported in users; if feasible discontinue OCs at least 4 weeks before surgery or in prolonged immobilization. Before resuming OCs, weigh risks of postsurgery TE complications against contraceptive needs. The RCGP prospective study reported higher incidence of superficial and deep vein thrombosis in users, the former correlated with progestogen dose. The data suggest varicose veins have little effect on development of deep vein thrombosis but substantially increase risk of superficial vein thrombosis, risk depending on severity of varicosities. **2.** *Ocular lesions:* Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OCs. Discontinue if there is unexplained, gradual or sudden, partial or complete loss of vision; proptosis or diplopia; papilledema; or evidence of retinal vascular lesions. Institute diagnostic and therapeutic measures. **3.** *Carcinoma:* Long-term estrogen in certain animals increases carcinomas and/or nonmalignant neoplasms, such as those of breast, uterus, cervix, vagina, ovary, liver, pituitary. Some synthetic progestogens, none currently in OCs, increase incidence of benign and malignant mammary nodules in dogs. There is evidence that estrogens increase risk of human endometrial carcinoma. In case-controlled studies, increased risk (2.2-13.9 times) associated endometrial carcinoma with prolonged estrogen for menopausal symptoms. Risk was independent of other known risk factors and depended on duration of use and dose. This is supported by increased endometrial cancer incidence since 1969 in areas with cancer-reporting systems, which may be related to rapidly expanding estrogen use. There is no evidence that natural estrogens are more or less hazardous than synthetics at equi-estrogenic doses. Of the first 30 cases of endometrial carcinoma in OC users under 40, those without predispositions (eg, irregular bleeding before OCs, polycystic ovaries) occurred nearly always with no-longer-marketed sequential OCs. No statistical association is reported suggesting increased endometrial cancer with combination or progestogen-only OCs, though individual cases were reported. Studies show no increased breast cancer with OCs or estrogens. However, greater risk was noted in OC users with benign breast disease or long-term (2-4 years) use. One study found greater risk of breast cancer with >4 years OC use prior to first full-term pregnancy. Risk of breast cancer with menopausal estrogens rose and further increased with duration of follow-up. OCs or estrogen were related to breast cancer in connection with other risk factors. Decrease in benign breast tumors in OC users is well known. Some studies suggested increased risk of cervical dysplasia, erosion, and carcinoma in long-term users. Cervical microglandular hyperplasia has been reported. In summary, there is no consistent evidence from human studies of increased cancer risk with OCs. Close surveillance is, however, essential. In undiagnosed persistent or recurrent abnormal vaginal bleeding, consider nonfunctional causes and take diagnostic measures to rule out malignancy. Carefully monitor users with strong family history of breast cancer or with breast nodules, fibrocystic disease, recurrent cystic mastitis, abnormal mammograms, or cervical dysplasia. **4.** *Hepatic adenomas, etc* have been associated with OCs. In 1 study OC formulations with high "hormonal potency" were associated with higher risk, as was age over 30. Though benign, these lesions may rupture and cause fatal hemoperitoneum in short- or long-term users. Two studies related risk to duration of use, being much greater after ≥4 years. Long-term OC users have an estimated adenoma incidence of 3-4/year/100,000. Consider such lesions in users with abdominal pain, tenderness, mass, or shock. About 25% present with abdominal masses, up to half with acute hemoperitoneum. Radiologic and lab studies may not be helpful; liver scans may show local defect. Hepatic arteriography or CAT may be useful. Hepatocellular carcinoma risk is increased 7-20 times in users for 8 or more years. Bile duct cancer may occur with OC use. OCs are contraindicated in case of past or present, benign or malignant liver tumors developing during use of estrogenic products. **5.** *Use in or immediately preceding pregnancy, birth defects, malignancy in offspring:* Estrogens or progestogens during early pregnancy may seriously damage offspring. Females exposed in utero to DES and other nonsteroidal estrogens may have increased risk of vaginal or cervical cancer. Though OCs may not enhance this risk, monitor such patients carefully. From 30-90% of DES-exposed women develop benign vaginal or cervical adenosis, which could be premalignant. DES-exposed daughters have more unfavorable pregnancy outcomes. Males so exposed may develop urogenital and sperm defects. Other estrogens may mimic DES. Reports relate fetal exposure to female sex hormones and congenital anomalies, eg, VACTERL for vertebral, anal, cardiac, tracheoesophageal, renal, and limb defects. There appears preferential expression by males. There was a 4.7-fold increased risk of limb-reduction defects in infants exposed in utero to sex hormones (OCs, hormonal withdrawal pregnancy tests, or treatment for threatened abortion). Some exposures were very short, being only a few days. Cardiovascular defects in children exposed to female hormones, including OCs, during early pregnancy occurred in 18.2/1,000 births, compared to 7.8 for those unexposed (P<.05). More twinning was reported in conceptions shortly after OC discontinuance. Female sex hormones were used for threatened or habitual abortion, but there is considerable evidence estrogens are ineffective and no evidence progestogens are effective. Triploidy and possibly other polyploidy may be increased in abortuses from women becoming pregnant soon post-OC. These almost always abort spontaneously. It is unknown if spontaneous abortion is increased soon after stopping OCs. Increased chromosome aberrations have been reported. Safety of OCs in pregnancy has not been demonstrated. Rule out pregnancy in using OCs and always consider it if withdrawal bleeding does not occur. If 2 consecutive periods are missed rule out pregnancy before continuing. If user deviates from regimen, consider pregnancy at first missed period and withhold OCs. If pregnancy is confirmed, apprise patient of potential fetal risks and discuss continuation of pregnancy. Users discontinuing for pregnancy should use alternative contraception for 3 months before attempting to conceive, though this is not based on precise information. Do not use progestogen-only or progestogen-estrogen drugs as pregnancy tests. **6.** *Gallbladder disease* risk, surgically confirmed, appeared after 2 years' use and doubled after 4-5 years in 1 study; it appeared in 6-12 months' use in another. **7.** *Carbohydrate and lipid metabolism:* Decreased glucose tolerance occurs in a significant number of users; carefully observe diabetic and prediabetic patients. Triglycerides, total phospholipids, and lipoproteins may increase. **8.** *Elevated blood pressure* (BP) occurs in some users in a few months, and degree may relate to progestogen dose. Hypertension prevalence in users is not higher in the first year, but increases with longer exposure; in the 5th year it is 2½—3 times the first year. Age is strongly correlated with hypertension in users. Those with history of elevated BP (hypertension), preexisting renal disease, history of toxemia or elevated BP in pregnancy, familial tendency to hypertension or its consequences, or history of excessive weight gain or fluid retention during menstrual cycles may be more likely to develop elevated BP on OCs and should be monitored closely. Even though elevated BP may be in "normal" range, do not ignore implications. Follow closely, particularly those with other risk factors for cardiovascular disease or stroke. High BP may or may not persist after stopping OCs. **9.** *Headache:* Onset or exacerbation of migraine or development of headache of a new pattern that is recurrent, persistent, or severe requires stopping OCs and evaluating cause. **10.** *Bleeding irregularities:* Breakthrough bleeding (BTB), spotting, and amenorrhea are frequent reasons for discontinuing. In BTB, as in all cases of irregular bleeding, consider nonfunctional causes. In undiagnosed persistent or recurrent bleeding, rule out pregnancy or malignancy. If pathology is excluded, time or change to another product may suffice. Change to a higher-estrogen OC only when necessary as this may increase thromboembolic risk. Those with history of oligomenorrhea or secondary amenorrhea or without established regular cycles may be anovulatory or amenorrheic after discontinuing. Those with such problems should be so informed and other methods encouraged. Post-use anovulation, possibly prolonged, may occur in women without previous irregularities. Higher incidence of galactorrhea and of pituitary tumors has been associated with postpill amenorrhea. One study reported 16-fold increased prevalence of pituitary prolactin-secreting tumors in patients with postpill amenorrhea when galactorrhea was present. **11.** *Ectopics:* In failures with combination OCs, the ratio of ectopic to intrauterine pregnancies is no higher than in nonusers. There may be increased risk of ectopics after discontinuing. **12.** *Breast feeding:* OCs may decrease quantity and quality of milk. A small fraction of OC steroids appears in milk. Effects if any on child are unknown. If feasible defer OCs until after weaning. **13.** *Infertility* may occur after discontinuing OCs. While impairment diminishes with time, there is still an appreciable difference in nulliparas aged 25-29 after 30 months; difference is negligible after 48 months. In nullips aged 30-34 impairment persists to 72 months and is more severe for 2-year users. In paras aged 25-34, difference is negligible after 30 months. **Precautions:** Conduct complete history and physical prior to OC use and annually thereafter. Include breasts, abdomen, and pelvis, with BP, Pap, and lab tests. Preexisting uterine leiomyomata may enlarge. Mental depression may occur; with positive history, observe carefully and discontinue if serious recurrence. Fluid retention may occur; prescribe cautiously and monitor carefully in convulsive disorders, migraine, asthma, or cardiac, hepatic, or renal dysfunction. History of jaundice in pregnancy increases risk of recurrence on OCs; observe carefully and discontinue if it occurs. Cholestatic jaundice occurred after OC and troleandomycin use. OC/cyclosporine combination may be hepatotoxic. Steroids may be poorly metabolized in liver dysfunction and should be given cautiously. Tryptophan metabolism may be disturbed and result in pyridoxine deficiency; megaloblastic anemia has been reported. Serum folate may be depressed and may be exacerbated in post-OC pregnancy. Advise pathologist of OC use when submitting specimens. Endocrine and liver function tests may be altered; repeat abnormal tests after stopping for 2 months. These have been observed: increased BSP retention and other abnormalities in liver function tests; increased prothrombin and coagulation factors VII, VIII, IX, X; decreased antithrombin III, increased platelet aggregability; increased thyroid-binding globulin leading to increased circulating total thyroid hormone (PBI, T^4); decreased T^3 uptake, unaltered free T^4, decreased pregnanediol excretion; reduced metyrapone response; increased blood transcortin, corticosteroid, triglyceride, phospholipid, reduced serum folate; impaired glucose tolerance; altered plasma trace minerals. Influence of prolonged OC use on pituitary, ovarian, adrenal, hepatic, or uterine function, or on immune response not established. OCs may mask onset of climacteric. Cervical chlamydial prevalence is increased; do not assume OC protects against chlamydial PID. *Information for patients.* See patient labeling. *Drug interactions:* OC effectiveness may be decreased and more BTB occur with rifampin, isoniazid, ampicillin, neomycin, penicillin V, tetracycline, chloramphenicol, sulfonamides, nitrofurantoin, griseofulvin, barbiturates, phenytoin, carbamazepine, primidone, phenylbutazone, analgesics, tranquilizers, antimigraines. OCs may alter effectiveness of oral anticoagulants, anticonvulsants, tranquilizers, tricyclic antidepressants, antihypertensives, theophylline, caffeine, vitamins, hypoglycemics, clofibrate, glucocorticoids, acetaminophen. **Adverse reactions:** Increased risk of the following serious adverse reactions has been associated with OCs: thrombophlebitis and thrombosis; pulmonary embolism; arterial thromboembolism; Raynaud's disease, myocardial infarction; coronary thrombosis; cerebral thrombosis; cerebral hemorrhage; hypertension; gallbladder disease; Budd-Chiari syndrome; benign adenomas and other hepatic lesions, with or without intra-abdominal bleeding, liver cancer, congenital abnormalities. There is evidence of association between the following and OCs, though confirmatory studies have not been done: mesenteric thrombosis; neuro-ocular lesions, eg, retinal thrombosis and optic neuritis. The following are believed drug related: nausea, vomiting, gastrointestinal symptoms, BTB, spotting, change in menstrual flow, dysmenorrhea, amenorrhea during and after use, infertility post-OC, edema, chloasma or melasma, which may persist post-OC, breast changes (tenderness, enlargement, secretion), weight changes, change in cervical erosion and secretion, endocervical hyperplasia, lactation decrease, cholestatic jaundice, migraine, enlarged uterine leiomyomata, allergic rash, mental depression, reduced carbohydrate tolerance, vaginal candidiasis, change in corneal curvature (steepening), intolerance to contact lenses. Association not confirmed or refuted for: premenstrual-like syndrome, cataracts, libido changes, chorea, appetite changes, cystitis-like syndrome, headache, paresthesia, nervousness, dizziness, auditory disturbances, rhinitis, fatigue, backache, hirsutism, scalp hair loss, erythema multiforme or nodosum, hemorrhagic eruption, herpes gestationis, hemolytic uremia, malignant hypertension, itching, vaginitis, porphyria, impaired renal function, acute renal failure, sometimes irreversible, PVCs, abnormal EKG, pulmonary hypertension, TTP, anemia, pancreatitis, hepatitis, colitis, Crohn's disease, gingivitis, dry socket, lupus erythematosus, rheumatoid arthritis, pituitary tumors (eg, adenoma) with amenorrhea and/or galactorrhea after use, malignant melanoma, endometrial, cervical, breast, and bile duct carcinoma. **Acute overdosage:** Serious ill effects have not been reported after large acute doses of OCs in young children. Nausea and withdrawal bleeding might occur. **Important note: Complete prescribing information should be consulted prior to use and is available on request.**

9/21/87 • P87-A4830V

SEARLE Searle & Co. San Juan, PR 00936

Address medical inquiries to: G.D. Searle & Co. Medical & Scientific Information Department 4901 Searle Parkway, Skokie, IL 60077

An idea whose time has come



OBSTETRICS/GYNECOLOGY REPORT

The depth of texts, the timeliness of journals . . .

OBSTETRICS/GYNECOLOGY REPORT, a **new** resource that works for **you**, is a synthesis of current information on the most important clinical developments in the field. The REPORT makes it **convenient** for the busy practitioner to stay up to date.

- Three issues every 12 months.
- Each issue focuses on two main topics within the specialty:

Vol. 1, No. 1	Infectious diseases Reproductive endo-fertility
Vol. 1, No. 2	Fetal monitoring Human papilloma virus
Vol. 1, No. 3	Breast disease Urinary incontinence

- Comprehensive coverage of the selected topics, from new developments to cases of interest.
- Approximately 150 pages per issue; no advertisements.
- Attractive, two-color format throughout.

Our editorial board of prominent physicians is committed to offering you an insightful, reliable, and practical resource that truly is beneficial to clinical practice.

Editor in Chief: **Frederick P. Zuspan, MD**

Editors:

Richard C. Bump, MD	**Ronald S. Gibbs, MD**
William T. Creasman, MD	**Mary G. Hammond, MD**
Philip J. DiSaia, MD	**Glenn Hurt, MD**
Patrick Duff, MD	**Mark B. Landon, MD**
Steven G. Gabbe, MD	**Edward J. Quilligan, MD**
Thomas J. Garite, MD	**Luther M. Talbert, MD**

Subscribe now at the low rate of $52.50 U.S. and $58.50 Canada and International.

Try the REPORT under our no-risk, money-back guarantee! To place an order or to request more information about OBSTETRICS/GYNECOLOGY REPORT, please contact the Journal Circulation Department ■ The C.V. Mosby Company ■ 11830 Westline Industrial Drive ■ St. Louis, MO 63146. In the U.S., call toll-free: 1-800-325-4177, ext. 394. Or call: (314) 872-8370, ext. 394.

OBR-010

Mosby

2V MEDTECH INTERNATIONAL CORP.

DIAGNOSIS 2V STAT TREATMENT

PRESENTS

2V STAT Medical Decision Support Software Covering 69 Specialities – Algorithms and Text.

PLUS

COMPUTER 80286/10 MHz Turbo CPU, 80 MB HD, EGA Color Monitor, Color Graphic Card, 40 MB Back-up Drive, and Printer.

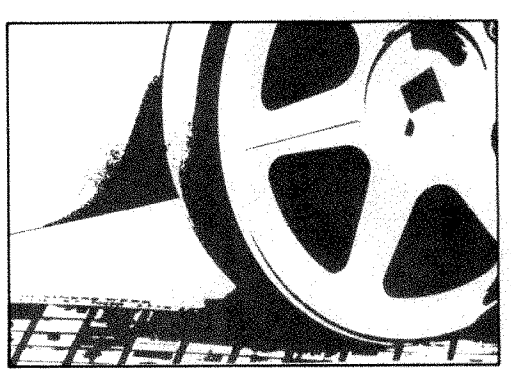
$5962
(for complete system)

ORDER NOW!
1•800•228•STAT
Technical Support Call 1•404•956•1855

Add volume to your practice and make an extra $500K with only a $5,962 one-time investment with 2V STAT including managerial support, brochures, +/- one-day seminar.

2480 Windy Hill Road, Suite 201 • Marietta, Georgia 30067
Fax 404•956•7068 Telex 551341 MedVideo Easy Link 622779660

This Publication is available in Microform.



University Microfilms International

Please send additional information
for _____ (name of publication)
Name_____
Institution_____
Street_____
City_____
State_____ Zip_____

300 North Zeeb Road, Dept. P.R., Ann Arbor, Mi. 48106

Make a clean break



to 3-day therapy*

Cure vulvovaginal candidiasis in just three days*

Broad spectrum of activity

- Cures infections due to *Candida albicans* and *Candida tropicalis* within 3 days.

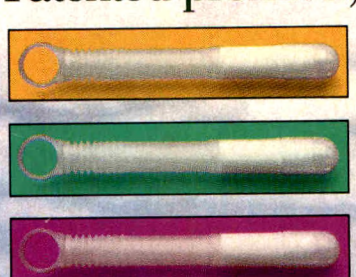
Documented 3-day efficacy

- Microbiological cure rates of 95% eight days following Femstat 3-day therapy.[1]
- Low relapse rates 30 days post therapy.

Femstat Prefill is:

- NOT a triazole/NO flu-like symptoms.
- Safe in second- and third-trimester pregnant patients.*

Patented prefilled, disposable applicators



- Disposable to reduce risk of reinfection.
- Prefilled for unit-dose accuracy.
- Designed for enhanced patient compliance.

*In pregnant patients (second and third trimesters only), therapy should be extended to six days.

vaginal cream 2%

Femstat® Prefill™

(butoconazole nitrate)



Make it your choice for unsurpassed efficacy and convenience.

1. Droegemueller W, Adamson DG, Brown D et al: Three-day treatment with butoconazole nitrate for vulvovaginal candidiasis. *Obstet Gynecol* 1984;64(4):530-534.

© 1999 Syntex Laboratories, Inc.

SYNTEX

SYNTEX LABORATORIES, INC.
PALO ALTO, CA 94304

vaginal cream 2%
Femstat® Prefill
(butoconazole nitrate)



BRIEF SUMMARY

INDICATIONS: FEMSTAT® (butoconazole nitrate) vaginal cream 2.0% is indicated for local treatment of vulvovaginal mycotic infections caused by Candida species. Confirm the diagnosis by KOH smears and/or cultures. FEMSTAT can be used with oral contraceptives and antibiotics. It is effective in non-pregnant women and during the second and third trimesters of pregnancy.

CONTRAINDICATIONS: FEMSTAT is contraindicated in patients hypersensitive to any of the ingredients.

PRECAUTIONS: General: If clinical symptoms persist, repeat microbiological tests to rule out other pathogens and confirm the diagnosis. Discontinue drug if sensitization or irritation occurs.

Information for the Patient: Do not discontinue prematurely during menstruation or because of symptomatic relief.

Carcinogenesis: Animal studies have not been done.

Mutagenesis: Mutagenicity studies were negative.

Impairment of Fertility: Animal studies showed no impairment of fertility.

Pregnancy Category C: Adverse effects were noted in animals treated with high oral doses. No studies were done in women during first trimester. Patients in the second or third trimester have shown no adverse effects attributable to the drug.

Nursing Mothers: Use with caution.

Pediatric Use: Safety and efficacy have not been established.

ADVERSE REACTIONS: Vulvar/vaginal burning in 2.3% of patients, vulvar itching in 0.9%, discharge, soreness, swelling, itching of fingers each in 0.2%. Complaints caused 1.6% to discontinue drug.

DOSAGE AND ADMINISTRATION:
Non-pregnant Patients: The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for three days. Treatment can be extended for an additional three days if necessary.

Pregnant Patients (second and third trimesters only): The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for six days.

CAUTION:
Federal law prohibits dispensing without prescription.

SYNTEX
SYNTEX LABORATORIES, INC.
PALO ALTO, CA 94304

Your comprehensive reference guide to a decade of valuable clinical information . . .

Ten-Year Cumulative Index (1978-1987) to the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY

Now you have easy access to 10 years of clinical information, indexed in one convenient source — the Ten-Year Cumulative Index to the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY.

This hard-cover index is a comprehensive guide to more than 30,000 pages of original articles, case reports, letters, and editorials published in the Journal from 1978 through 1987 (volumes 130 to 157). These influential, peer-reviewed papers are indexed both by subject and by author.

The Cumulative Index features:
- more than 800 pages
- both a subject and an author index
- 4,331 subject headings, containing 24,126 references
- 22,456 author entries

The Cumulative Index is a great time-saver for locating information on clinical topics of immediate or historical significance in obstetrics and gynecology. There's no need to search for index issues from each year.

Order this key reference guide for your personal library!

YES! Please send me the Ten-Year Cumulative Index to the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY (1978-1987). I've indicated my method of payment below.

☐ $64.50 U.S. ☐ $69.00 International
(prices include shipping)

Payment must accompany your order.

☐ Check enclosed (U.S. funds, drawn on a U.S. bank, payable to The C.V. Mosby Company)
☐ MasterCard ☐ VISA

Card # _____ Exp. date _____
Signature _____
Name _____
Address _____
Apt/Suite/Dept _____
City _____ State _____
ZIP/PC _____ Country _____
Please allow six weeks for delivery. **ABS-267**

Return to:
The C.V. Mosby Company
Journal Circulation Department
11830 Westline Industrial Drive
St. Louis, MO 63146 U.S.A.

Heuristic determination of relevant diagnostic procedures in a medical expert system for gynecology

Steven L. Small, PhD,[a] and Eberhard K. Muechler, MD[b]

Pittsburgh, Pennsylvania, and Rochester, New York

Many professions including medicine have standard operating procedures for the performance of their tasks. In the construction of expert systems, knowledge engineers have exploited this fact in devising heuristic rules that mimic the standard practice among such personnel (i.e., experts). This article suggests that the expert system designer should not stop at the level of the standard operating procedure heuristic but should instead investigate the reasons that the standard procedures have become standard. Because the experts in a field often do not understand the reasons for the standard operating procedures of their profession, this effort not only rewards the system designer but the expert as well. Because medical training does not always emphasize the logical reasoning underlying certain standard operating procedures, the ability to perform this reasoning is especially important in medicine. Further, a medical expert system for consultation or education would make a valuable impact by incorporating such knowledge and inference rules. This article investigates the development of a computerized medical expert system that applies the principles of artificial intelligence by limiting the number of questions and tests to find the solution for an ill-defined complex problem. Finally, we describe a logic program that tests the basic ideas. (Am J Obstet Gynecol 1989;161:17-24.)

Key words: Artificial intelligence, medical expert system, diagnostic reasoning in gynecology

The purposes for building expert systems in medicine are either (1) clinical advice for physicians who are experts or nonexperts in the particular area of the program, or (2) teaching students or postdoctoral trainees of the methods of practice in that area. Because these systems may reflect accurately the long-standing methods of medical education and practice in their emphasis on standard operating procedures, perhaps the use of automated reasoning could be used to improve this practice. Both consultation and educational programs might be substantially improved by more extensive reasoning than can be obtained by the typical standard operating procedure (SOP) heuristics of these systems.

Existing medical expert systems that are aimed at the diagnostic instead of the treatment phase seem to use variations on two similar approaches: (a) the findings/diagnosis matrix and (b) the findings/conclusion rule set. Most of the type a systems do not represent causality in any direct way and instead rely on correlations between sets of findings and conclusions. Usually they are not considered to be artificial intelligence systems, but their designers do call them expert systems. Our attention was called to this approach by a large system to diagnose problems of infertility in couples.[1] The system scored a wide range of findings and attempted to rule in and rule out diagnoses from a large number included in the data base. The type b systems (artificial intelligence medical (AIM) systems) represent causality directly, and the different chains of reasoning from the given findings to the possible conclusions are scored by their likelihood of being correct. The likelihood for any chain of reasoning depends on numerical combination of the individual likelihoods for each reasoning step. In both type a and type b systems, the scoring systems are applications of mathematical physics. A group of abstract speculations are written as equations in a computer program and the computer solves the equations by using a numerical value system for reaching conclusions.

These medical expert systems incorporate vast amounts of knowledge centered on the different medical findings and the conclusions that can be drawn from these findings. However, in actual practice the availability of different findings depends on the physician's ability to ask the proper questions, perform procedures; or request that procedures be performed. Furthermore, the physician is expected to perform the required diagnostic procedures in some rational order and to perform only those procedures that are actually required to make the correct diagnosis. This situation suggests that the diagnostic procedures themselves be given a focal place in the representation of medical knowledge for diagnosis. The gynecologic domain of infertility is used in this presentation. It represents a well-defined field with medical experts (reproductive

From the Department of Computer Science and the Decision Systems Laboratory, University of Pittsburgh,[a] and the Department of Obstetrics and Gynecology, University of Rochester.[b]

Reprint requests: Eberhard K. Muechler, MD, 1561 Long Pond Road, Rochester, NY 14626.

endocrinologists) who daily perform the complex tasks of decision making with patients and teach patterns of abstract thinking to students and residents.

The system to be described uses knowledge about diagnostic procedures as a fundamental source of heuristic knowledge for problem solving. Thus it broadens the search space from the space of diagnostic findings to one that includes the examinations and tests that obtain those findings. The heuristic rules weigh the advantages and disadvantages of different procedures at each stage of the search for an accurate diagnosis.

Heuristics are strategies (rules of thumb) that are applied to a defined set of observations that are obtained by patient history, physical findings, and laboratory tests assigned a value system to reach conclusions and recommendations. Artificial intelligence uses heuristics in computer programs to mimic the ability to solve problems by medical experts. In cases of female infertility the most relevant facts in the history focus on the menstrual cycle, a history of pain, infection, contraceptive use, and previous pelvic surgery. The observations of physical examination are usually confined to a limited set of findings obtained by inspection and palpation, such as appearance and color of the cervix and vagina; size, position, and regularity of uterus, ovaries, and tubes; presence or absence of pain on palpation, change in consistency of structures that are usually not palpable (ligaments). In addition to this information, a set of biochemical laboratory procedures (e.g., blood count, blood chemistry, and hormone values) and biophysical tests (e.g., ultrasonography, hysterosalpingogram, endometrial biopsy, hysteroscopy, and laparoscopy) define the process of data gathering that leads the medical expert to conclusions and decisions. The cost factor of different procedures is subconsciously incorporated by medical experts in assigning a rank order in the decision-making process. Note that "cost" is defined in the most general sense of the word, including morbidity and mortality as the most important costs, with pain, inconvenience, and financial factors as secondary costs. However, the financial cost of a procedure itself implicitly represents to some extent the overall risk factor of the procedure.

Diagnostic procedure reasoning

Our goal has been to explore the diagnostic process to construct a computer program that can reason over a range of gynecologic problems. Diagnostic process is defined as the step-by-step procedure of recommending diagnostic tests and acting on their results. This cycle continues until some test gives a definitive result. Performing the tests themselves is considered adjunctive to the diagnosis process, and is left to the laboratory or physician. The medical problems included in the study are meant to be indicative of typical gynecologic presentations and should not be considered special in any way. If the notions presented do not seem applicable to domains other than the sample domain, then we have not been successful in our approach.

A major decision in the approach to this problem concerned what information was to be given explicitly to the program and what information the program should infer for itself. This issue was confronted early, since a first diagnostic test is recommended for almost all gynecologic patients in almost all circumstances—the pelvic examination. Most gynecologists would claim that this test should always be done as part of an initial patient examination, regardless of the other aspects of this examination. It seemed to us that despite the widespread agreement on the need for this examination as an initial diagnostic procedure, the reasons should be investigated more carefully. If some test seems universally accepted as a standard part of some diagnostic sequence, it must have some specific value in that role, underlying its acceptance, that can be uncovered.

It should be noted that this reasoning is not specific to the pelvic examination or to gynecologic diagnosis. An analysis of the medical history section of the normal physical examination leads to the same questions. Why does the physician take a patient history before performing an exploratory laparotomy when a patient is initially seen with abdominal pain? Likewise, when a computer goes down, why does the system programmer examine the programs that were running on the machine, instead of immediately changing some of the circuit boards? Clearly, the expert medical doctor or computer fault diagnostician considers various costs, such as financial cost or the amount of time required, in a stepwise approach to solving the problem. If a low-cost approach does not succeed, then a higher cost approach should be tried.

Because standard operating procedures exist for troubleshooting in expert domains, and these can be used heuristically in computer programs to advise troubleshooters, it might be advantageous to investigate these procedures and determine why they were initially devised. With that knowledge, a much better expert computer program can be built that incorporates an ability to reason at a deeper level than one based on SOPs. In a system for medical diagnosis, such an approach would lead the designer to consider various levels of explanation back to the relevant physiology and cell biology. The simple medical diagnosis program described in this paper uses information at only a single level removed from normal clinical SOPs. However, for a system to be useful it must be fluent in the more basic explanations.

Instead of focusing exclusively on the causal relationships that pertain in a collection of diagnostic findings, our program uses additional knowledge about the procedures that obtain these findings. It reasons *which* procedures should be performed and *why* they should be performed to obtain the findings that are needed. Thus the course of the overall diagnostic procedure is

driven by the determination of the relevant medical tests or procedures that should be performed at a given point in the process. By making this determination a rational process instead of one based on standard operating procedure, the underlying reasons for performing or not performing a procedure at a particular time, such as cost or effectiveness at evaluating competing diagnoses, can be addressed explicitly. Often these reasons are not even realized explicitly by physicians who make decisions based on them.

The foregoing application of artificial intelligence to gynecology was designed through collaboration between the computer scientist and the medical expert in reproductive endocrinology. In this scenario the medical expert provides the reasoning for abstract decision making and the computer scientist uses mathematical rules of value to arrive at conclusions that simulate the thought process.

As an initial test of these ideas, a small program has been implemented to recommend diagnostic procedures and to use the results of these procedures either to recommend other procedures or to make a final diagnosis. The gynecologic domain in this example consists of the common symptoms of pelvic pain and infertility. The medical expert and the computer should arrive at a limited number of tests and procedures in a logical sequence without excessive cost. The program has been coded in a Horne clause theorem proving system based on Prolog[2] and implemented in Lisp.[2,3]

For each diagnostic procedure the system incorporates a number of facts describing the different classes of findings that that procedure can be used to determine. The following predicate states that the pelvic examination can be used to determine the position of a patient's uterus (anteflexed or retroflexed):

(procedure pelvic-exam uterine-position)

In addition, for each procedure the program includes certain information about the different costs associated with that procedure. The following predicate is representative:

(cost pelvic-exam pain small)

This assertion indicates that the pelvic examination has a small amount of pain associated with it. The program uses symbolic descriptions (e.g., small) instead of numerical coefficients in all user predicates, although ad hoc numerical computations do drive the reasoning.

At least four different cost parameters appear to be considered in the process of choosing which diagnostic procedures to perform at a particular point in evaluating a patient: risk of morbidity, financial cost, pain and suffering, and time expenditure. The overall cost of a procedure is a weighted algebraic sum of these individual factors. In different domains and in certain contexts, each of these factors becomes extremely important to decision making in the real world. The *risk of morbidity* might be always considered paramount, but consider a case in which the diagnoses themselves involve tremendous morbidity. *Financial cost* may in some circumstances depend on the wishes of the payer, which may or may not be a patient. *Pain* may rarely be considered by the physician, but depending on the importance of the procedure, may be important for the patient. The *expenditure of time* does not matter much in most diagnoses in gynecology. Consider the time required to analyze arterial oxygen or carbon dioxide, procedures that are important and common in people with respiratory problems, if the patient shows severe respiratory distress and will suffocate without immediate intervention.

The four cost parameters themselves do not direct the decision-making process toward the correct class of procedures, although they do aid in the decision from among a class of procedures that might be appropriate at a given time. Another needed heuristic must correlate the costs with the diagnostic process, represented by the following information: the need for new findings, the procedures that can obtain those findings, and the plausible competing diagnoses at the current point in the overall stage of reasoning. A physician knows which findings would help to differentiate among the leading diagnoses; indeed, this knowledge has been encoded in many good AIM systems.[4-9] The physician also knows which procedures can provide this information or make suggestions about it. With this knowledge, a program should make good decisions.

Results

Our example program contains an oversimplified set of predicates about these issues. For each possible diagnosis, the data base contains predicates about the classes of findings required to make that diagnosis. The following predicate is typical:

(dxneed chronic-PID tubal-patency)

This assertion indicates that the program needs some value for the parameter *tubal-patency* to make a diagnosis of chronic pelvic inflammatory disease associated with infertility. In fact, the heuristics of the program use these data as suggestions; a finding regarding tubal patency would be helpful for the diagnosis but not necessary. As described above, the data base also contains predicates such as the following, which are used as one might expect:

(procedure HSG tubal-patency)

This datum indicates that the tubal patency of a patient can be assessed with the hysterosalpingogram procedure. These two sorts of facts, along with cost information, are used in conjunction with the leading potential diagnoses to choose an appropriate procedure to recommend. The findings and diagnoses are scored by an ad hoc numerical scheme, but certain numerical

Table I. Diagnoses and tests included in the AIM system

Diagnoses
Leiomyoma
Chronic pelvic inflammatory disease
Endometriosis
Uterine polyps
Cervical polyps
Chronic cystourethritis
Ileitis
Colitis
Tests
Pelvic examination
Uterine sound
Hysterosalpingogram
Ultrasonography
Laparoscopy

values (thresholds) are assigned symbolic names for use in the heuristics.

The currently operating computer system comprises approximately 50 rules and approximately 100 basic facts. The relevant diagnoses and tests are listed in Table I. To recommend a diagnostic procedure, the system uses logic to try to "prove" that a certain procedure should be recommended. Each such proof must be based on proving a number of other smaller notions, until eventually a combination of simple facts suffices to complete the picture. The system uses two primary heuristic rules for recommending procedures, both of which will be presented here. Note that each rule calls on the system to perform a number of other tasks (i.e., to prove that a number of conditions pertain) before making the recommendation. These secondary rules are not illustrated here.

The data base of the system stores the names of the procedures that it can recommend, such as the pelvic examination, hysterosalpingogram, or laparoscopy. The system attempts to recommend all of these procedures, and is allowed to use any available rules to do so. However, the resulting recommendation always consists of a single procedure because of the nature of the rules themselves (shown below), which prefers first, useful procedures, and second, ones that are of low cost.

The basic heuristic for recommending a diagnosis consists of the following:

1. Find two diagnoses that are plausible
2. Find one class of findings that are needed by both
3. Find a procedure that can get this finding that has not already been performed, and
4. Ensure that the cost of this procedure is low

A *diagnosis* is considered *plausible* if its score has reached a certain threshold. A diagnosis is considered *suggested* or *probable* if its score has reached one or another higher threshold. A suggested or probable diagnosis warrants a more costly procedure than does a plausible one.

The computer program code for this first heuristic rule illustrates the predicates used and the order in which they are evaluated. The horn clause below contains a conclusion on the left and a conjunction of preconditions on the right:

```
((recommend ?proc) < (diagnosis ?dx1
                      plausible)
                     (diagnosis ?dx2
                      plausible)
                     (NEQ ?dx1 ?dx2)
                     (dxneed ?dx1 ?param)
                     (dxneed ?dx2 ?param)
                     (procedure ?proc
                      ?param)
                     (status procedure ?proc
                      available)
                     (sumcost ?proc ?cost)
                     (maximumthreshold
                      sumcost ?cost minimal))
```

This rule can be written in English as follows:

If: Some diagnosis dx1 is plausible;
and
Some diagnosis dx2 is plausible;
and
Diagnoses dx1 and dx2 are not the same; and
Finding param helps in the diagnosis dx1; and
Finding param helps in the diagnosis of dx2; and
Procedure proc can determine finding param; and
Procedure proc has not yet been performed and
The cost of procedure proc is cost; and
Cost cost is minimal; then

Conclude: Procedure proc is recommended

Currently, a simple polynomial equation computes the cost of a particular procedure and the coefficients for this polynomial are not context dependent. The symbolic name of a cost (e.g., minimal) represents a threshold on a numerical parameter. Note that each of the conditions in the "if" leads to the use of additional rules (e.g., showing that some diagnosis is "plausible"). It is important to understand the reclusive nature of a reasoning system based on logical axioms and proof techniques. In the two heuristic rules shown, a number of additional rules are involved to achieve the goal. For example, rules exist to find competing diagnoses. These particular rules comprise no more than a numerical evidence scheme as in Mycin,[4] with mappings from numbers to labels (e.g., "plausible" or "definitive"). The reclusive algorithm ensures all diagnoses included in the system (see Table I) are exhaustively searched until the diagnoses with the needed attribute (e.g., "plausible") are found.

In using the first heuristic, the system attempts first

to prove that the diagnoses of chronic pelvic inflammatory disease and leiomyoma are plausible. If one of these could not be proved, the system tries reclusively another diagnosis until finding the two needed. In fact, one computational argument against proof-based systems is the slow nature of exhaustive search.

Sometimes a physician bypasses the lowest cost procedures in favor of a procedure of significantly higher cost because of an intuitive feeling that a particular diagnosis can be confirmed. What this probably represents is something that has been incorporated in INTERNIST[6] for some time, namely, that the difference in score between the top two diagnoses may be more important than the absolute score of either.[10] This second main heuristic rule is applied as follows:

1. Find the two highest ranking diagnoses.
2. Make sure their scores are very far apart.
3. Find a class of findings that can make the top diagnosis definitive.
4. Find a procedure that can get this finding.

This situation occasionally occurs in the gynecology domain, when some patient presentation resembles a classic pattern so closely that diagnostic surgery such as laparoscopy is performed immediately without the lower cost pre-procedures that ordinarily precede it. This is illustrated in the sample trace shown in the Appendix, when laparoscopic surgery becomes the procedure of choice early in the diagnostic process because of an overwhelming system preference for one diagnosis (i.e., endometriosis) over all others. Our program performs the same inference that is expected from a medical expert who uses his complex thought process for proceeding with the differential diagnosis and arriving at a specific conclusion that is accurate and cost effective.[11]

The program code for this heuristic rule might be helpful for understanding the nature of the system:

```
((recommend ?proc) < (SETVALUE ?topdx
                      (bestdx 1))
                     (SETVALUE ?seconddx
                      (bestdx 2))
                     (dxvalue ?topdx ?topval)
                     (dxvalue ?seconddx
                      ?secondval)
                     (freqcode spread large
                      ?spread)
                     (spreaddx ?topval
                      ?secondval ?spread)
                     (dxneed ?topdx ?param)
                     (finding ?param ?topdx
                      definitive)
                     (procedure ?proc
                      ?param)
                     (status procedure ?proc
                      available))
```

This rule could be written in English as follows:

If: Some diagnosis topdx is currently the best; and
Some diagnosis seconddx is currently second; and
Diagnosis topdx has value topval; and
Diagnosis seconddx has value secondval; and
Diagnosis values differing by at least spread are far apart; and
The topval and secondval differ by more than spread; and
Finding param is needed to diagnose topdx; and
Finding param can make topdx definitive; and
Procedure proc determines finding param; and
Procedure proc has not yet been performed; then
Conclude: Procedure proc is recommended

The computer program trace in the Appendix illustrates the application of both this rule and the preceding one in a diagnostic problem.

Comment

Standard operating procedures are used in medicine to enable an economy of memory in medical experts. Because such economy may be necessary because of the vastness of medical knowledge, the construction of expert systems may aid the physician in organizing and presenting this knowledge. The construction of expert systems may aid the physician in organizing and presenting this knowledge. The expert system should incorporate the knowledge underlying the heuristic SOPs of the physician in addition to the higher level consolidated rules. One such form of knowledge concerns the risks and costs of diagnostic procedures, which underlie the standard sequences of medical tests involved in deciding diagnoses. Another concerns the physiologic mechanisms that different procedures aim to uncover. All of this knowledge, some of which is not readily at the hands of medical experts, needs to be incorporated in AIM systems.

REFERENCES

1. Schild W, Gavish B, Lunenfeld B. Computer assisted medical diagnosis with an application to endocrinology. Technical Report 014. Haifa, Israel: IBM Israel Scientific Center, 1974:1-75.
2. Roussel P. PROLOG—Manuel de Reference et d'Utilisation, Groupe d'Intelligence Artificelle, UER de Luminy, Université d'Aix-Marseille II, 1975:1-36.
3. Allen JF, Giuliano M, Frisch AM. The HORNE reasoning system. Technical report 126. Rochester, New York: Department of Computer Science, University of Rochester, 1983:1-50.
4. Shortliffe EH. Computer based medical consultations: MYCIN. North Holland: Elsevier, 1976.
5. Miller PL. ATTENDING: critiquing a physician's man-

agement plan. IEEE Trans Pattern Anal Machine Intell 1983;5:449.
6. Miller RA, Pople HE Jr, Myers JD. INTERNIST-1, an experimental computer-based diagnostic consultant for general internal medicine. N Engl J Med 1982;307:468.
7. Patil RS. Causal representation of patient illness for electrolyte and acid-base diagnosis. Technical report #267. Laboratory for Computer Science. Cambridge: Massachusetts Institute of Technology, 1981:1-137.
8. Davis R. Applications of meta-level knowledge to the construction, maintenance, and use of large knowledge bases. Technical report #76-7. Heuristic Programming Project. Palo Alto: Stanford University, 1976:1-27.
9. Shortliffe EH, Fagan LM. Expert systems research: Modeling the medical decision making process. Technical memo #82-3. Heuristic Programming Project. Palo Alto: Stanford University, 1982:1-17.
10. Pople HE Jr. Heuristic methods for imposing structure on ill-structured problems: the structuring of medical diagnosis. In: Szolovits P, ed. Artificial intelligence in medicine. AAAS Symposia Series. Boulder, Col: Westview Press, 1982:119-85.
11. Small SL, Muechler EK. Representing mutually exclusive knowledge in a property hierarchy for a reasoning system in clinical gynecology. In: Proceedings of the ninth annual symposium on computer applications in medical care. Baltimore: 1985:304-8.

Appendix: A program trace

This trace shows the application of the two rules illustrated in the body of the paper. First, the program recommends a diagnostic test by applying the rule that suggests low-cost tests to discriminate among plausible examinations in suggesting diagnostic surgery because of the rule that permits the attempt to verify diagnoses that appear to be far more pausible than that of the others. Thus in the second case, the system does not follow the standard operating procedure, which would recommend a procedure to test the patency of the fallopian tubes (HSG) before attempting to validate the principal hypothesis (endometriosis) at greater cost.

```
  % gyn
(procedure history)
Please enter results of history:
→infertility
     adding: ((dxvalue endometriosis 10) <)
     adding: ((dxvalue chronic-PID 10) <)
     adding: ((dxvalue leiomyoma 2) <)
→ dysmenorrhea
      adding: ((dxvalue endometriosis 20) <)
      adding: ((dxvalue chronic-PID 12) <)
      adding: ((dxvalue leiomyoma 8) <)
→ spotting
      adding: ((dxvalue endometriosis 26) <)
      adding: ((dxvalue chronic-PID 6) <)
      adding: ((dxvalue leiomyoma 14) <)
→ nausea
      adding: ((dxvalue endometriosis 32) <)
      adding: ((dxvalue chronic-PID 8) <)
      adding: ((dxvalue leiomyoma 4) <)
→pain-with-intercourse
     adding: ((dxvalue endometriosis 34) <)
     adding: ((dxvalue chronic-PID 14) <)
     adding: ((dxvalue leiomyoma 6) <)
→←
t
```

We tell the system that the patient has an infertility problem with dysmenorrhea, spotting, nausea, and painful intercourse.

```
(prove' (recommend?x) )
  (q-1) (recommend?x)
  (Edited out: System fails on an inappropriate rule)
    (q-2) (diagnosis ?dx19 plausible)
      (q-3) (dxvalue ?dx19 ?y10)
      (r-3) (dxvalue endometriosis 34)
      (q-3) (minimum threshold dx 34 plausible)
      (r-3) (minimum threshold dx 34 plausible)
    (r-2) (diagnosis endometriosis plausible)
    (q-2) (diagnosis ?dx29 plausible)
  (Edited out: System tries endometriosis again but fails)
    (r-2) (diagnosis chronic-PID plausible)
    (q-2) (NEQ endometriosis chronic-PID)
    (r-2) (NEQ endometriosis chronic-PID)
    (q-2) (dxneed endometriosis ?param9)
    (r-2) (dxneed endometriosis s-s-spot-histology)
    (q-2) (dxneed chronic-PID-s-s-split-histology)
    (r-2) (dxneed endometriosis serosal-surface-spot-color)
    (q-2) (dxneed chronic-PID serosal-surface-spot-color)
    (r-2) (dxneed endometriosis serosal-surface-spot-presence)
    (q-2) (dxneed chronic-PID serosal-surface-spot-presence)
  (Edited out: Failed search for common needs)
    (r-2) (dxneed endometriosis uterine-tenderness)
    (q-2) (dxneed chronic-PID uterine-tenderness)
    (r-2) (dxneed chronic-PID uterine-tenderness)
    (q-2) (procedure ?x uterine-tenderness)
    (r-2) (procedure pelvic-exam uterine-tenderness)
    (q-2) (status procedure pelvic-exam available)
    (r-2) (status procedure pelvic-exam available)
    (q-2) (sumcost pelvic-exam ?cost9)
    (r-2) (sumcost pelvic-exam 7.1)
    (q-2) (maximum threshold sumcost 7.1 minimal)
    r-2 (maximum threshold sumcost 7.1 minimal)
  (r-1) (recommend pelvic exam)
((recommend pelvic exam))
```

Try to recommend the next step. The system employs the first heuristic rule listed above. Note that a "q" is an attempt to prove something, and an "r" is the successful result. Thus in trying to show that some diagnosis (called dx19) is plausible leads to the successful result that indeed endometriosis is plausible (henceforth, dx19 = endometriosis). The same occurs again with result that dx29 = chronic PID. To show that someone has endometriosis requires the histologic report of ectopic endometrium (parameter 9 = serosal-surface-spot-histology). To show chronic pelvic inflammatory disease requires showing tenderness to the uterus. Since the pelvic exam has not been done, and since it has low cost (the lowest in all categories), it is recommended.

```
(procedure 'pelvic-exam)
Please enter results of pelvic exam:
→ uterine-mobility-limited
      adding: ((dxvalue endometriosis 40) <)
      adding: ((dxvalue chronic-PID 20) <)
→ uterine-position-retroflexed
      adding: ((dxvalue endometriosis 46) <)
→←
t
```

The results of the pelvic exam are entered.

```
(prove' (recommend?x) )
  (q-1) (recommend?x)
    (q-2) (SETVALUE ?topdx1 (bestdx 1))
    (r-2) (SETVALUE endometriosis (bestdx 2))
    (q-2) (SETVALUE ?seconddx 1 (bestdx 2))
    (r-2) (SETVALUE chronic-PID (bestdx 2))
    (q-2) (dxvalue endometriosis ?topval 1)
    (r-2) (dxvalue endometriosis 46)
    (q-2) (dxvalue chronic-PID ?secondval 1)
    (r-2) (dxvalue chronic-PID 20)
    (q-2) (freqcode spread large ?spread 1)
    (r-2) (freqcode spread large 24)
    (q-2) (spreaddx 46 20 24)
    (r-2) (spreaddx 46 20 24)
    (q-2) (dxneed endometriosis ?param 1)
    (r-2) (dxneed endometriosis s-s-spot-histology)
    (q-2) (finding s-s-spot-histology endometriosis definitive)
    (r-2) (finding s-s-spot-histology endometriosis definitive)
    (q-2) (procedure ?x s-s-spot-histology)
    (r-2) (procedure laparoscopy s-s-spot-histology)
    (q-2) (status procedure laparoscopy available)
    (r-2) (status procedure laparoscopy available)
    (r-1) (recommend laparoscopy)
  ((recommend laparoscopy))
```

Here the system employs the other main heuristic rule of the system. It tries to find the two diagnoses with the top scores. Since their scores differ by a substantial margin, the system does not recommend the procedure with the lowest cost, but instead recommends the procedure that will be definitive in affirming the highest ranked diagnosis (or in ruling it out). To obtain histology of "serosal surface spots" requires laparoscopy. Thus the system can prefer a higher cost procedure if the benefits (finding the answer) are great enough.

```
q
% ↑ D
```

Breech presentation after cesarean section: Always a section?

Ella Ophir, MD, Moshe Oettinger, MD, Arie Yagoda, MD, Yacov Markovits, MD, Nathan Rojansky, MD, and Harry Shapiro, MD

Nahariya, Israel

A retrospective review of 71 breech deliveries after previous cesarean was done to determine the need for repeat cesarean section. Twenty-four (33.8%) women were allocated to the elective repeat cesarean section group and forty-seven (66.2%) patients were allocated to a trial of labor group. Thirty-seven (78.7%) were delivered of their infants vaginally. A total of 37 of the 71 women (52.1%) had successful vaginal deliveries. Neonatal morbidity did not differ for women who were delivered vaginally or by cesarean section. Maternal febrile morbidity was significantly higher in the cesarean section group than in the vaginal delivery group ($p < 0.001$). On the basis of these data, a trial of labor seems reasonable in carefully selected cases of breech presentation after a previous cesarean section. (Am J Obstet Gynecol 1989;161:25-8.)

Key words: Breech presentation, previous cesarean section, trial of labor

The optimal mode of delivery for a breech pregnancy has remained a subject of controversy. The first prospective report in 1978 concluded that it seems reasonable to allow vaginal delivery in carefully selected cases of term frank breech presentations.[1] Many other investigators have contributed to the overall improvement in outcome in breech labor and delivery.[2-4] The summation of these principles into a single protocol has resulted in a modern approach to the breech fetus.

For patients who arrive in labor with a breech presentation and have a scarred uterus from a previous cesarean section, the consensus is that the patient should have a repeat cesarean section.[5] However, this point of view is not supported by an abundance of data and may change in the future. The purpose of our report is to present our experience in allowing women with breech presentation after previous cesarean section a trial of labor.

Material and methods

During the 11-year inclusive period between 1977 and 1987, there were 43,396 deliveries; 78 deliveries were with singleton breech presentation after previous cesarean section in 32 to 42 weeks of pregnancy. It has been our policy since 1977 to offer a trial of labor to all women with one previous cesarean section and breech presentation in the current pregnancy unless there was a medical or obstetric complication that contraindicated labor. Inclusion criteria for a trial of labor included: singleton pregnancy, frank and nonfrank breech presentation, and estimated fetal weight between 1500 and 3800 gm.

Exclusion criteria included previous classical cesarean section, more than one previous cesarean section, inadequate pelvic dimensions on x-ray pelvimetry and clinical examination, and hyperextension of the fetal head grade III or IV, diagnosed by abdominal x-ray films.[6] We use x-ray pelvimetry routinely in the secundigravid patient (first labor after cesarean), but only selectively in parous patients. Fetal weight estimation was performed by clinical and ultrasonographic methods.[7]

All material and neonatal charts were retrospectively reviewed. To focus on the impact of mode of delivery, antepartum deaths, infants weighing <1500 gm, and infants with congenital anomalies incompatible with life were excluded. Of the 78 patients with breech presentation and a prior cesarean section, seven patients were excluded for this reason. The total number of patients studied were categorized into three study groups (1, 2, 3), based on the patient's mode of delivery by protocol selection and on the actual route of delivery. Statistical analysis was by χ^2 analysis. A p value of <0.05 was considered significant.

From the Department of Obstetrics and Gynecology, Nahariya Hospital, Western Galilee Regional Hospital.

Received for publication June 28, 1988; revised November 6, 1988; accepted January 30, 1989.

Reprint requests: Ella Ophir, MD, Department of Obstetrics and Gynecology, Nahariya Hospital, Western Galilee Regional Hospital, P.O.B. 21 Nahariya 22100, Israel.

Table I. Indications for elective cesarean section (group 1)

Indications	*No. of patients*
Contracted and borderline pelvis by x-ray pelvimetry and clinical examination	6
More than one previous cesarean section	5
Estimated fetal weight >3800 gm	9
Hyperextension of fetal head, grades III and IV	1
Severe preeclamptic toxemia, placenta previa, and antepartum hemorrhage	3
Total	24

Results

From January 1977 to December 1987, 71 women with previous cesarean section and singleton breech presentation in labor were studied. Twenty-four (33.8%) women were allocated to the elective repeat cesarean section group (group 1) and 47 (66.2%) to the trial of labor (group 2). Of the 47 patients allocated to the trial of labor group, 37 (78.7%) were delivered vaginally and 10 (21.3%) required repeat cesarean section (group 3).

Group 1: Elective repeat cesarean section. Of the 24 women allocated to the elective cesarean section group, all were delivered according to protocol. Indications for elective cesarean section are summarized in Table I. There were 18 multigravid patients and six secundigravid women (first pregnancy after cesarean section). All women allocated to the abdominal delivery group underwent a transverse lower-segment cesarean section. Anesthesia for these patients consisted of general anesthesia in 21 cases (87.5%) (for technical reasons of anesthesiologists) and a lumbar epidural anesthesia in 3 cases (12.5%).

Immediate neonatal outcome for each group was determined by the 1- and 5-minute Apgar scores, birth trauma, neonatal mortality, and morbidity. There were no neonatal deaths in this group. All 24 women received some systemic antibiotics during the postoperative course. However, 10 patients had febrile morbidity. Five cases of morbidity were attributed to endometritis, and three were caused by a urinary tract infection. One patient had deep vein thrombosis of the leg and one had a midline wound infection. Factors relating to maternal outcome are listed in Table II.

Group 2: Trial of labor, vaginally delivered. Of the 71 study-patients, 47 (66.2%) were selected to undergo a trial of labor. Thirty-seven (78.7%) of those patients, were delivered vaginally. There were 23 secundigravid (62.2%) and 14 (37.8%) multigravid patients. Intravenous or intramuscular analgesic (narcotic) was administered to all women. Pudendal block anesthesia was used in six of the vaginal breech deliveries.

Table II. Maternal outcome by group

	Study group		
	1 (n = 24)	*2 (n = 37)*	*3 (n = 10)*
Hospital days (mean)	8.2	3.6	9.6
Febrile morbidity (urinary tract infection, endometritis, wound infection, deep venous thrombosis)	10	2	2
Anemia requiring blood transfusion	3	1	1
Dehiscence	2	0	1
Cesarean hysterectomy	0	1	0
Vaginal hematoma	0	1	0
Total maternal morbidity*	15	5	4

*$p < 0.001$, $\chi^2 = 21.08$, $df = 2$.

Five of the patients required breast stimulation with an Egnell breast pump. None of the patients in this study required oxytocin stimulation. Before 1987 we did not use oxytocin stimulation after cesarean section. Now we administer oxytocin to patients who are undergoing a trial of labor when indicated for obstetric reasons.

One patient had induction of labor because of premature rupture of the membranes. Two women received augmentation of labor for a prolonged latent phase of labor, and two received augmentation of labor for protracted cervical dilatation in the active phase of labor. Fetal heart rate monitoring and uterine contraction monitoring were used on a continuous basis in all labors. No patterns of the fetal heart rate were judged severe enough to warrant cesarean section for fetal distress.

Delivery was by assisted breech delivery with Piper forceps for the aftercoming head (35 patients) and assisted breech delivery with manual maneuvers (2 patients). Two of the 37 vaginal deliveries were complicated by the occurrence of a nuchal arm. In one case the infant sustained mild brachial plexus injury. The infant's right upper-extremity functions improved by the time he was discharged on the fourth postpartum day. At 1 month of age, only mild right shoulder muscle weakness was present. This weakness was not apparent on reexamination at 6 and 12 months of age. Neonatal outcome for all infants is listed in Table III.

Immediate postpartum intrauterine examination was routinely done to determine uterine dehiscence in this group. There was a uterine rupture in one grandmultipara.

Case report. A 37-year-old woman, para 10, presented in labor at 38 weeks by last menstrual period with frank breech at −1 station and cervical dilatation of 3 cm.

The previous cesarean section for fetal distress was performed in her second pregnancy. She had eight normal deliveries after the cesarean section. Spontaneous labor progressed normally with a normal fetal heart rate tracing. The patient had an uneventful trial of labor and was delivered with assisted breech and forceps for the aftercoming head of a 2980 gm infant with Apgar scores of 7 and 9 at 1 and 5 minutes, respectively. Because of the previous cesarean section, postpartum intrauterine examination was done. A low transverse uterine rupture was found. Because of persistent postpartum bleeding, an exploratory laparotomy was performed. The woman also desired sterilization. During the laparotomy a low transverse uterine rupture extending to the round ligament was found. A hysterectomy was necessary for hemorrhage. The patient had an uncomplicated postoperative course.

Maternal outcome of this group is given in Table II.

Group 3: Cesarean section after trial of labor. Ten patients (10 of 47, 21.3%), six secundigravid and four multigravid women, required cesarean section after being selected for trial of labor. There were two patients delivered by cesarean section because of fetal distress.

Failed augmentation and induction of labor accounted for the majority of trial of labor "failures" in this group. Three patients (1 multigravid and 2 secundigravid) received breast stimulation for a prolonged latent and active phase of labor. Three secundigravid and one multigravid patient received nipple stimulation for induction of labor (premature rupture of membranes (three cases) and postdated pregnancy (one case). All of these women failed to progress satisfactorily in labor and were delivered by cesarean section.

Cord prolapse occurred in the first stage of labor in one patient with footling presentation. The diagnosis was made clinically and the woman underwent a cesarean section. Her infant did well.

Maternal and neonatal outcome of group 3 is given in Tables II and III. Overall, 66.2% of study patients undergoing trial of labor (47 of 71) had a successful trial of labor (78.7%, 37 of 47) and vaginal delivery (52.1%, 37 of 71).

There were 10 patients among the study groups with nonfrank breech presentation. All except two were delivered successfully by vaginal delivery. Among the neonatal outcome (p measures) statistical significance was not reached in any category.

The duration of hospitalization was longer in the cesarean section group, but these data were influenced by the hospitalization of the mothers after abdominal delivery.

Mothers and infants are usually discharged 3 to 4 days after vaginal delivery and 7 to 8 days after cesarean section. Maternal morbidity was significantly less in patients who had a vaginal delivery compared with patients undergoing cesarean delivery ($p < 0.001$). Four mothers in the cesarean section group received blood transfusions. One woman in the vaginal delivery group required a blood transfusion.

Table III. Neonatal outcome by study group

	Study group		
	1 ($n = 24$)	*2* ($n = 37$)	*3* ($n = 10$)
Birth weight (gm) (mean)	3520	3210	3325
Apgar score at 1 min*			
1-4	0	0	1
5-7	3	5	1
8-10	21	32	8
Apgar score at 5 min†			
1-4	0	0	0
5-7	1	1	1
8-10	23	36	9
Neonatal morbidity‡ (hypoglycemia, hyperbilirubinemia, mild respiratory distress syndrome, brachial plexus injury)	7	7	1

*$0.5 > p > 0.1$; $\chi^2 = 7.51$, $df = 4$.
†$0.5 > p > 0.1$; $\chi^2 = 1.044$, $df = 4$.
‡$0.5 > p > 0.1$; $\chi^2 = 1.65$, $df = 2$.

Comment

Review of the literature from the British Commonwealth and United States shows that one third to two thirds of patients with a previous cesarean section will progress to a successful and safe vaginal delivery if properly selected and allowed a trial of labor.[8-11] Sixty-one (85.9%) of the 71 cases in our study were delivered according to protocol; 78.7% (37 of 47 patients) had a successful trial of labor.

During the study period 1504 women had a history of one or more cesarean sections and singleton vertex presentations in current pregnancies. The total number of patients with one prior cesarean section was 1260; 244 patients had two or more prior cesarean deliveries. Of the 1504 patients, 372 (24.8%) were selected to undergo a repeat cesarean section, and 1132 (75.2%) were allowed a trial of labor. Overall, 893 patients (78.88%) from the trial-of-labor group had a successful trial of labor and 239 patients (21.12%) failed the trial of labor and underwent cesarean section. The total vaginal delivery rate was 59.37% (893 of 1504).

The breech presentation and presence of uterine scar do not appear to significantly influence the likelihood of a successful trial of labor.

There were 10 patients in this study group with nonfrank breech presentations. The case of cord prolapse occurred in the first stage of labor and the fetus was delivered by cesarean section and did well. Many investigators report that nonfrank breech presentation is associated with a significantly higher incidence of cord

prolapse.[12, 13] The incidence of cord prolapse may be as high as 10%. However, cord prolapse does not occur in 90% of these cases; when it does occur, it is usually associated with a good fetal outcome. Cord prolapse in the nonfrank breech presentation, although an indication for cesarean delivery, usually does not have as severe an outcome as in vertex or frank breech presentations. Because the cord is prolapsed between the fetal legs, it often is not markedly compressed during subsequent contractions.[5] Two studies have failed to demonstrate any difference in outcome in frank and nonfrank breech deliveries.[4, 14]

Results of our study suggest that the neonatal outcome was not significantly influenced by the mode of delivery. As indicated in other reports, neonatal injury after delivery from breech presentation (comparison between cesarean section and carefully performed assisted breech vaginal delivery) is similar.[15] There were no maternal or perinatal deaths in any of the 47 trials of labor. The incidence of maternal morbidity after cesarean section compared with that after vaginal delivery was high.

The overall febrile morbidity in the group undergoing a trial of labor, including failures, was lower than in the group having cesarean section delivery. The apparent dehiscence incidence was higher at cesarean section than after vaginal delivery. This is probably related to the accuracy of diagnosis after a vaginal delivery compared with the direct visualization at laparotomy.[16]

The major complication of uterine rupture in the case presentation may have been related to grand multiparity, but the association with previous cesarean section, grand multiparity, and breech presentation cannot be ignored.

Despite modern operative techniques and systemic antibiotic regimens, women undergoing cesarean section experience intraoperative complications, blood loss, and infections at a higher rate than those associated with vaginal delivery. Maternal risks of morbidity multiply when the potential anesthetic and operative complications encountered in subsequent abdominal deliveries are considered. The favorable outcome of the neonate who is delivered vaginally, and the established maternal risks associated with cesarean section support the concept that in selected breech presentations pregnancies after a previous cesarean section, a vaginal delivery can be performed without increased risk to either mother or infant.

There is no apparent advantage of cesarean section for the neonate. The experience of Green et al.[17] in the Montreal study clearly demonstrates that the increase in cesarean section rate did not affect breech outcome. Cesarean sections are performed to protect the mother or the fetus. Elective repeat cesarean section frequently does not achieve either goal.[18]

In summary, a selective protocol for management of breech presentation after previous cesarean section seems acceptably safe. A trial of labor decreases the total number of cesarean births. It appears that if there is risk associated with allowing a trial of labor, it is small or less than that associated with repeat cesarean section. Consideration of these principles may aid both the clinician and the patient when deciding to allow a trial of labor in the management of breech presentation after previous cesarean section.

REFERENCES

1. Collea JV, Rabin SC, Weghorst GR, et al. The randomized management of term frank breech presentation. Vaginal delivery vs. cesarean section. Am J Obstet Gynecol 1978;131:186.
2. Friedman EA. Labor: clinical evaluation and management. 2nd ed. New York: Appleton-Century Crofts, 1978:168-75.
3. Collea JV, Chein C, Quilligan EJ. The randomized management of term frank breech presentation. A study of 208 cases. Am J Obstet Gynecol 1980;137:235.
4. Gimovsky ML, Wallage RL, Schifrin BS, Paul RH. Randomized management of the non-frank breech presentation at term: a preliminary report. Am J Obstet Gynecol 1983;146:34.
5. Cruikshank DP. Breech presentation. Clin Obstet Gynecol 1986;29:258.
6. Ballas S, Toaff R. Hyperextension of the fetal head in breech presentation: radiological evaluation and significance. Br J Obstet Gynaecol 1976;83:201.
7. Rojansky N, Schwartz J, Chazanchuk T, Shapiro H. Fetal weight estimation. A comparison of ultrasonographic and clinical methods. In: Schenker JG, Weinstein S, eds. The intrauterine life—management and therapy. Amsterdam: Elsevier Science, 1986:225-32.
8. Lavin J, Stephens R, Miodovnik M, Barden T. Vaginal delivery in patients with a prior cesarean section. Am J Obstet Gynecol 1982;59:135.
9. McCarrey JA. The management of patients previously delivered by cesarean section. J Obstet Gynaecol Br Commonw 1969;76:137.
10. Notzon FC, Paul JP, Taffel SM. Comparisons of national cesarean section rates. N Engl J Med 1987;316:386.
11. Flamm BL, Lim OW, Jones C, Fallon D, Newman LA, Mantis JK. Vaginal birth after cesarean section: results of a multicenter study. Am J Obstet Gynecol 1988;158:1079.
12. Seeds S, Cefalo R. Breech delivery. Clin Obstet Gynecol 1985;25:145-56.
13. Karp LE, Doney JR, McCarry T, et al. The premature breech: trial of labor or cesarean section? Obstet Gynecol 1979;53:88-92.
14. Woods JR. Effect of low birth weight breech delivery on neonatal mortality. Obstet Gynecol 1979;53:735-40.
15. Rovinsky JI, Miller JA, Kaplan S. Management of breech presentation at term. Am J Obstet Gynecol 1973;11:497.
16. Paul RH, Phelan JP, Yeh SY. Trial of labor in the patient with a prior cesarean birth. Am J Obstet Gynecol 1985;151:297.
17. Green JE, McLean F, Paul SL, et al. Has an increased cesarean section rate for term breech deliveries reduced the incidence of birth asphyxia, trauma and death? Am J Obstet Gynecol 1982;142:643.
18. Flamm BL, Dunnett C, Fischermann E, Quilligan EJ. Vaginal delivery following cesarean section: use of oxytocin augmentation and epidural anesthesia with internal tocodynamic and internal fetal monitoring. Am J Obstet Gynecol 1984;148:759.

Vaginal birth after cesarean delivery in the twin gestation

Thomas H. Strong, Jr., MD, Jeffrey P. Phelan, MD, Myoung Ock Ahn, MD, MPH, and Albert P. Sarno, Jr., MD, Maj., USA

Los Angeles, California

The pregnancy outcomes of 56 women with a twin gestation and a prior cesarean birth were analyzed to determine whether a trial of labor was a reasonable consideration. Of these patients, 31 (55%) underwent an elective repeat cesarean delivery and 25 (45%) attempted vaginal delivery. Of those who attempted vaginal delivery, 18 (72%) were vaginally delivered of both infants. The dehiscence rate among women with twin pregnancies who attempted a trial of labor was 4% compared with 2% in women with singleton pregnancies. There were no significant differences in maternal or neonatal morbidity or mortality rates in trial of labor versus no trial of labor groups. We conclude in this limited population that a trial of labor in a twin gestation after a previous cesarean delivery appears to be a reasonable consideration. The usual safeguards for attempted vaginal delivery in the twin gestation should be followed. (AM J OBSTET GYNECOL 1989;161:29-32.)

Key words: Trial of labor, vaginal birth after cesarean section

Vaginal birth after cesarean delivery is considered an acceptable alternative to routine elective repeat cesarean delivery.[1] In some cases, vaginal birth after cesarean delivery may represent the safest delivery method for the pregnant woman and her fetus. Initially a choice available to those patients who satisfied limited entry criteria such as a singleton pregnancy with a vertex presentation and one prior low-transverse uterine scar, studies suggest that favorable maternal and neonatal outcomes can be expected for many women who do not satisfy these criteria.[1, 2]

Inherent in cesarean birth are increased maternal morbidity and mortality rates and greater expense for the patient with a cesarean delivery.[3] Some authors have attempted to broaden the acceptance criteria for vaginal birth after cesarean delivery (multiple prior cesarean deliveries, unknown uterine scar, and breech presentation), but no study has focused on vaginal birth after cesarean delivery as an alternative for women with a prior cesarean delivery and a twin gestation.[1] Twins, which occur in approximately 1% of all pregnancies,[4] represent a small but significant group of patients who might be considered candidates for a trial of labor after a prior cesarean delivery. But vaginal birth after cesarean delivery in twin gestations has been rarely described.[5-8] A review of the literature disclosed only 23 cases of a trial of labor after a prior cesarean delivery and all achieved a vaginal delivery.[5-8] Therefore, the purpose of this study is to present detailed information on the pregnancy outcomes of 56 women with a prior cesarean delivery and a twin gestation.

From the Department of Obstetrics and Gynecology, University of Southern California School of Medicine.

The opinions herein are those of the authors and are not necessarily those of the U.S. Army or the Department of Defense.

Received for publication June 27, 1988; revised January 1, 1989; accepted February 8, 1989.

Reprint requests: Jeffrey P. Phelan, MD, Director, Maternal-Fetal Medicine, Queen of the Valley Hospital, 1135 S. Sunset Ave., Suite 402, West Covina, CA 91790.

Material and methods

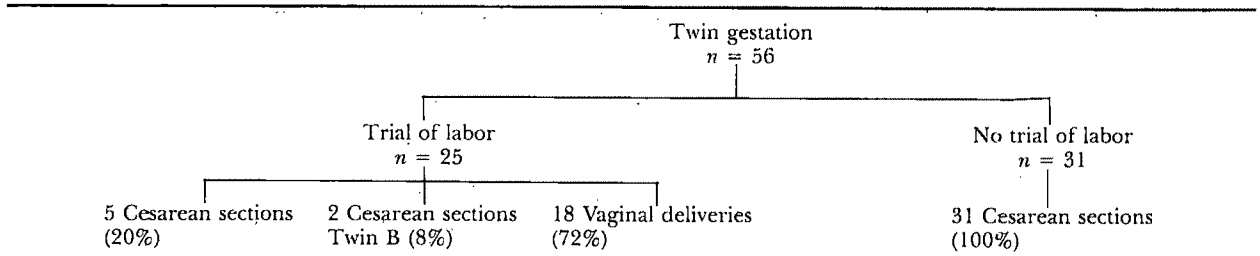
The records of all gravid women with at least one prior cesarean birth who were seen at the Labor and Delivery Suite of the University of Southern California School of Medicine from July 1, 1982, to June 30, 1986, were reviewed. A trial of labor was considered in those women with a twin gestation and a prior cesarean delivery. Trial of labor was not considered if immediate operative intervention was needed or if a malpresentation was diagnosed in twin A. All other women were offered a trial of labor. A variety of maternal and fetal outcome variables were collected and data were analyzed by the Fischer exact test. Comparisons were made between the trial of labor and nontrial of labor groups.

Results

A total of 6250 gravid women with a prior cesarean delivery were delivered of infants during the study period. Of these, 62 (1%) had a twin gestation. Fifty-six of these patients had sufficient information available for review and analysis.

Among the 31 patients (55%) who did not undergo a trial of labor, a repeat cesarean delivery was undertaken. The indications for the repeat cesarean delivery in the group without a trial of labor were: elective, 14 (45%); malpresentation (twin A), 11 (35%); pregnancy-induced hypertension, 3 (10%); and other, 3 (10%). Of the 25 (45%) who attempted a vaginal delivery, 18 (72%) were delivered of both infants vaginally (Table I). A repeat cesarean delivery was undertaken in seven patients (28%) because of the following indications:

Table I. Outcome of delivery



malpresentation of twin A diagnosed in the active phase of labor, 4 (57%); fetal distress in twin B, 2 (29%); and failure to progress, 1 (14%). Of those who attempted a vaginal delivery, the number of prior cesarean deliveries was one (21, 84%) and two (4, 16%). There were 18 patients (64%) who had an unknown scar. Seven patients (28%) received oxytocin for induction or augmentation of labor, with a dose range of 3.0 to 22.8 mU/minute and a mean of 5.7 mU/minute. Of these, six (86%) were vaginally delivered.

The indications for the primary cesarean deliveries of the 25 women with twin gestations who attempted vaginal delivery are illustrated in Table II. Unlike singleton pregnancies, successful vaginal delivery rates appeared to be unrelated to the reason for the primary cesarean delivery.

The mean birth weight and estimated gestational age among the group with repeat cesarean delivery were 36.2 weeks and 2488 gm, respectively. Likewise, among the group with trial of labor mean birth weight and estimated gestational age were 33.1 weeks and 2283 gm, respectively. The mean birth weight of the trial of labor "A" twins was 2309 gm versus 2281 gm for the trial of labor "B" twins. Among the B twins in the group with trial of labor, eight were nonvertex presentations. Two of eight (25%) were delivered by complete breech extraction.

After delivery, the uterus was examined for evidence of rupture or dehiscence. One patient was noted to have a single 4 cm transverse dehiscence in the lower uterine segment after a vaginal delivery of a vertex-breech set of twins. The rupture or dehiscence rate of the 25 women with twin gestations who attempted a trial of labor was 4% (1 of 25) as compared with a rate of 2% for the singleton vaginal birth after cesarean delivery patients who were delivered of infants during the same study period.

There were six neonatal deaths among the women who underwent trial of labor. All were attributable to extreme prematurity. The mean gestational age of this group was 22.3 weeks (range, 21 to 24 weeks). Among the neonatal deaths, no anomalies were noted. One hysterectomy was performed secondary to placenta accreta and occurred during a repeat cesarean delivery (Table III). There were no significant differences in maternal or neonatal morbidity and mortality rates in any comparison of trial of labor versus no trial of labor.

Comment

Previous investigators have routinely excluded women with a prior cesarean delivery and a twin gestation from a trial of labor. A concern expressed by Kaltreider and Krone[9] was that uterine overdistention, which can occur in twin gestations, would increase the risk of uterine rupture. However, a review of 23 sets of twins delivered vaginally during the early 1960s failed to demonstrate such an occurrence.[5-8] Moreover, neither Pedowitz and Schwartz[10] nor Phelan and associates[11] were able to substantiate a relationship between uterine overdistension and uterine rupture.

In a 30-year review of the English language literature, two thirds of singleton candidates for vaginal birth after cesarean delivery who were allowed a trial of labor were delivered of infants vaginally, with a 0.7% rupture rate.[3] In the current study, 72% of women with a twin gestation and prior cesarean delivery who were given a trial of labor were vaginally delivered of both infants, with a dehiscence rate of 4% and no uterine rupture. This compares favorably with the success and dehiscence rates at our institution of singleton pregnancies with a prior cesarean delivery over the same study period (80% and 2%, respectively). It is noteworthy that the vaginal delivery rate of the prior cesarean delivery patients with a twin gestation who were delivered of at least one twin vaginally also was 80%. The reason for the single dehiscence in our study group could be related to the combination of multiple prior cesarean deliveries, oxytocin augmentation, midforceps rotation of twin A, and Piper forceps placement of twin B. However, this seems unlikely in light of the recent evidence of Ahn and associates[12] and the data of Horenstein et al.[13]

Paul and associates[14] suggest that "the indication for a prior cesarean delivery bears little influence as it re-

Table II. Previous indications for cesarean sections and subsequent trial of labor outcome (n = 25)

Prior indication	Number	Vaginal deliveries	
		No.	%
Arrest of labor	9	8	89
Malpresentation	6	6	100
Macrosomia	2	1	50
Twins	2	1	50
Distress	1	0	0
Other	5	2	40

Table III. Pregnancy outcome for twin gestations in women with a prior cesarean delivery

	Trial of labor (%)	No trial of labor (%)
Neonatal outcome		
Number	50	62
Mean estimated gestational age	33.1	36.2
Meconium	3 (6)	
Apgar <7 (1-minute)	6 (12)	10 (16)
Apgar <7 (5-minute)	3 (6)	4 (6.5)
Mean birth weight	2283	2488
<1000 gm	7 (14)	3 (5)
1001-2499 gm	20 (40)	22 (35)
≥2500 gm	23 (46)	37 (60)
Birth trauma	0	0
Neonatal death	6 (12)	3 (5)
Maternal outcome		
Number	25	31
Hemorrhage	5 (20)	10 (32)
Dehiscence	1 (4)	0 (0)
Hysterectomy	0 (0)	1 (3)*

*Performed because of placenta accreta.

lates to the success of achieving a vaginal delivery in a current pregnancy." With this principle in mind, all women without an obstetric or medical contraindication to labor during the study period were offered a trial of labor. Unlike patients with one prior cesarean delivery, the vaginal delivery rate, however, was not similarly affected by the prior indication for cesarean delivery (Table II) or the use of oxytocin. The relatively high success rate of twins being delivered vaginally after a previous cesarean delivery because of arrested labor is probably a result of the smaller size of the twin fetus as compared with the term singleton.

Other authors suggest that a trial of labor in women with multiple prior cesarean deliveries[1, 15] or an unknown scar type[1, 2] is reasonable and not associated with an increased risk of morbidity or mortality. In accordance with these findings, patients with twin gestations were also permitted a trial of labor. This practice was not associated with an increased risk of maternal or fetal morbidity or mortality.

Neonatal mortality among the entire group with twin gestation and prior cesarean delivery was 8%. This compares favorably with the reported frequency of twin neonatal mortality rates.[16] In that report, it was noted that as the gestational age increased, neonatal mortality decreased. This is in keeping with our findings in which all neonatal deaths were secondary to extreme prematurity.

As experience increases with patients with vaginal birth after cesarean delivery, a greater number of the patients with a twin gestation will desire a trial of labor. If a trial of labor is considered in these patients, continuous electronic fetal monitoring of both twins would appear prudent. Candidates for attempted vaginal delivery should be limited to those pregnancies in which twin A is in a vertex presentation. After the delivery of twin A, real-time ultrasonography should be used to evaluate the lie, presentation, and heart rate of twin B. A concern among practitioners who manage patients with prior cesarean deliveries involves the risks of intra- or extrauterine manipulations. Whereas the number of such manipulations performed in our population was too small to clarify this issue, information from this report and others[16, 17] suggest that version is not an unreasonable consideration. Therefore, if twin B is a malpresentation, exernal cephalic version, internal podalic version, or outright cesarean delivery would appear to be acceptable management alternatives.[17] Cesarean delivery of twin B should be reserved for those circumstances in which fetal distress is present and vaginal delivery cannot be safely accomplished within a reasonable time.

In summary, the present series corroborates the work of others[5-8] in that vaginal birth after delivery in the twin gestation is not associated with an increased risk of perinatal or maternal morbidity and mortality. Though a trial of labor in the twin gravid woman with a prior cesarean delivery remains controversial, our contemporary experience suggests that it is a reasonable consideration in the properly selected patient. If attempted vaginal delivery is undertaken, the usual safeguards for a trial of labor in a twin gestation should be followed.

REFERENCES

1. Phelan JP, Clark SL, Diaz F, Paul RH. Vaginal birth after cesarean. AM J OBSTET GYNECOL 1987;157:1510-5.
2. Beall M, Eglington GS, Clark SL, Phelan JP. Vaginal delivery after cesarean section in women with unknown types of uterine scar. J Reprod Med 1984;29:31-4.
3. Lavin JP, Stephens RJ, Miodovnik M, Barden TP. Vaginal

delivery in patients with a prior cesarean section. Obstet Gynecol 1982;59:135-48.
4. Benirschke K. Multiple gestation. In: Creasy RK, Resnik R, eds. Maternal-Fetal medicine: principles and practice. 1st ed. Philadelphia: WB Saunders, 1984:511.
5. Brady K, Read JA. Vaginal delivery of twins after previous cesarean section. N Engl J Med 1988;319:118-9.
6. O'Connell W. Vaginal delivery following cesarean section. Poc Med Surg 1966;74:343-5.
7. Allahbadia N. Vaginal delivery following cesarean section. AM J OBSTET GYNECOL 1963;85:241-8.
8. Brown ADH, McGrath J. Vaginal delivery after previous cesarean section: a survey of 800 cases at the Rodunda Hospital, Dublin. J Obstet Gynaecol Br Emp 1965;72:557-63.
9. Kaltreider D, Krone W. Delivery following cesarean section. Clin Obstet Gynecol 1959;2:1029-42.
10. Pedowitz P, Schwartz RM. The true incidence of silent rupture of cesarean section scars. AM J OBSTET GYNECOL 1957;74:1071-80.
11. Phelan JP, Eglington GS, Horenstein JM, Clark SL, Yeh S-Y. Previous cesarean birth: trial of labor in women with macrosomic infants. J Reprod Med 1984;29:36-40.
12. Ahn MO, Phelan JP, Sarno AP, Strong TH. Midpelvic deliveries in vaginal births after cesarean deliveries. In: Procedings of the Society of Perinatal Obstetricians [Abstract]. Las Vegas, Nevada, February 4-6, 1988.
13. Horenstein JM, Phelan JP. Previous cesarean section: the risks and benefits of oxytocin usage in a trial of labor. AM J OBSTET GYNECOL 1985;151:564-9.
14. Paul RH, Phelan JP, Yeh S. Trial of labor in the patient with a prior cesarean birth. AM J OBSTET GYNECOL 1985;151:297-304.
15. Phelan JP, Ahn MO, Diaz F. Twice a cesarean always a cesarean? Obstet Gynecol 1989;73:161-5.
16. Chervenak FA, Chervenak J. Multiple gestations. In: Phelan JP, Clark SL, eds. Cesarean delivery. New York: Elsevier Science Publishing, 1988.
17. Phelan JP, Clark SL, Porreco RP, VanDorsten JP. Finding alternatives to cesarean section, a symposium. Contemp Ob/Gyn 1988;31(1):191-210.

Pharmacokinetics of orally administered ritodrine

Steven N. Caritis, MD, Raman Venkataramanan, PhD, Margaret Cotroneo, RN, and Jye-Ping Chiao, MS
Pittsburgh, Pennsylvania

The oral dosing regimen for ritodrine was based in large part on kinetic data obtained in nonpregnant subjects. There are limited kinetic data after oral administration of ritodrine in pregnancy. The purpose of the present study was to compare ritodrine kinetics in pregnant and nonpregnant women, evaluate the effect of feeding on ritodrine absorption in pregnant women, and determine if the plasma concentration of ritodrine is proportional to the dose administered in nonpregnant women. Plasma concentrations after a single 20 mg dose of ritodrine were significantly greater in fasting nonpregnant women than in fasting pregnant women. The area under the concentration time curve was 1372 ± 385 and 1001 ± 257 ng/ml/min, respectively. In pregnant women ingesting 20 mg of ritodrine, plasma concentrations were not significantly different in the fed or fasted state; plasma concentrations peaked at 11 ng/ml and were less than 3 ng/ml within 4 hours. In nonpregnant subjects the concentration of ritodrine in plasma was proportional to the dose. After ingestion of 10, 20, or 30 mg of ritodrine, the area under the curve was 751 ± 253, 1372 ± 385, and 2148 ± 571 ng/ml/min, respectively. These data indicate that ritodrine concentrations in pregnant women after a 20 mg oral dose are low. Increases in dosage will probably result in proportional increases in plasma concentration. The maximal dose of ritodrine recommended for prevention of recurrent preterm labor should be increased. (AM J OBSTET GYNECOL 1989;161:32-5.)

Key words: Ritodrine, kinetics, oral dosage, pregnancy

Ritodrine was approved for the treatment of preterm labor in 1980. Regimens for both intravenous and oral administration were in large part developed from kinetic studies in nonpregnant women and male volunteers. Pregnancy is characterized by changes in plasma volume, plasma protein concentration, renal blood flow, intestinal transit time, and a multitude of other factors that can affect ritodrine absorption, distribution, or elimination.[1, 2] The highest dose of oral ritodrine recommended for continuing therapy of preterm labor is 120 mg/day, but this recommendation is not based on kinetic studies in pregnant women. Smit et al.[3] and Van Lierde et al.[4] have suggested that the plasma concentrations achieved with this dosage of rito-

From the Department of Obstetrics and Gynecology, University of Pittsburgh School of Medicine, and Department of Pharmaceutical Sciences, University of Pittsburgh School of Pharmacy.
Received for publication September 14, 1988; revised December 1, 1988; accepted January 30, 1989.
Supported by a grant from Astra Pharmaceutical Products, Inc.
Reprint requests: Steve N. Caritis, MD, Magee-Womens Hospital, Forbes Avenue and Halket Street, Pittsburgh, PA 15213.

Table I. Comparison of ritodrine pharmacokinetics in pregnant and nonpregnant subjects

	Pregnant	*Nonpregnant*
AUC (ng/ml/min)	1001 ± 257	1372 ± 385*
Concentration (ng/ml)		
At peak	10.8 ± 5.6	13.4 ± 5.2
At 4 hr	2.1 ± 0.5	2.3 ± 0.8
Time to peak (min)	58 ± 38	30 ± 14*

AUC, Area under the curve.
*Asterisk indicates value differs significantly from that of pregnant subjects.

Table II. Effect of feeding on the pharmacokinetics of ritodrine in pregnant subjects

	Fed	*Fasted*
AUC (ng/ml/min)	847 ± 280	1001 ± 257
Concentration (ng/ml)		
At peak	8.3 ± 5.6	10.8 ± 5.6
At 4 hr	1.7 ± 0.9	2.1 ± 0.6
Time to peak (min)	78 ± 45	58 ± 38
Half-life (min)	166 ± 115	149 ± 76

AUC, Area under the curve.

drine are unlikely to sustain labor inhibition. Indeed, we have shown that 120 mg/day of ritodrine is significantly less effective than 30 mg/day of terbutaline in preventing recurrence of preterm labor.[5] It is unclear if adequate ritodrine concentrations could be obtained with the present formulation merely by increasing the oral dosage above 120 mg/day. Also, it is unclear if ritodrine absorption could be enhanced in pregnancy by administering the drug before meals. The purpose of the present study was threefold: (1) to define the kinetics of orally administered ritodrine in pregnant and nonpregnant subjects, (2) to evaluate the effect of feeding on ritodrine absorption in pregnant women, and (3) to determine if plasma concentration increased proportionally with an increase in the dose of ritodrine.

Material and methods

After approval by the Research Review and Human Experimentation Committee of Magee-Womens Hospital, we obtained informed consent from 10 nonpregnant and 10 pregnant nonlaboring women for participation in this study. Excluded from the study were women with a contraindication to β-agonist medication and women receiving theophylline or other tocolytic therapy.

Pregnant women at gestational ages of 20 to 34 weeks received 20 mg of ritodrine orally on different days, once after an overnight fast, and once 60 minutes after eating a standardized breakfast. Breakfast consisted of one soft boiled egg, one slice of toast, one 4-ounce cup of juice, and one 8-ounce glass of nonfat milk. Fasting nonpregnant women received either 10, 20, or 30 mg of ritodrine orally in random order on different days. In all women at least 48 hours elapsed between each study. On the day of study, women did not ingest caffeine. Blood (7 ml) was removed from an indwelling cannula (heparin lock) before and at 11 times in the 6 hours after drug ingestion (10, 20, 30, 45, 60, 90, 120, 180, 240, 300, and 360 minutes). Blood was collected in heparinized syringes and immediately centrifuged at 2000 rpm. The supernatant plasma was frozen at −20° C until analyzed for ritodrine concentration by high-performance liquid chromatography with electrochemical detection.[6] The area under the plasma concentration versus time curve from time 0 to the last measured point was calculated by the trapezoidal method.[7] Terminal disposition rate constant and disposition half life were calculated according to standard techniques.[7]

Differences in pharmacokinetic parameters were compared by paired or nonpaired *t* test or by repeated measures analysis of variance where appropriate.[8] Data are expressed as mean ± SD.

Results

The pharmacokinetics of ritodrine in 10 pregnant and 10 nonpregnant subjects are compared in Table I. All subjects received 20 mg of ritodrine while fasting. The area under the curve was significantly greater in nonpregnant women. Peak plasma concentrations were also greater in nonpregnant subjects but not significantly so. Four hours after ritodrine ingestion, ritodrine concentration was 3 ng/ml or less in all subjects. The time to peak concentration was significantly shorter in nonpregnant subjects.

The effect of feeding on ritodrine pharmacokinetics in pregnant women is summarized in Table II. All 10 subjects received 20 mg of ritodrine once in the fed and once in the fasted states. The area under the concentration time curve, and the peak plasma concentrations were greater and the absorption was more rapid during fasting than after eating. However, none of these variables was significantly different in the fed or fasted state. Four hours after ingestion of ritodrine, plasma concentrations were 3 ng/ml or less in all subjects. The elimination half-life was not altered by fasting.

Table III describes the relationship between ritodrine dosage and plasma concentration in 10 nonpregnant subjects. The area under the curve and peak plasma concentration increased proportionally to the dose. The ritodrine concentration was also proportional to the dose administered 4 hours after ingestion.

Table III. Relationship between ritodrine dosage and plasma concentration in nonpregnant subjects

	10 mg	*20 mg*	*30 mg*
AUC (ng/ml/min)	751 ± 253	1372 ± 385	2148 ± 571
Concentration (ng/ml)			
At peak	6.6 ± 2.9	13.4 ± 5.2	20.1 ± 8.2
At 4 hr	1.3 ± 0.9	2.3 ± 0.8	3.4 ± 1.4
Time to peak (min)	29 ± 13	30 ± 14	50 ± 38

AUC, Area under the curve.

The time to achieve peak concentrations did not differ significantly in the three groups.

Comment

We have demonstrated significant differences between pregnant and nonpregnant subjects in the kinetics of orally administered ritodrine. After oral ingestion, ritodrine plasma concentrations were lower in pregnant women as was the area under the concentration time curve. Pregnant women absorbed ritodrine significantly slower than nonpregnant women. Lower plasma concentrations in pregnant women may be the result of poorer absorption, greater elimination, or distribution into a greater volume than in nonpregnant subjects. From the present study we cannot determine which of these factor(s) is responsible for the differences in pregnant and nonpregnant subjects.

Ritodrine absorption was not enhanced to any significant degree by the presence of food. In fasting pregnant women, peak plasma concentration after a 20 mg dose averaged only 11 ng/ml (range, 4 to 21 ng/ml). The concentration decreased rapidly and averaged approximately 2 ng/ml 4 hours after ingestion of the drug. With repeated doses of ritodrine, the peak concentration will increase somewhat as will the concentration at 4 hours. In the study of Smit et al.,[3] plasma concentrations in pregnant women receiving ritodrine for at least 3 days averaged 19 ng/ml at peak (range, 10 to 31 ng/ml) and 7 ng/ml 4 hours after ingestion (range, 5 to 10 ng/ml).

We performed dose proportionality studies in nonpregnant rather than pregnant subjects for ethical reasons. We did not wish to subject pregnant women to three different studies in which blood was withdrawn, and we were concerned that the 30 mg dose might cause side effects. We demonstrated proportionality between ritodrine dosage and plasma concentration in nonpregnant subjects. Linear pharmacokinetics of ritodrine have been demonstrated in pregnant and nonpregnant subjects receiving intravenous ritodrine over a very wide dose range.[4, 9] We have assumed that proportionality between oral dosage and plasma concentration exists in pregnant women because factors such as plasma volume and clearance that alter ritodrine kinetics in pregnancy are unlikely to effect the relationship between dose and plasma concentration. A relationship between oral dosage and plasma concentration has been demonstrated for doses of 5 and 10 mg of ritodrine,[4] and there is no evidence that this relationship should not exist with doses up to 30 mg.

The lowest concentration of ritodrine achieved during intravenous therapy ranges from 15 to 31 ng/ml.[9] Concentrations of 80 ng/ml or more are commonly required to initially inhibit preterm labor.[9] The concentration of ritodrine required to sustain labor inhibition has not been established. However, it is clear that 120 mg/day, that is, 20 mg orally every 4 hours, is insufficient to prevent recurrent preterm labor in a substantial percentage of patients.[5] This clinical observation, coupled with the large variability in plasma concentrations between subjects in this study and in the study of Smit et al.,[3] suggest that higher ritodrine dosages can be used to prevent recurrent preterm labor. Not every patient may require a higher dosage, but those with poor absorption or rapid clearance of ritodrine will. The intravenous tocolytic dosage of ritodrine is established by incremental increases in the infusion rate until contractions are inhibited or side effects occur. A similar approach appears to be reasonable for orally administered drug. A starting dose of 120 mg is unlikely to lead to side effects and will be effective in some women. If a response is not achieved, the dose can be increased accordingly.

Higher oral doses of ritodrine will not address the problem of inconvenience caused by a dosing frequency of every 4 hours. Similar problems exist with terbutaline, which is also administered orally every 4 hours.[9] A more desirable formulation of ritodrine has recently been developed.[10] This sustained release capsule can be administered every 8 hours and provides concentrations of 18 and 11 ng/ml 4 and 8 hours after ingestion of an 80 mg dose. This formulation is not yet available in this country.

In summary, we have demonstrated that the plasma concentration of ritodrine after an oral dose of 20 mg is lower in pregnant than in nonpregnant women. The concentration of ritodrine in pregnant women ingesting a 20 mg dose is low. The maximal dosage of 120 mg/day is unlikely to adequately sustain labor inhibition in many women.

REFERENCES

1. Cummings AJ. A survey of pharmacokinetic data from pregnant women. Clin Pharmacokinetics 1983;8:344-54.
2. Caritis SN, Lin LS, Venkataramanan R, Wong LK. Effect of pregnancy on ritodrine pharmacokinetics. Am J Obstet Gynecol 1984;159:328-32.
3. Smit DA, Essed GGM, deHaan J. Serum levels of ritodrine during oral maintenance therapy. Gynecol Obstet Invest 1984;18:105-12.
4. Van Lierde M, Desager JP, Harvengt C, Thomas K. Ritodrine serum levels: influence of dose and route of administration. Int J Clin Pharmacol 1984;22:382-5.
5. Caritis SN, Toig G, Heddinger LA, Ashmead G. A double-blind study comparing ritodrine and terbutaline in the treatment of preterm labor. Am J Obstet Gynecol 1984;150:7-14.
6. Lin LS, Caritis SN, Wong LK. Analysis of ritodrine by HPLC electrochemical detection. J Pharm Sci 1984;73:131.
7. Gibaldi M, Perrier D. Pharmacokinetics. New York: Marcel Dekker, 1985.
8. Zar JH. Biostatistical analysis. Englewood Cliffs, New Jersey: Prentice-Hall, Inc, 1974.
9. Caritis SN, Lin LS, Toig G, Wong LH. Pharmacodynamics of ritodrine in pregnant women during preterm labor. Am J Obstet Gynecol 1983;147:752-9.
10. Essed GGM, deHaan J, Stuyker Boudier HAJ, van Elferen LWM. A new administration form of ritodrine: ritodrine plasma levels with sustained-release capsules in a steady state. Gynecol Obstet Invest 1987;23:10-5.

Neuromuscular blockade with magnesium sulfate and nifedipine

Scott W. Snyder, MD, and Michael S. Cardwell, MD
Columbia, Missouri

A patient who received tocolysis with nifedipine developed neuromuscular blockade after 500 mg of magnesium sulfate was administered. This reaction demonstrates that nifedipine can seriously potentiate the toxicity of magnesium. Caution should be exercised when these two tocolytics are combined. (Am J Obstet Gynecol 1989;161:35-6.)

Key words: Nifedipine, magnesium, drug interaction, neuromuscular blockade

Nifedipine has been used as a tocolytic in the treatment of premature labor and has been compared favorably to ritodrine.[1] Because both magnesium sulfate and nifedipine are calcium channel blockers, some interaction might be expected as both are undoubtedly involved in the alteration of the movement of calcium across membranes in the myometrium.

Recently a report appeared that described hypotension in preeclamptic patients who received both agents: nifedipine for the treatment of pregnancy-induced hypertension and magnesium sulfate as an anticonvulsant. We report a patient who developed skeletal muscle neuromuscular blockade with a small dose of magnesium sulfate after tocolysis with nifedipine.

Case report

This 22-year-old patient, gravida 4, para 2, was seen with contractions and effacement of the cervix at 32 weeks' gestation. The patient had one previous first-trimester loss and two previous premature births of an 1872 gm infant and a 2152 gm infant who survive. The patient had had some contractions in the 2 weeks before admission but no descent of the presenting part or effacement of the cervix.

On the night of admission, contractions had become regular and of 45 seconds' duration. The cervix was 50% effaced, a change from examination 4 days earlier. The patient was hydrated with 1000 ml lactated Ringer's solution without change in labor. Nifedipine was started and the patient received 60 mg orally over the next 3 hours. This resulted in a marked decrease in duration and frequency of contractions. A maintenance regimen of 20 mg every 8 hours was begun. Contractions recurred 12 hours later. Because we thought a maximum maintenance dose of nifedipine was being administered, magnesium sulfate was chosen. After 500 mg of intravenous administration of magnesium, the patient developed jerky movements of the extremities, complained of having difficulty swallowing, had paradoxical respirations, and was unable to lift her head from the pillow. The magnesium was discontinued and the muscle weakness resolved over the next 25 minutes.

Contractions ceased and were not a problem

From the Department of Obstetrics and Gynecology, University of Missouri Health Sciences Center.
Received for publication September 28, 1988; revised January 10, 1989; accepted February 8, 1989.
Reprint requests: Scott W. Snyder, MD, Department of Obstetrics and Gynecology, N606 University of Missouri Health Sciences Center, Columbia, MO 65212.

throughout the remainder of the 3-day hospitalization. The patient was discharged while taking nifedipine, 20 mg every 6 hours. The pregnancy continued and the patient was delivered of a 3490 gm boy at 38 week's gestation.

Comment

The mechanism of action of both nifedipine and magnesium in the suppression of uterine contractility is obscure. Both agents block intracellular flow of ionized calcium in both skeletal and smooth muscle. Smooth muscle relaxation may be more profound than that of skeletal muscle because of limited reserves of calcium in endoplasmic reticulum of smooth muscle. In skeletal muscle, magnesium has clear prejunctional and postjunctional effects, and at high levels can produce neuromuscular blockade. Magnesium has been shown to potentiate both depolarizing and nondepolarizing neuromuscular blockers. Although some conflicting reports of potentiation of neuromuscular blockers by nifedipine on animal in vitro and in vivo muscle preparations have appeared,[2] we are not aware of any reports of serious interactions in humans, despite the relative frequency with which the combination of these agents is seen in practice.

We think that our patient's reaction suggests that nifedipine potentiates the neuromuscular blockade effect of magnesium sulfate. We recommend that nifedipine and magnesium not be used for tocolysis in the same patient. Choosing an agent with another mechanism of action, for example, β-sympathomimetics, may be a safer and more logical choice when an additional tocolytic seems indicated.

REFERENCES

1. Read MD, Wellby DE. The use of a calcium antagonist (nifedipine) to suppress preterm labour. Br J Obstet Gynaecol 1986;93:933-7.
2. Bikhazi GB, Leung I, Flores C, et al. Potentiation of neuromuscular blocking agents by calcium channel blockers in rats. Anesth Analg 1988;67:1-8.

Usefulness of continuous fetal heart rate monitoring during cesarean section

Boris M. Petrikovsky, MD, PhD, Mario Cohen, MD, and M. Leon Tancer, MD

Brooklyn, New York, and Farmington, Connecticut

Cesarean section performed because of fetal distress routinely leaves the fetus unmonitored for a variable period of time (perioperatively and intraoperatively). We present in this report two cases in which continuous fetal heart rate monitoring during cesarean section modified the obstetric management for the benefit of the mother and of the fetus. (Am J Obstet Gynecol 1989;161:36-7.)

Key words: Cesarean section, fetal heart rate, fetal distress

Fetal distress is one of the major indications for the performance of primary cesarean sections. Overall, there has been a greater than threefold increase in the number of cesarean sections during the last decade in the United States; fetal distress represents the reason for 13.2% of all cesarean sections.

It has been a routine practice to discontinue fetal heart rate monitoring before the performance of a cesarean section. Thus the fetus, already compromised, remains unmonitored throughout the entire surgical procedure, and the obstetric team (obstetrician, anesthesiologist, and neonatologist) is unaware of the current fetal status.

Beginning in June 1985, we extended intrapartum fetal heart rate monitoring to the intraoperative period in cases in which indications for cesarean section arose during labor. This report deals with the practical usefulness of intraoperative fetal heart rate monitoring.

From the Department of Obstetrics and Gynecology, Maimonides Medical Center and the State University of New York at Downstate, and the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of Connecticut Health Center.
Received for publication June 16, 1987; accepted May 9, 1988.
Reprint requests: Boris M. Petrikovsky, MD, Department of Obstetrics and Gynecology, School of Medicine, State University of New York, Stony Brook, NY 11794-8091.

Case reports

Case 1. A 32-year-old patient, gravida 2, para 1, was admitted to the hospital for a trial of vaginal delivery after a previous cesarean section. The patient was moderately obese.

Labor progressed uneventfully until 6 cm of cervical dilation was reached. At this point, the internal fetal

heart rate monitor registered moderate variable decelerations. A pelvic examination was performed and umbilical cord prolapse was ruled out. Conservative measures (such as changes in maternal position and oxygen) were not successful. Twenty minutes later, variable decelerations became severe; scalp pH was 7.20. At this point, a decision was made to perform a cesarean section. The internal scalp electrode was left in situ and fetal heart rate monitoring was continued. The fetal heart rate returned to normal after the tracheal intubation was completed and remained normal throughout the entire procedure.

When the peritoneal cavity was entered, the surgeons found multiple adhesions between the loops of the small bowel, omentum, and the uterus, which necessitated meticulous and time-consuming dissection. The identification of the vesicouterine fold and dissection of the bladder flap was also technically difficult.

The neonate was born in satisfactory condition. There were two loops of umbilical cord around the fetal neck. The Apgar score was 8 at 1 minute and 9 at 5 minutes. Cord venous pH was 7.28. Fetal weight was 4200 gm. Estimated blood loss was 700 ml.

Case 2. A 26-year-old patient, gravida 1, para 0, was admitted to the hospital in active labor with spontaneously ruptured membranes. Pelvic examination revealed the cervix to be 3 cm dilated and fully effaced. An internal electrode was placed and registered late decelerations and diminished beat-to-beat variability. The scalp pH was 7.18. A decision was made to perform an emergency cesarean section.

The fetal heart rate tracing improved after the tracheal intubation and remained stable up to the time of the uterine incision, which permitted the surgeon to proceed up to this point without haste. Immediately after the myometrial incision, the fetal heart rate dropped abruptly without a tendency to recover. Expeditious delivery of the fetus was undertaken by the senior operator.

The neonate was born with mild hypoxia. The 1-minute Apgar score was 5; the 5-minute score was 8. The venous cord pH was 7.21, and the estimated blood loss was 950 ml.

Comment

Schaefer et al.[1] stated that indications for undertaking cesarean section may serve as a clue to the physician as to possible complications. Thus the complication rate for emergency operations is almost five times higher than that for elective cesarean sections. These authors registered a 9.5% increase in the occurrence of the following complications during cesarean sections performed in a "rushed" fashion: lacerations of the isthmic part of the uterus, extension of the initial uterine incision with bleeding, and excessive blood loss necessitating blood transfusion.[1]

Major surgical complications under these circumstances were noted in 2.5% of the cases and included bladder injury, intestinal injury, laceration of one or both uterine arteries, and extension of the uterine incision into the broad ligament with subsequent development of hematoma.

Increased surgical speed and vigorous attempts to deliver the fetus may also result in neonatal trauma. Direct fetal injuries either by knife or scissors that necessitate repair by plastic surgeons, femoral fractures, peripheral nerve injury, and other injuries have been described in the literature. The unanimous opinion of all involved in researching cesarean complications is that their advoidance depends largely on the use of a controlled technique. We think that intraoperative fetal heart rate monitoring helps to achieve this goal. Thus a reassuring fetal heart rate pattern (case 1) allowed surgeons to proceed throughout the entire procedure in a patient at high risk (obesity, intraabdominal adhesions, large fetus) without haste.

If the fetal heart rate deteriorates at some point during cesarean section, the surgeon and the neonatologist are aware of it immediately because of continuous monitoring and can take appropriate measures to deliver the fetus as soon as possible and be fully prepared for the immediate resuscitation. Two steps in the course of the cesarean section require special attention, abdominal preparation and myometrial incision, because they are commonly associated with fetal heart rate changes.[2]

REFERENCES

1. Schaefer G, Graber E. Complications in obstetric and gynecologic surgery. New York: Harper & Row, 1981.
2. Petrikovsky BM, Cohen M, Fastman D, Tancer ML. Electronic fetal heart rate monitoring during cesarean section. Int J Gynaecol Obstet 1988;26:203-7.

Risk factors for vulvar lichen sclerosus

Mario Sideri, MD,[a] Fabio Parazzini, MD,[b] Maria Teresa Rognoni, MD,[a] Carlo La Vecchia, MD,[b, c] Eva Negri, ScD,[b] Salvatore Garsia, MD,[a] Elena Arnoletti, MD,[a] and Gabriela Cecchetti, MD[b]

Milan, Italy, and Lausanne, Switzerland

To assess risk factors for vulvar lichen sclerosus data were collected in a case-control study of 75 cases and 225 age-matched control subjects. Questions concerned personal characteristics and habits, gynecologic and obstetric data, general indicators of sexual habits, and selected dietary habits. The risk of vulvar lichen sclerosus was greater in parous women than nulliparous women, but there was little tendency for the risk to increase with number of births; the finding was not significant when only married women were considered. There was no material difference between cases and control subjects in relation to major indicators of sexual habits (age at first intercourse and number of sexual partners). A smaller proportion of cases was married, but no difference was observed in the distribution of cases and controls with reference to education, smoking habits, body mass index, and previous history of diabetes. There was no association between retinoids and risk of vulvar lichen sclerosus, but intake of carotenoids was inversely and strongly associated with vulvar lichen sclerosus. This apparent protection did not materially change after adjustment for socioeconomic status and other potential confounding factors. (Am J Obstet Gynecol 1989;61:38-42.)

Key words: Risk factors, vulvar lichen sclerosus, reproductive factors, diet

Lichen sclerosus is an uncommon disease whose diagnostic features are lichenoid, slightly atrophic lesions with characteristic histologic features. An immunologically mediated pathogenesis has been claimed[1] on the basis of a reported higher frequency of autoimmune disorders[2] and specific HLA antigens in lichen sclerosus cases.[3] Immunologic factors may well explain part of the approximate tenfold greater frequency in women than in men,[4] because most autoimmune diseases are more frequent in women. Hormonal factors also may contribute to this elevated frequency in women.

The clinical observation that lichen sclerosis is more common in women with a history of repeated vaginitis[4] or in men with phimosis has suggested potential infective mechanisms.[5] However, little epidemiologic evidence is available to quantify the role of these factors. Therefore we considered the relationship between various environmental factors and vulvar lichen sclerosus, with data from a case-control study conducted in Lombardy, Northern Italy.

Subjects and methods

Between January 1985 and May 1987 trained interviewers identified and questioned women with vulvar lichen sclerosus and control subjects using a structured questionnaire. Despite the sensitive nature of the interview, the participation rate was practically complete (one case and two eligible controls refused the interview).

The cases were all women with histologically confirmed diagnosis of vulvar lichen sclerosus, according to the classification of the International Society for the Study of Vulvar Disease, who were admitted during the study period to the First Obstetrics and Gynecology Clinic of the University of Milan. A total of 75 subjects, aged 28 to 72 years (median age, 56 years), met these criteria.

The control subjects were patients admitted for acute conditions to the Ospedale Maggiore (including the four major teaching and general hospitals in Milan) and several specialized university clinics serving a catchment area comparable to that of the hospital where cases had been identified. Three controls were age-matched with each case in 5-year intervals; a total number of 225 subjects were interviewed. Of these, 29% were admitted for traumatic conditions (mostly fractures and sprains), 26% had nontraumatic orthopedic disorders (mostly low back pain and disk disorders), 20% were admitted for acute abdominal diseases that generally required surgery, and 25% had other illnesses such as disorders of the ear, nose, throat, or teeth.

Information was obtained concerning personal characteristics and habits, gynecologic and obstetric data, related medical history, and general indicators of sexual habits (age at first intercourse, total number of sexual partners). The subjects were also questioned about

From the First Obstetrics and Gynecology Clinic, University of Milan,[a] the "Mario Negri" Institute for Pharmacological Research,[b] and the Institute of Social and Preventive Medicine, University of Lausanne.[c]

Received for publication March 7, 1988; revised June 15, 1988; accepted January 10, 1989.

Reprint requests: Fabio Parazzini, MD, Istituto di Ricerche Farmacologiche, "Mario Negri," Via Eritrea, 62, 20157 Milan, Italy.

Table I. Distribution of 75 cases of vulvar lichen sclerosus and 225 controls according to obstetric and gynecologic factors (Milan, Italy, 1985 to 1987)

	Cases	*Controls*	*Relative risk*	*95% Confidence interval*
Parity				
0	6	47	1*	
1-2	46	121	3.0	1.2-7.2
≥3	23	57	3.2	1.2-8.2
χ^2_1 trend			4.28 (p = 0.04)	
Spontaneous abortions				
0	57	174	1*	
≥1	18	51	1.1	0.6-2.0
Menopausal status				
Premenopausal	10	42	1*	
In menopause	6	19	1.3	0.4-4.2
Postmenopausal	59	164	1.5	0.7-3.2
χ^2_1 trend			1.17 (NS)	
Age at menopause (yr)†				
<45	11	37	1*	
45-49	21	52	1.4	0.6-3.2
≥50	33	94	1.2	0.5-2.6
χ trend			0.06 (NS)	

*Reference category.
†Postmenopausal women only.

Table II. Distribution of 75 cases of vulvar lichen sclerosus and 225 controls according to selected indicators of sexual habits (Milan, Italy, 1985 to 1987)

	Cases	*Controls*	*Relative risk*	*95% Confidence interval*
Age at first intercourse (yr)				
≤17	7	9	1*†	
18-22	29	85	0.4	0.2-1.3
≥23 or never	39	131	0.4	0.1-1.1
χ^2_1 trend			2.10 (NS)	
No. of sexual partners				
0-1	64	192	1*‡	
≥2	11	33	1.0	0.5-2.2

*Reference category.
†Adjusted for number of sexual partners by the Mantel-Haenszel procedure.
‡Adjusted for age at first intercourse by the Mantel-Haenszel procedure.

their frequency of consumption per week of the major sources in the Italian diet of retinoids (milk, liver, and meat) and carotenoids (carrots and green vegetables).

Data analysis

Odds ratios (as estimators of relative risks) and 95% confidence intervals[6] were computed with use of an unmatched approach; when appropriate, relative risks were tested for trend.[7] Potential confounding factors were controlled individually with stratification and the Mantel-Haenszel procedure.[8] However, since adjustments for age or other factors did not materially change most of the relative risks, unadjusted estimates were chosen for presentation except where indicated in the text. Indexes of monthly retinol and β-carotene intake were computed using Italian Department of Agriculture standard tables of nutrient values.[9] Obviously, because the limited dietary information did not include all possible sources of dietary vitamin A, these computed indexes should be interpreted merely as approximated estimates of intake.

Results

The relationship between reproductive, gynecologic factors and vulvar lichen sclerosus is considered in Table I. The risk of vulvar lichen sclerosus was greater for parous than nulliparous women, but there was little tendency for the risk to increase with number of births (point estimates, 3.0 for women with one or two births and 3.2 for those with three or more births). This finding was significant in the overall series but not when only married women were considered. The estimated relative risks were respectively 1.8 (95% confidence

Table III. Distribution of 75 cases of vulvar lichen sclerosus and 225 controls according to tertiles of dietary retinoid and carotenoid intake (Milan, Italy, 1985 to 1987)

	Cases	*Controls*	*Relative risk*	*95% Confidence interval*
Retinoid tertiles				
1 (<115*)	20	79	1†	
2 (115-129)	34	67	2.0	1.1-3.8
3 (≥130)	21	79	1.1	0.5-2.1
χ_1^2 trend			0.02 (NS)	
Carotenoid tertiles				
1 (<95*)	33	66	1†	
2 (95-169)	31	72	0.9	0.5-1.6
3 (≥170)	11	87	0.3	0.1-0.5
χ_1^2 trend			12.76 ($p < 0.001$)	

*In thousand international units per month.
†Reference category

Table IV. Distribution of 75 cases of vulvar lichen sclerosus and 225 controls according to selected variables, (Milan, Italy, 1985 to 1987)

	Cases	*Controls*	*Relative risk*	*95% Confidence interval*
Marital status				
Married	55	138	1*	
Widowed-divorced	20	66	0.8	0.4-1.4
Never married	—	21		
Education (years)				
<7	40	150	1*	
≥7	25	75	3	0.7-2.2
Smoking habits				
Never smoked	65	175	1*	
Ever smoked	10	50	0.5	0.3-1.1
Body mass index (kg m^{-2})				
<25	48	145	1*	
25-30	20	57	1.1	0.6-1.9
≥30	7	23	0.9	0.4-2.3

*Reference category.

interval 0.7-4.5) and 1.7 (95% confidence interval 0.7-4.9) in women with one or two and three or more births (χ_1^2 for linear trend = 0.96, NS). No correlation emerged with number of spontaneous abortions and age at first pregnancy (data not presented). Similarly, there was no association between vulvar lichen sclerosus, menopausal status, and age at menopause (Table I).

There was no material difference between cases and controls in relation to major indicators of sexual habits (age at first intercourse and number of sexual partners, Table II).

The distribution of cases and controls according to the estimated indexes of monthly intake of retinoids (preformed vitamin A) and caroteinoids (provitamin A) is presented in Table III. There was no apparent relation between retinoid intake and the risk of vulvar lichen sclerosus but women in the upper category of carotenoid intake were at significantly lower risk.

A smaller proportion of cases was unmarried or had ever smoked (the latter finding was not statistically significant), but there were no differences between the distribution of cases and controls with regard to education or body mass index (Table IV). A previous diagnosis of diabetes was reported by a similar percentage of wowen with vulvar lichen sclerosus (8%) and the control group. No cases or controls had a history of clinically diagnosed immune connective disorders (e.g., systemic lupus erythematosus).

Comment

Two different patterns emerged from this exploratory epidemiologic investigation of vulvar lichen sclerosus. First, there were significant associations with marital status (since approximately 10% of controls but

none of the cases were never married) and with nulliparity. The possibility of selection bias being responsible for this could not be excluded, since unmarried women are probably less careful about problems of the external genital organs. It is generally difficult to quantify the role of selection bias in studying a disease when its diagnosis can be noticeably influenced by subjective factors, although in this study no major differences in socioeconomic indicators emerged between cases and controls. Furthermore, cases and controls were chosen from hospitals and institutions that covered broadly comparable catchment areas; participation in the study was almost complete.

The association with parity may be considered with regard to hormonal factors. Lichen sclerosus is more frequent in women and is generally diagnosed in the perimenopausal age (when estrogen levels are proportionally elevated and progesterone levels are decreased in relation to the inconsistent pattern of ovulation). However, in our study menopausal status and age at menopause were not related to the frequency of vulvar lichen sclerosus. These results and the observation that therapy with female hormones had no effect on the clinical course of the disease suggest that female hormones do not play an important role in the pathogenesis of vulvar lichen sclerosus. Conversely, the observation that women who had ever smoked had a lower risk of vulvar lichen sclerosus might be linked to the elevated androgen levels in smokers.[10] Although not statistically significant, this association has a plausible biologic explanation because testosterone has been advocated as the first-choice treatment for vulvar lichen sclerosus.[11]

The second major finding of this study was the negative correlation with carotenoids. This association could not be simply explained by confounding, because allowance for potential confounders (including indicators of socioeconomic and marital status) failed to account for it. Furthermore, at the time of data collection, the possible relationship between diet and vulvar lichen sclerosus was unknown to the interviewers and to the subjects interviewed, and only a small part of the questionnaire was related to dietary factors. Hence, the interviewers and the patients' attention was probably not concentrated on those aspects.

A limitation of this study is the small number of dietary variables collected. However, because the food items analyzed represent the major sources of dietary vitamin A in the Italian diet, we believe that the estimates are probably at least reasonably correlated with the real values. There is no obvious biologic explanation of this finding. The association with carotenoids but not retinoids could be related to the fact that serum levels of the former, but not the latter, are directly related to dietary intake.[12] In vitro, vitamin A inhibits human fibroblasts and collagen production.[13] Thus a deficiency of vitamin A has been claimed in the pathogenesis of skin diseases characterized by excess collagen formation. Because of this, etretinate has been proposed for the treatment of vulvar lichen sclerosus.[14-16]

The clinical observation of previous vaginitis in patients with vulvar lichen sclerosus[4] suggested that infections may play a provocative or localizing role. However, number of sexual partners and age at first intercourse (two well-established indirect indicators of the risk of sex-related diseases) were not associated with the risk of vulvar lichen sclerosus in this series.

On the basis of clinical data, an elevated frequency of autoimmune disorders has been repeatedly associated with vulvar lichen sclerosus.[1, 2] We did not collect a detailed history regarding autoimmune disorders, but no cases or controls had a history of clinically diagnosed important immune-related connective disorders such as systemic lupus erythematosus. Finally, in relation to other disorders, the absence of association with diabetes agreed with the results of a previous case-control study conducted in Britain.[2]

We thank Ms. Maria Nigro and Ms. Judy Baggott for editorial assistance.

REFERENCES

1. Ridley CL. Lichen sclerosus et atrophicus. Br Med J [Clin Res] 1987;295:1295.
2. Harrington CI, Dunsmore IR. An investigation into the incidence of auto-immune disorders in patients with lichen sclerosus and atrophicus. Br J Dermatol 1981; 104:563.
3. Harrington CI, Gelsthorpe K. The association between lichen sclerosus et atrophicus and HLA-B_{40}. Br J Dermatol 1981;104:561.
4. Rook A, Wilkinson DS, Ebling F. Textbook of dermatology. Vol. 2, 3rd ed. Oxford: Blackwell, 1979:1229.
5. Chalmers RJG, Burton PA, Bennett RF, Goring CC, Smith PJB. Lichen sclerosus et atrophicus. A common and distinctive cause of phimosis in boys. Arch Dermatol 1984; 120:1025.
6. Miettinen O. Estimability and estimation in case-referent studies. Am J Epidemiol 1976;103:226.
7. Mantel N. Chi-square tests with one degree of freedom, extensions of the Mantel-Haenszel procedure. J Am Stat Assoc 1963;58:690.
8. Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. JNCI 1959;22:719.
9. Co.N.AL. ABC per l'educazione alimentare. Manuale. Milano: Cooperativa Libreria Editrice Scienze Agrarie Alimentari Veterinarie, 1983.
10. Barbieri RL, McShane PM, Ryan KJ. Constituents of cigarette smoke inhibit human granulosa cell aromatase. Fertil Steril 1986;46:232.
11. Friedrich EG Jr. Vulvar disease. 2nd ed. Philadelphia: Saunders, 1983:134.
12. Peto R. The marked difference between carotenoids and retinoids: methodological implications for biochemical epidemiology. Cancer Surv 1983;2:327.

13. Nelson DL, Balian G. The influence of retinoic acid on collagen synthesis by skin fibroblasts. J Invest Dermatol 1983;80:357.
14. Neuhofer J, Fritsch P. Treatment of localized scleroderma and lichen sclerosus with etretinate. Acta Derm Venereol (Stockh) 1984;64:171.
15. Romppanen U. Long-term results of etretinate therapy in vulvar dystrophy. Curr Ther Res 1986;40:442.
16. Mork NJ, Jensen P, Hoel PS. Vulval lichen sclerosus et atrophicus treated with etretinate (Tigason). Acta Derm Venereol (Stockh) 1986;66:363.

Fetal liver length in normal and isoimmunized pregnancies

Alistair B. Roberts, MD, Jennifer M. Mitchell, and Neil S. Pattison, MD
Auckland, New Zealand

The length of the right lobe of the fetal liver was measured by means of ultrasound examined on 53 occasions in 21 isoimmunized pregnancies. Fetal blood samples were taken in all patients within 24 hours. Comparisons were made with 350 measurements of liver length in normal pregnancies. A good correlation was found between liver length and fetal hemoglobin level ($r = 0.794$, $p < 0.001$) and between liver length and reticulocyte count ($r = 0.721$, $p < 0.001$). At the time of first sample, all fetuses with a hemoglobin of <100 gm/L had a liver length that was greater than the ninetieth percentile. Liver length measurement seems to be a useful indicator of the degree of fetal anemia in isoimmunized pregnancies. (Am J Obstet Gynecol 1989;161:42-6.)

Key words: Fetal liver, ultrasound, rhesus

Ultrasound examination now plays a major role in the assessment and management of isoimmunized pregnancies. Many ultrasound signs have been advocated as indicators of fetal anemia. These include the presence of fetal ascites, skin edema, pericardial effusion, the measurement of umbilical vein diameter, abdominal circumference, placental size, amniotic fluid volume, fetal activity, and Doppler studies of the fetal aorta and umbilical vein.[1-4] Overall, the use of ultrasound studies to assess the severity of fetal disease has been disappointing.[4] Most signs only appear in the severely anemic fetus. The correlation between optical density of the amniotic fluid (ΔOD 450 nm) and fetal hemoglobin is also poor, especially before 24 weeks.[5]

Therefore many units rely on determining the severity of fetal anemia by direct measurement of fetal hemoglobin or hematocrit level after a sample of fetal blood is obtained. Specimens of fetal blood are usually obtained by percutaneous umbilical blood sampling under ultrasound guidance.[6] Treatment is then usually instituted with the use of an intravascular transfusion technique[7, 8] or a combined intravascular intraperitoneal approach. However, these are invasive procedures and carry significant risks of fetal loss or increased maternal sensitization and antibody production.[4] There is a need for a noninvasive method of assessing the degree of fetal anemia.

Enlargement of the fetal liver in isoimmunized pregnancies has proved a useful subjective sign of increased fetal liver hemopoiesis. The size of the liver has been measured indirectly by abdominal circumference or diameter. Vintzileos et al.[9] showed that direct measurement of the right lobe of the fetal liver was more useful than abdominal circumference

This study was designed to measure the right lobe of the fetal liver in isoimmunized pregnancies and correlate this measurement with fetal hemoglobin. We felt it was important to have normal fetal liver measurements from 15 weeks' gestation, and for this reason collected fetal liver measurements during normal pregnancies from 15 to 40 weeks.

Patients and methods

Normal patients. Three hundred fifty measurements of fetal liver length were made between 15 and 40 weeks' gestation in 350 patients; measurements were taken as part of a clinically requested ultrasound examination. The women studied had regular menstrual cycles with certain last menstrual periods and a scan done before 20 weeks that agreed with their menstrual dates. Delivery occurred within 2 weeks of the pre-

From the National Women's Hospital.
Received for publication September 27, 1988; revised December 20, 1988; accepted January 11, 1989.
Reprint requests: A. B. Roberts, MD, National Women's Hospital, Claude Road, Auckland 3, New Zealand.

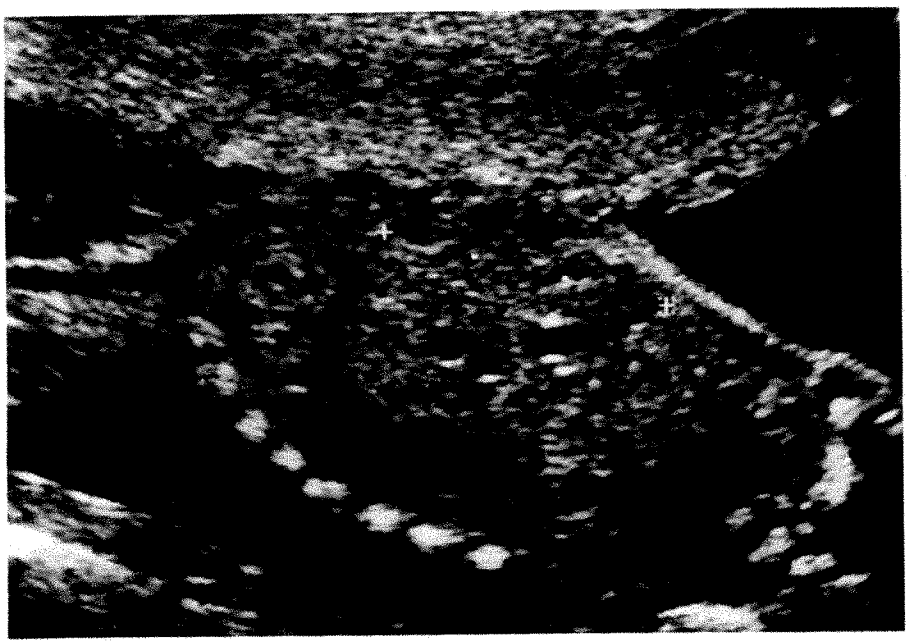


Fig. 1. Ultrasound picture demonstrates method of measuring right lobe of fetal liver at 18 weeks' gestation.

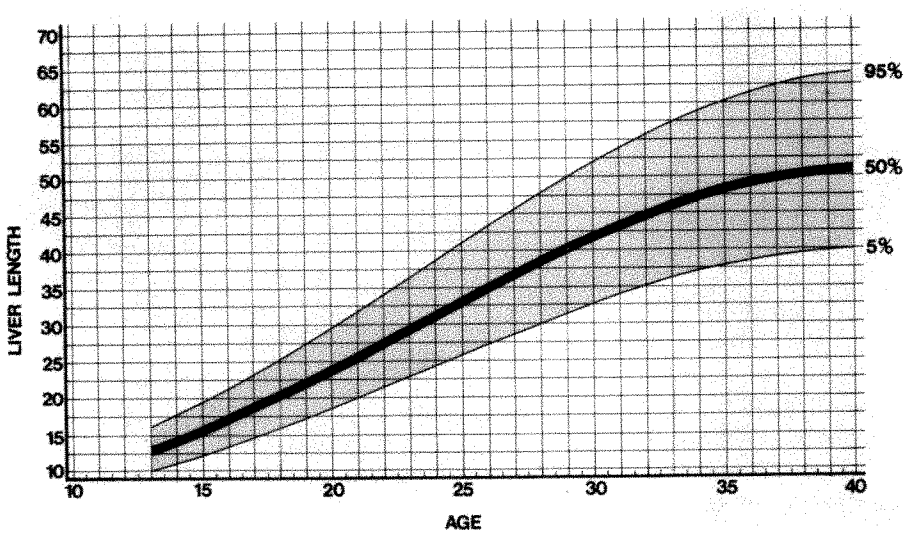


Fig. 2. Relationship between fetal liver length and gestational age in 350 normal pregnancies. Fifth and ninety-fifth percentile confidence limits are indicated.

dicted date of delivery, and neonatal Dubowitz scores were compatible with gestational age.

Fetal liver length was measured by means of the following technique, which is similar to that described by Vintzileos et al.[9] The fetus was scanned until a longitudinal view of the abdominal aorta was achieved. The scanning plane was moved parallel to visualize the right lobe of the liver. The liver was measured from the dome of the right hemidiaphragm to the tip of the right lobe. Fetal position sometimes made it difficult to measure the right lobe, but no particular position of the fetus was selected for exclusion. Ultrasound measurements were made by means of Diasonics sector scanner with a 3.5 or 5 MHz transducer or a Toshiba 100-convex 3.75 MHz transducer. Freeze-frame capability was available and on-screen calipers were used for measurements (Fig. 1). The reproducibility of the measurement was assessed by two independent observers in 29 patients. There was no significant difference between observers ($r = 0.976$, $p < 0.001$). The interobserver variation was less than 10% in 95% of cases.

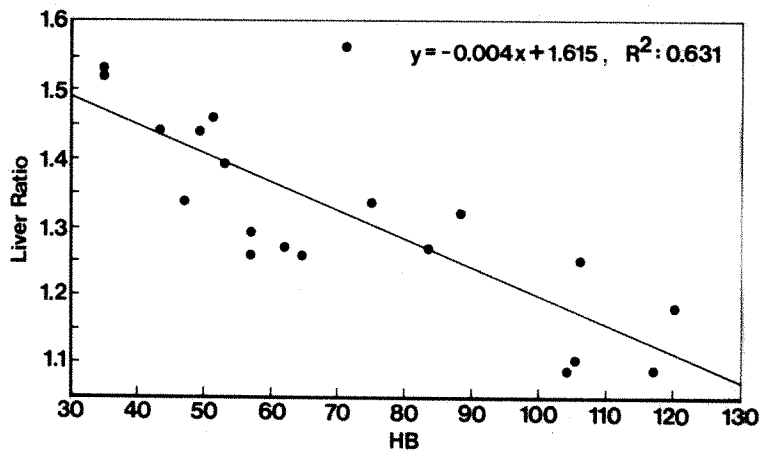


Fig. 3. Relationship between liver length ratio and fetal hemoglobin in 21 patients at time of first sample.

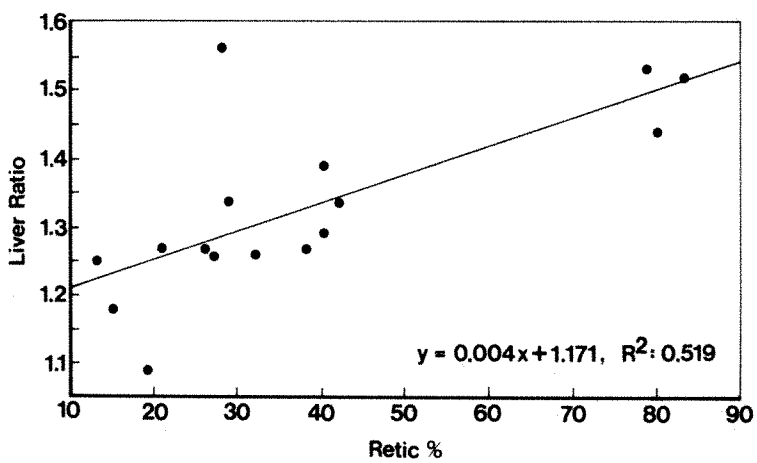


Fig. 4. Relationship between liver length ratio and fetal reticulocyte count at time of first sample.

Isoimmunized pregnancies. Twenty-one pregnant women with severe isoimmunization were studied. The antibodies present were anti-Kell in two women and anti-D in the remainder. Three patients had hydropic fetuses at the time of presentation with gross ascites and significant subcutaneous edema. Patients were referred to the National Women's Hospital after amniocentesis, when the optical density difference (ΔOD 450 nm) was elevated. After detailed ultrasound examination, including measurement of the fetal liver, fetal blood sampling was performed with a 20 cm, 22-gauge needle, directed under ultrasound guidance, into the umbilical vein or artery. The cord was usually sampled within 1 cm of the placental insertion, although occasionally other sites were used, including a free loop of cord or rarely the cord insertion into the fetus. Three milliliters of fetal blood were taken for analysis, including blood grouping. Coombs testing, hemoglobin (urgent), hematocrit, reticulocyte count, Kleihauer, bilirubin, and total protein. Anemic fetuses received a transfusion and fetal blood sampling, and transfusion was repeated at an interval of 1 to 4 weeks. Measurements of fetal liver length were made before fetal blood sampling (within 24 hours) in all 21 patients with the first sample and in 32 subsequent fetal blood samples and transfusions. Fifty-three blood samples in 21 patients were included in the analysis.

For purposes of comparison, liver length is expressed as liver length ratio (actual liver length over mean for gestation).

Results

The relationship between fetal liver length and gestational age is shown in Fig. 2 with fifth and ninety-fifth percentile confidence limits. The predicted limits were obtained by fitting a polynomial regression model for gestational age to a logarithm of liver length and then transforming the resultant prediction intervals

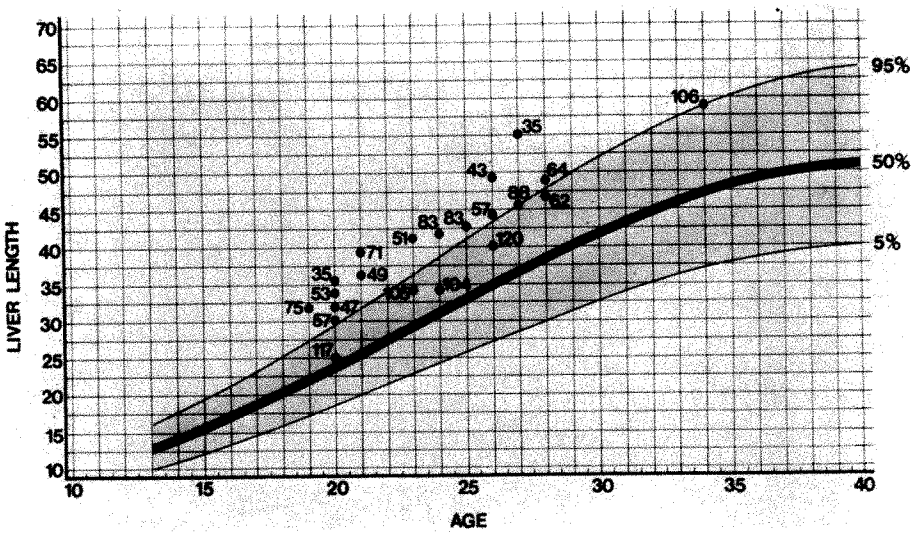


Fig. 5. Actual fetal liver length plotted against normal graph at time of first sample. Numbers beside each point denote fetal hemoglobin.

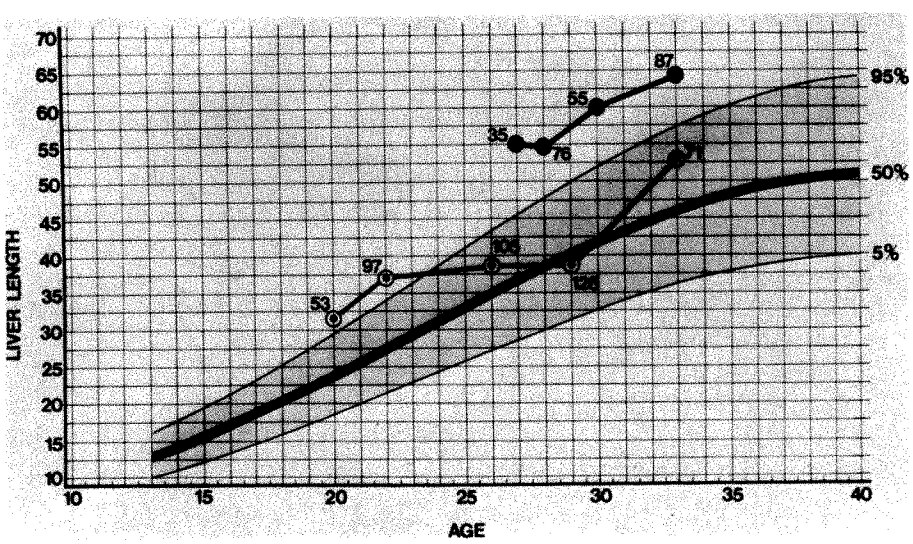


Fig. 6. Actual liver length plotted against normal graph in two cases.

back to the original scale. A quadratic model was found to fit the data well ($R^2 = 0.88$).

Isoimmunized pregnancies. There was a significant relationship ($p < 0.001$) between fetal liver length and fetal hemoglobin level in all 21 patients at the time of first sample ($r = 0.794$) (Fig. 3). There was also a significant relationship ($p < 0.001$) between fetal liver length and the percentage of reticulocytes in the fetal circulation ($r = 0.721$) (Fig. 4). At the time of subsequent transfusion, the relationship between liver length and hemoglobin was significant ($r = 0.631, p < 0.001$), although the relationship with the reticulocyte count was less good ($r = 0.445, p < 0.004$). At the time of the first fetal blood sample, all fetuses with a fetal hemoglobin level of <100 gm/L had a liver length greater than the ninetieth percentile (Fig. 5). Fetal transfusion and correction of fetal anemia led to a relative reduction in fetal liver length (Fig. 6). No difference was found between pregnancies with Kell and D antibodies.

Comment

Fetal liver length measurements in normal pregnancies in this series confirm the data of Vintzileos et al.[9] and more recently those of Murao et al.[10] There is a discrepancy between our data and those of Vintzileos et al. before 24 weeks, which is probably explained by the small numbers that Vintzileos et al. had at the lower gestational age. Our study confirms the usefulness of

liver length in the evaluation of the isoimmunized pregnancy. The study enlarges on the data from Vintzileos et al. by directly comparing liver length with fetal hemoglobin level and reticulocyte count. This has obvious significance for triggering referrals to centers that perform fetal blood sampling and transfusion. This is a particular problem in New Zealand where patients are referred from a long distance and there is a need for a specific measurement or sign that can be used for referral. We currently use amniocentesis, and an optical density of greater than 0.2 is used to trigger referrals. However, the correlation between fetal hemoglobin and optical density is poor.[5] It is now possible to suggest that any fetal liver measurement greater than the ninetieth percentile in a patient with antibodies should be considered for transfer. Confusion may arise, since there are other reasons for enlargement of the fetal liver. Some fetuses of pregnant women with diabetes have significantly enlarged livers, although this is usually in the third trimester. Other possibilities include fetal or maternal infections or fetal anemia from other causes. It will probably be most useful before 30 weeks of gestation when it is rare to have fetal liver enlargement for other reasons. It is difficult to be sure how reproducible measurement of fetal liver length will be. We tested the reproducibility between two experienced observers on the same patient with no knowledge of the others results. The reproducibility was good. The reproducibility of the measurement depends on the experience of the operator and more important on the ultrasound equipment used. The improvements in ultrasound technology have made visualization of the fetal liver possible. Variations in quality of equipment will undoubtedly lead to problems with reproducibility. The position of the fetus can also be a problem. If the right lobe of the fetal liver is posterior, it can be difficult to identify the tip of the right lobe.

Measurement of the size of the fetal liver seems to be more useful before the first transfusion than subsequently, and this may be partly gestation dependent. The percentage of hemopoesis that is extramedullary decreases with gestational age, and it is probable that switching to increased extramedullary hemopoesis may occur later with increasing gestational age. Therefore, liver length measurement may be more useful before 30 weeks, although it is our experience that even in the third trimester, a liver size that is well within the normal range has not so far been associated with any significant anemia.

In conclusion, our studies confirm the usefulness of measuring fetal liver length in the assessment of isoimmunized pregnancies. Further studies are needed to test whether the measurement is reproducible enough to be useful on a larger scale.

We thank Professor A. Scott, Statistics Department, Auckland University, for help with the statistical analysis.

REFERENCES

1. Warren P, Gill RW, Fisher CC. Doppler studies in rhesus isoimmunization. Semin Perinat 1987;11:375-8.
2. De Vore GR, Mayden K, Tortora M. Dilatation of the fetal umbilical vein in rhesus hemolytic anemia: a predictor of severe disease. Am J Obstet Gynecol 1981;141:464-8.
3. Rightmire DA, Nicolaides KH, Rodeck CH, Campbell S. Fetal blood velocities in rhesus isoimmunization: relationship to gestational age and to fetal hematocrit. Obstet Gynecol 1986;68:233-6.
4. Chitkara U, Wilkins I, Lynch L, Mehalek K, Berkowitz RL. The role of sonography in assessing severity of fetal anemia in Rh and Kell-isoimmunized pregnancies. Obstet Gynecol 1988;71:393-8.
5. Nicolaides KH, Rodeck CH, Mibashan RS, Kemp JR. Have Liley charts outlived their usefulness? Am J Obstet Gynecol 1986;155:90-4.
6. Daffos F, Capella-Pavlovsky M, Forestier F. Fetal blood sampling during pregnancy with use of a needle guided by ultrasound. A study of 606 consecutive cases. Am J Obstet Gynecol 1985;153:655-61.
7. Bang J, Bock JE, Troule D. Ultrasound guided fetal intrauterine transfusion for severe rhesus haemolytic disease. Br Med J 1982;1:373-4.
8. De Crespigny LCh, Robinson HP, Quinn M, Doyle C, Cauchi M. Ultrasound guided fetal blood transfusion for severe rhesus isoimmunization. Obstet gynecol 1985; 66:529-32.
9. Vintzileos AM, Campbell WA, Storlazzi E, Mirochnick MH, Escoto DT, Nochimson DJ. Fetal liver ultrasound measurements in isoimmunized pregnancies. Obstet Gynecol 1986;68:162-7.
10. Murao F, Takamori H, Hata K, Hata T, Kitao M. Fetal liver measurements by ultrasonography. Int J Gynecol Obstet 1987;25:381-5.

Does midtrimester ΔOD_{450} of amniotic fluid reflect severity of Rh disease?

Uma Ananth, MD, and John T. Queenan, MD
Washington, D.C.

Thirty-two patients with Rh-immunized pregnancies had amniocenteses between 16 and 20 weeks' gestation. Serial amniotic fluid values of delta optical density at 450 nm were studied to predict the severity of Rh disease. A value >0.15 indicated severe Rh immunization. Values <0.09 indicated mild or no Rh disease; delta optical density at 450 nm between 0.09 and 0.15 require further evaluation to determine the trend. Midtrimester amniocentesis is a simple technique that helps to determine which patients may or may not require fetal blood sampling. (Am J Obstet Gynecol 1989;161:47-9.)

Key words: Midtrimester, amniotic fluid analysis, ΔOD_{450}, Rh immunization

Erythroblastosis fetalis that occurs as a result of Rh immunization continues to be a significant contributor to perinatal mortality despite the widespread use of Rh-immune globulin. Before Liley's[1] systematized approach to management of the Rh problem by serial amniotic fluid values of delta optical density measurements at 450 nm (ΔOD_{450}), 25% of all Rh-immunized pregnancies were lost. Preterm delivery to avoid intrauterine death was the principal method of management. Many Rh-positive fetuses died in utero as a result of erythroblastosis fetalis and others died in the nursery. Some Rh-negative babies died because of prematurity when they were delivered early unnecessarily. Amniotic fluid analysis allows determination of the severity of Rh disease in utero and thus permits intrauterine transfusion or delivery of the affected fetus before death occurs in utero and allows the unaffected fetuses to remain in utero to gain valuable maturity. The Liley approach is extremely effective in preventing fetal death in the third trimester.[2, 3]

In cases of very severe Rh immunization, fetal death can occur in the second trimester. Because intrauterine intravascular fetal transfusion is capable of saving fetuses in the early second trimester, it is important to evaluate the reliability of amniotic fluid ΔOD_{450} in predicting fetal condition in the second trimester. Therefore, this study was undertaken to determine whether the amniotic fluid ΔOD_{450} value reflects the severity of Rh disease in the early second trimester and thereby indicates the need for intrauterine transfusion.

From the Department of Obstetrics and Gynecology, Georgetown University School of Medicine.
Received for publication July 9, 1988; accepted January 30, 1989.
Reprint requests: Uma Ananth, MD, Department of Obstetrics and Gynecology, Georgetown University School of Medicine, 3800 Reservoir Rd., N.W., Washington, D.C. 20007.

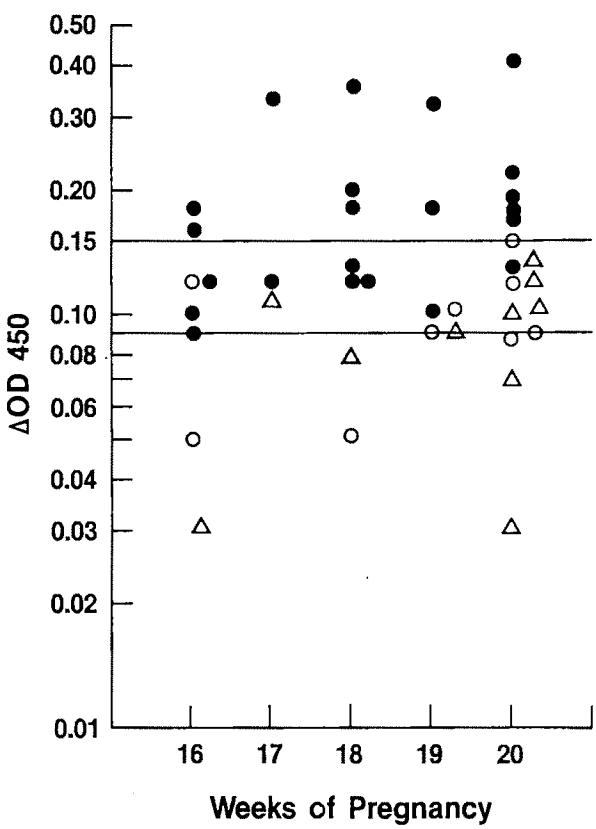


Fig. 1. Distribution of amniotic fluid ΔOD_{450} values from Rh-immunized pregnancies between 16 and 20 weeks' gestation. ○, Unaffected pregnancies; △, mildly affected pregnancies; ●, severely affected pregnancies.

Material and methods

Thirty-two patients with Rh immunization who were between 16 and 20 weeks' gestation at the time of initial amniocentesis were studied. The criteria for selection included a maternal antibody titer ≥1:64 or a history of previous stillbirth. Study patients were healthy

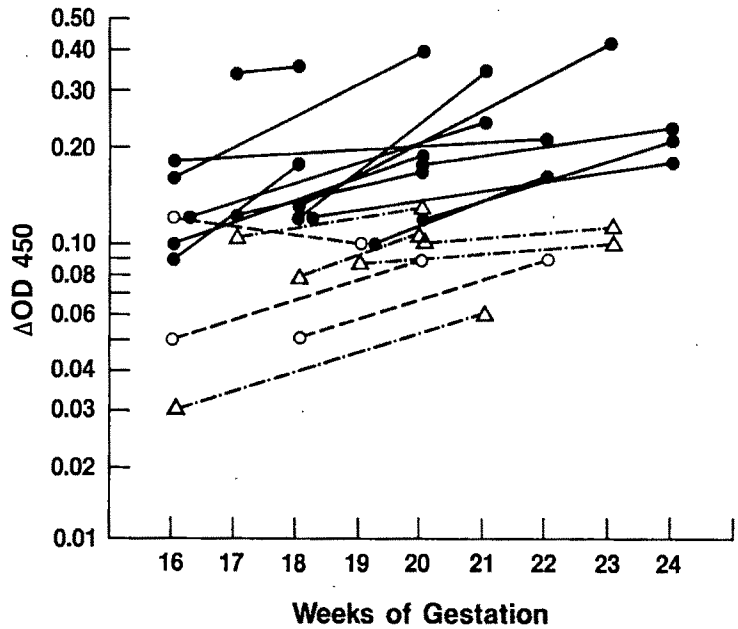


Fig. 2. Trend of amniotic fluid ΔOD_{450} values.

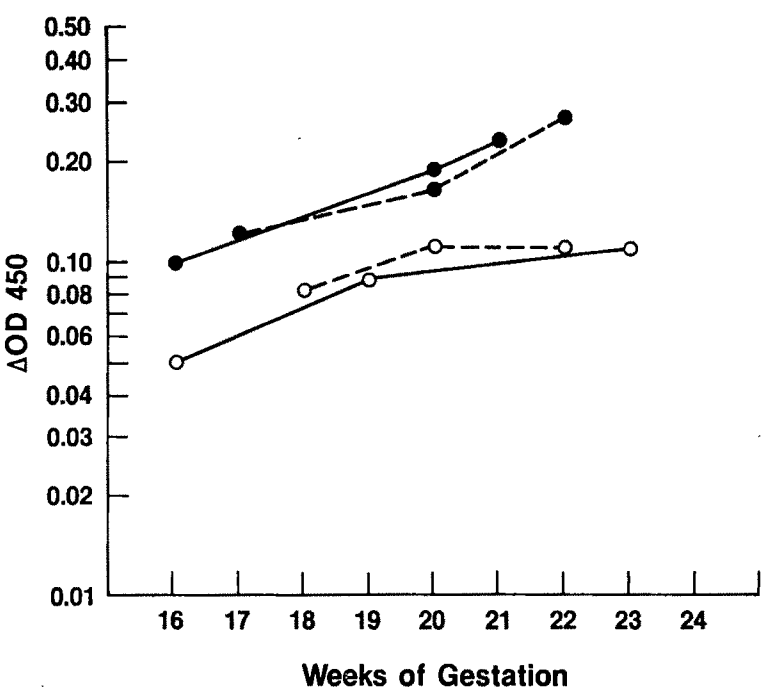


Fig. 3. Serial amniotic fluid ΔOD_{450} values in two patients in their two consecutive pregnancies. *Continuous* and *interrupted lines* represent different patients.

women between 30 and 40 years of age who had amniocenteses for genetic purposes or for determining amniotic fluid ΔOD_{450}.

Each sample of amniotic fluid was centrifuged at 3000 rpm for 10 minutes immediately after collection, and samples were then analyzed in a spectrophotometer to obtain a continuous recording between 300 and 700 nm. The deviation in optical density (ΔOD) was obtained by drawing an arbitrary line between 375 and 525 nm and measuring the difference at 450 nm, as described by Liley.[1,2] Samples demonstrating significant peaks at 412, 540, and 575 nm were excluded

from the study because they indicated contamination from other heme pigments and could have altered the bilirubin curve.[4] Twenty-one of the 32 patients underwent a second amniocentesis 1 to 6 weeks after the first examination and were studied to examine the trend of ΔOD_{450} values. Two of the patients were pregnant twice during the period of our study and are discussed here.

Results

Fig. 1 shows the distribution of 41 ΔOD_{450} values obtained from the 32 patients studied between 16 and 20 weeks' gestation. The values were divided into three groups based on the outcome. Group 1 consisted of unaffected pregnancies; these patients were delivered of Rh-negative babies. Group 2 comprised mildly affected pregnancies, defined in our study as fetuses who were delivered at term, possibly requiring phototherapy but no exchange transfusions. Group 3 included severely Rh-immunized pregnancies, defined as fetal death, or infants who required intrauterine transfusions or exchange transfusions.

It is evident from Fig. 1 that the amniotic fluid ΔOD_{450} values for groups 1 and 2 are evenly distributed between 0.03 and 0.15. For group 3, all values lie above 0.08. Therefore, there is an area of overlap between 0.09 and 0.15 in which all three groups are represented.

Fig. 2 shows the trend of ΔOD_{450} values in the 21 patients who underwent repeat amniocenteses between 1 and 6 weeks after the first examination. The values for all three groups of patients show an upward trend.

Fig. 3 illustrates the trend in ΔOD_{450} values for the two patients who were pregnant twice during the study. In their first pregnancies, the serial ΔOD_{450} values showed an upward trend, rising above the critical value of 0.15. Both first pregnancies ended in fetal death in the second trimester. Their subsequent pregnancies resulted in healthy Rh-negative babies, reflected by serial ΔOD_{450} values <0.09.

Comment

We have previously established[5] normal values of ΔOD_{450} in the early trimester and have shown that the values are distributed between 0.01 and 0.15, with a slight but statistically significant upward trend from 16 to 20 weeks' gestation.[5] The findings in this study concur with those of our earlier study since a similar upward trend is demonstrated (Fig. 2).

The normal values for ΔOD_{450} in the third trimester show a downward trend from the twenty-seventh week until term. This trend forms the basis for the management of Rh disease in the third trimester. The accuracy of predicting severe Rh disease from a value in the third trimester is excellent.[3] However, it has been shown that Liley's graph cannot be extrapolated into the second trimester.[5, 6] Investigators[6] have demonstrated a poor correlation between amniotic fluid ΔOD_{450} values and fetal hemoglobin in the midtrimester. This has led them to conclude that amniotic fluid ΔOD_{450} has no predictive value in the second trimester. Therefore they advocated the routine use of percutaneous umbilical blood sampling in all Rh-immunized pregnancies. From a clinical standpoint the controversial issues are: (1) Can a single amniotic fluid ΔOD_{450} value or serial values predict which fetus has severe Rh disease early enough for fetal transfusion to be effective? (2) Can amniotic fluid ΔOD_{450} predict which fetuses have mild or no disease so that fetal umbilical blood sampling and intrauterine transfusions are not necessary?

From this study it is clear that a single amniotic fluid ΔOD_{450} value >0.15 or an amniotic fluid trend rising above 0.15 indicates severe disease. These patients can be selected for percutaneous umbilical blood sampling to confirm the degree of fetal anemia and whether direct intravascular transfusion is indicated. Values <0.09 indicate unaffected or mildly affected fetuses. These fetuses require further evaluation with serial amniocentesis to verify the trend. In cases in which the values fall between 0.09 to 0.15, careful follow-up with serial amniotic fluid ΔOD_{450} values is required to establish the amniotic fluid trend that determines the severity of Rh disease.

This relatively simple technique allows the identification of fetuses that need intravascular transfusion and avoids performing the more invasive procedure of percutaneous umbilical blood sampling on a routine basis.

REFERENCES

1. Liley AW. Liquor amnii analysis in the management of pregnancy complicated by rhesus sensitization. AM J OBSTET GYNECOL 1961;82:1359.
2. Liley AW. Errors in assessment of hemolytic disease from amniotic fluid. AM J OBSTET GYNECOL 1963;86:376.
3. Bowman JM. The management of Rh-isoimmunization. Obstet Gynecol 1978;52:1.
4. Queenan JT. Amniotic fluid analysis in modern management of the Rh problem. 2nd ed. Hagerstown, Maryland: Harper & Row, 1977:73.
5. Ananth U, Warsof SL, Coulehan JM, Wolf PH, Queenan JT. Midtrimester amniotic fluid delta optical density at 450 nm in normal pregnancies. AM J OBSTET GYNECOL 1986; 155:664.
6. Nicolaides KH, Rodeck CH, Mibashan RS, Kemp JR. Have Liley charts outlived their usefulness? AM J OBSTET GYNECOL 1986;155:90.

Increased frequency of detection of human papillomavirus deoxyribonucleic acid in exfoliated cervical cells during pregnancy

Robert F. Rando, PhD,[a, b] Steven Lindheim, MD,[b] Lisa Hasty, MD,[b] Thomas V. Sedlacek, MD,[b] Mark Woodland, MD,[b] and Catherine Eder, BA[a]

Philadelphia, Pennsylvania

Exfoliated cells of the uterine cervix obtained from women during pregnancy and at the time of their first postpartum examination were used to monitor the prevalence of human papillomavirus infections in this population and to study the natural fluctuations in viral expression. When deoxyribonucleic acid hybridization analysis alone was used to monitor the presence of human papillomavirus infection, 20.9% of our study population had results that were positive for human papillomavirus deoxyribonucleic acid during their first-trimester examinations. A dramatic increase in the percentage of women with positive results for human papillomavirus deoxyribonucleic acid was observed at the time of the patients' third-trimester examinations (46%). The overall increase in human papillomavirus-positive patients was a combination of a small number of patients who had positive results on their first examination and negative results on their second examination, and a larger number of patients who had negative results on their first-trimester examination and positive results for human papillomavirus deoxyribonucleic acid in the exfoliated cervical cells at the time of their third-trimester examination. The total percentage of patients with positive results for human papillomavirus deoxyribonucleic acid in their cervical cells at one or both assay points during pregnancy was 52.5%. Samples obtained at the postpartum examination demonstrated a dramatic decrease in the number of samples positive for human papillomavirus deoxyribonucleic acid (17.5%). This result was a combination of a large decrease in human papillomavirus-positive patients coupled with a small increase in detectable levels of human papillomavirus deoxyribonucleic acid in cervical samples from patients who had negative results on their previous examination. This study demonstrates a very high level of detectable human papillomavirus deoxyribonucleic acid in exfoliated cervical cells obtained during pregnancy and shows that the detectable levels of human papillomavirus deoxyribonucleic acid fluctuate during pregnancy. (AM J OBSTET GYNECOL 1989;161:50-5.)

Key words: Human papillomavirus (HPV), deoxyribonucleic acid (DNA), uterine cervix, pregnancy, hybridization

Surveillance of reported cases of clinically apparent anogenital human papillomavirus (HPV) infections indicated a steady rise in the incidence of new cases between 1966 to 1983.[1] In 1976 Meisels and Fortin[2] helped to heighten interest in human papillomaviruses when they reinterpreted the morphologic features of dysplasia of the uterine cervix as manifestations of HPV infections. Interest was further stimulated when HPV deoxyribonucleic acid (DNA) was identified in premalignant and malignant anogenital lesions.[3] To date more than 50 distinct types of HPVs have been described. At least 12 distinct types have been detected in anogenital tract and oral cavity lesions. Several studies have reported that HPV type 16 and HPV type 18 are found in association with a majority of high-grade dysplastic lesions of the cervix, carcinoma in situ, and cervical carcinomas.[3]

Two recent studies estimated that 10% to 20% of random populations that had cytologically "normal" cervices were in fact infected with HPV.[4, 5] Burk et al.[6] detected HPV DNA in 29% of a population of women who were referred to a colposcopy clinic and had normal Papanicolaou smears. Wickenden et al.[7] detected HPV infections in 10.5% of a population from a sexually transmitted disease clinic who had cytologically normal cervices. It is interesting that a study conducted among pregnant women[8] detected HPV DNA in 28% of the study population.

The enhanced detection of viral infections has been reported in patients with impaired cell-mediated immunity.[9, 10] It has also been noted that HPV infections and HPV-associated neoplasia of the anogenital tract

From the Departments of Pathology[a] and Obstetrics and Gynecology,[b] Pennsylvania Hospital.

Received for publication July 22, 1988; revised October 9, 1988; accepted January 10, 1989.

Reprint requests: Robert F. Rando, PhD, Department of Pathology, Preston Bldg., Rm. 758, Pennsylvania Hospital, 8th and Spruce St., Philadelphia, PA 19107.

are more readily detected in immunodeficient patients than in the general population.[9, 10] During pregnancy, a natural altered or depressed immunologic state has been suggested.[8] It has also been reported that clinically apparent HPV infections of the female genital tract seem to occur more frequently during pregnancy and regress rapidly after delivery.[11] Schneider et al.[8] reported the detection of HPV DNA in exfoliated cervical cells in 28% of a pregnant population compared with 12.5% for nonpregnant control patients; a higher detection level occurred in samples obtained later in pregnancy.

In addition to an immunodepressed condition, hormonal effects during pregnancy may play a role in the enhancement of viral infection or expression. Long-term users of oral contraceptives show an increased risk for the development of HPV-associated neoplasia.[12, 13] There are also reports of hormonal stimulation of HPV-directed transcription in vitro[14] and proliferation of viral growth with β-estradiol in vivo (Krieder J, personal communication).

This article describes a prospective study designed to evaluate the basal level of cervical HPV infections in a population of pregnant women, and to determine whether there are fluctuations in the detectable levels of HPV DNA over the course of pregnancies through the first postpartum examination.

Material and methods

Sample collections. From April 1987 until August 1987 all new patients at Pennsylvania Hospital's obstetric clinic were recruited for this study. This population of women was young (mean age = 23.45 years), sexually active, and from a low socioeconomic group. At the time of the patient's first-trimester examination, a sample of exfoliated cervical cells was obtained and used for both cytologic and DNA hybridization analysis. Similar specimens of exfoliated cervical cells were obtained for DNA hybridization analysis during a third-trimester visit (28 to 34 weeks) and at the time of the patient's first postpartum checkup (no later than 6 weeks after delivery).

DNA extraction. Exfoliated cervical cells were suspended in phosphate-buffered saline solution, concentrated by low-speed centrifugation (1500 rpm), resuspended in 1 ml of cell lysis buffer (10 mmol/L Tris hydrochloride, pH 8.0, 0.6% sodium dodecyl sulfate, 10 mmol/L ethylenediaminetetraacetic acid). Ribonuclease A (10 μg/ml) and proteinase K (50 μg/ml) were added to the cell lysis buffer and the samples were incubated at 37° C for 2 hours. Total cellular DNA was purified by extracting the lysed cellular material twice with an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1). The extracted nucleic acids were then precipitated by adjusting the salt concentration to 0.3 mol/L sodium acetate and adding 2.5 volumes of cold (−20° C) ethanol. The DNA was concentrated by centrifugation, washed twice with 70% ethanol, dried under vacuum, resuspended in 1 ml of a buffer containing 10 mmol/L Tris hydrochloride (pH 7.5) and 1

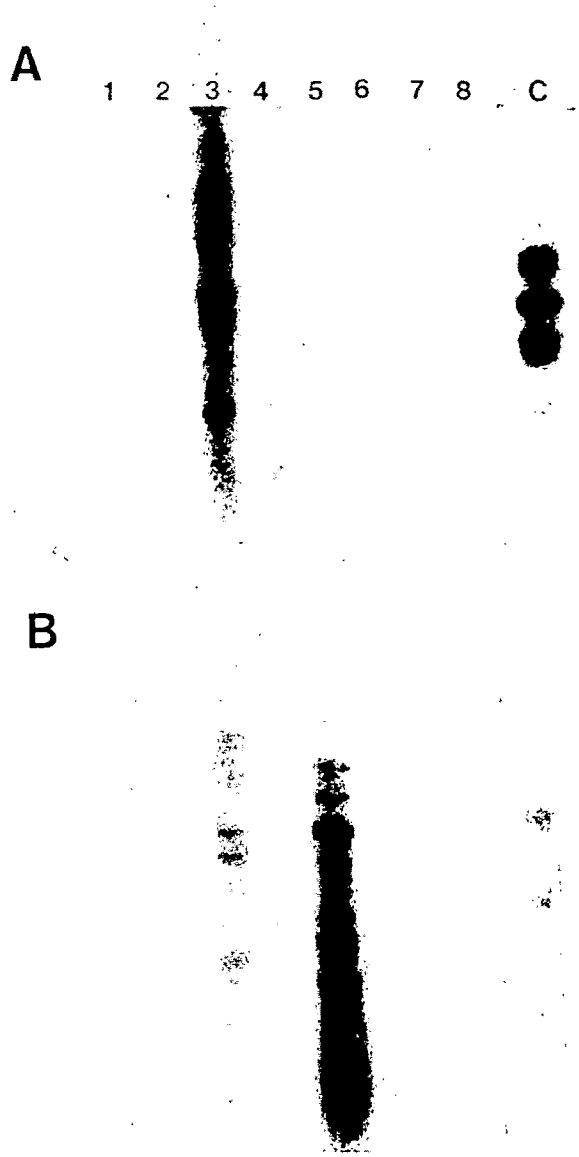


Fig. 1. Detection of HPV DNA in samples obtained from exfoliated cervical cells by Southern blot hybridization. DNA was extracted, cleaved with restriction enzymes, electrophoresed, and transferred onto nylon filter membranes as described in "Material and methods." The procedure described was performed in triplicate. One of each of the three triplicate filters was hybridized with a combination of ^{32}P-labeled molecular probes corresponding to HPV-16/18 (**A**) or HPV-31/33/35 (data not shown) or HPV-6/11 (**B**). DNA extracted from the samples analyzed in lane *3* hybridized better with the HPV-16/18 probes. DNA extracted from the sample in lane *5* hybridized better with the HPV-6/11 probes. Lane **C** contained 20 pg of an HPV-16 recombinant clone that was cleaved with *Pst* I.

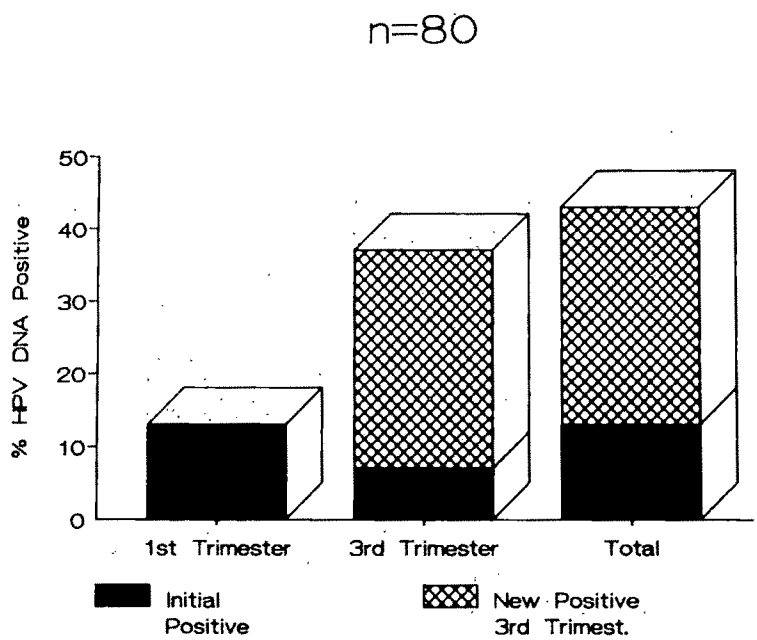


Fig. 2. Graphic representation of HPV-positive cervical samples obtained from patients compliant through their third-trimester examination. The number of patients with positive results in the third trimester is a combination of patients who had positive results in the first trimester (minus those patients who had positive results at their first-trimester examination but negative results for HPV DNA in their cervical cells during their third-trimester examination) combined with the number of new patients with negative results in the first trimester but who had HPV detected during their third-trimester examination. The *bar* labeled *total* represents patients who were HPV DNA-positive in one or both of their examinations.

Table I. Correlation of Papanicolaou smear versus DNA hybridization analysis for the detection of HPV during first trimester of pregnancy

	Papanicolaou smear		
	*Positive**	*Negative†*	*Total*
DNA pos.	6	17	23
DNA neg.	8	79	87
TOTAL	14	96	110

*Papanicolaou-positive smears included those described as condylomatous, hyperkeratosis, parakeratosis, or squamous atypia.

†Fourteen patients were classified as having benign atypia. Of those 14 patients, 5 had positive results for HPV DNA.

mmol/L ethylenediaminetetraacetic acid; the concentration was determined by spectrophotometric analysis at 260 nm.

Hybridization analysis. Total DNA (10 to 15 μg) extracted from patient samples was cleaved with the restriction endonuclease *Pst* I as recommended by the manufacturer (New England Biolabs). The resulting DNA fragments were fractionated by electrophoresis in a 0.8% agarose gel and then transferred to nylon filter membranes (Amersham Hybond-N). DNA hybridization analysis was performed with modifications of the Southern blot technique as previously described.[15] Molecular probes corresponding to HPV types 6, 11, 16, 18, 31, 33, and 35 were amplified and purified using standard laboratory methods.[16] The HPV genomes were purified from flanking bacterial plasmid DNA and then radiolabeled with ^{32}P-deoxycytidine triphosphate.[15, 16]

Results

Exfoliated cervical cells obtained during the first-trimester examination from 110 patients were examined by cytologic and DNA hybridization analysis for the presence of HPV infection. A comparison of the Papanicolaou smear and DNA hybridization analysis for the detection of HPV is described in Table I.

Thirty-one of the 110 patients (28.2%) had positive results for the presence of HPV in their cervical cells. Of these 31 HPV-positive patients, eight (7.2%) had positive results by Papanicolaou smears alone, six (5.4%) had positive results by both criteria, and 17 (15.4%) had only positive hybridization analysis results (Table I). A total of 23 of 110 (20.9%) had positive DNA hybridization analysis results.

DNA hybridization analysis was performed in triplicate for the first-trimester patient samples. The tripli-

Table II. Correlation of sexual partners with detection of HPV DNA

	No. of sexual partners			
	1-2		*≥3*	
	n	%	*n*	%
HPV DNA pos.* (n = 38)	13/38	34.2	25/38	65.7
HPV DNA neg. (n = 28)	11/28	39.2	17/28	60.7
			$\chi^2 = 0.187$ $p > 0.05$	

*Any patient who had HPV DNA detected during their first-trimester, third-trimester, or both examinations were considered HPV DNA pos.

cate hybridization reactions used mixtures of HPV types 16 and 18, HPV types 31, 33, and 35, or HPV types 6 and 11 for molecular probes (Fig. 1). Of the 23 patient samples that contained HPV DNA at the first-trimester examination, HPV-16/18 was detected in 14 (60.8%) samples, HPV-31/33/35 was detected in six (26%), and HPV-6/11 was detected in one (4.3%) sample. In the remaining two samples (8.7%), HPV DNA was detected but not typable with the available molecular probes.

Samples of exfoliated cervical cells were obtained from 80 of the original 110 patients during their third trimester-visit. Thirteen of these 80 patients (16.2%) had positive HPV DNA results during their first-trimester examination. DNA hybridization analysis was performed on these samples. The results indicate an increase in the percentage of patients with positive results for HPV DNA in the third trimester, compared with the first-trimester results (Fig. 2). This increase was reflected in a moderate regression of detectable levels of HPV DNA in patients with positive results for HPV DNA during the first-trimester visit and negative results at the third-trimester visit (7.5%), coupled with a large increase of patients with negative results for HPV DNA during the first trimester and positive results at the third trimester (37.5%). The total number of patients with positive HPV DNA results during their third-trimester of pregnancy was 37 of 80 (46.2%). The total number of patients who had positive results for HPV DNA at either or both examinations was 43 of 80 (53.7%). No significant correlation was observed when information concerning the smoking habits (obtained from 64 patients who remained compliant through their third-trimester examination) was compared with the presence of HPV DNA in the patients cervical cells (data not shown). In addition, information concerning the number of sexual partners (obtained from 66 patients compliant through the third trimester) was compared with the detection of HPV DNA in the patients

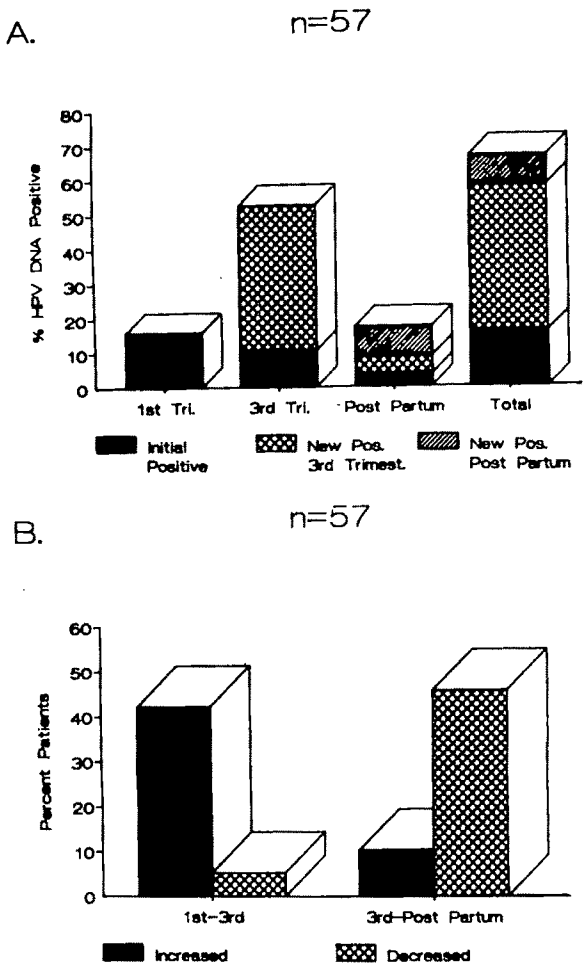


Fig. 3. Graphic representation of HPV DNA analysis for those patients who were compliant through their postpartum examination. The total percentage of patients positive at the three assay points is depicted in the top panel **(A)**. The percentage of patients who changed with respect to the detection of HPV DNA in their cervical cells from first to third trimester or from third trimester to postpartum examinations is depicted in the bottom panel **(B)**.

cervical cells (Table II). No significant difference was observed between patients who reported having one or two sexual partners compared with those who reported more than two partners and the detection of HPV DNA in the patients cervical cells.

Exfoliated cervical cells were obtained from 57 patients who remained compliant through their first postpartum examination (Fig. 3). DNA hybridization results indicate a decrease in the number of patients with detectable HPV infections between the third trimester of pregnancy and the first postpartum examination. The observed decrease was a combination of a small increase (10.2%) of HPV-positive patients between the third trimester and first postpartum examination coupled with a large decrease (45.6%) over this same time period. The total percentage of patients with positive

results at the time of their postpartum examination was 17.5%; 66% of patients had positive results at one of the three assay points.

Comment

Use of DNA hybridization (Southern blot) analysis as a diagnostic tool has confirmed the observations of several recent studies[1, 2, 3] that routine cytologic examination alone will not detect all types of cervical HPV infections. However, neither will DNA hybridization analysis alone detect all HPV infections. A significant number of patients (eight of 110, or 7.2%) with cytologically abnormal features consistent with HPV infection did not have HPV detected by DNA hybridization analysis in the first trimester.

The HPV infection level detected with DNA hybridization criteria alone for samples obtained during the first trimester (23 of 110 or 20.9%) was lower than the infection level of 29% reported for a population that was cytologically normal but referred to a colposcopy clinic,[6] and much higher than the level of 10.5% reported for women with normal cytologic and colposcopic examinations in a sexually transmitted disease clinic.[7] This level was also lower than the level reported by Schneider et al.[8] for a general population of pregnant women (28%). The difference between the first-trimester examination results reported here (Table I) and the 28% reported by Schneider et al.[8] could be a result of differences in the population size, the sensitivities of the different assay techniques used, the nature of the Pennsylvania Hospital clinic population, and the period during pregnancy at which samples were obtained.

Of the 80 patients who were compliant until their third-trimester examination, 37 (46.2%) had positive results for cervical HPV infection and 42 (52.5%) had positive HPV DNA results in one or both of their analyzed samples (Fig. 2). It has been reported that detection of HPV DNA is greater in cervical samples obtained later in pregnancy than in those obtained in the early stages.[8] In addition to the large progression in the number of HPV DNA-positive patients from the first to the third trimester, there was a concomitant decrease of detectable HPV DNA levels (6.2%) in the study population. It is possible that experimental error accounted for some of the samples that changed categories over time, but the number of cases that did change indicates that a certain level of natural fluctuation in viral expression occurs in these patients.

The observed decrease in detection of HPV DNA in the samples obtained from patients during their postpartum examinations (Fig. 3) reflects a difference in detectable levels of HPV DNA in the pregnant and nonpregnant cervical samples. We cannot determine from this study whether the difference in detectable levels of HPV DNA was a result of a higher number of HPV-infected cells during pregnancy or a higher level of viral DNA per infected cell. The increase in detection during pregnancy could represent an increase in viral expression in response to host immunosuppression during pregnancy, or to changes in the hormonal melieu bathing the epithelial cells of the cervix during pregnancy, or both. It has been reported that regulatory elements on the viral genome are responsive to cellular factors[17] and to glucocorticoid hormones in vitro.[14] It also has been observed that β-estradiol can stimulate the growth of experimental condylomas induced with HPV-11 in nude mice (Krieder J, personal communication).

Obvious fluctuations of the HPV status in maternal epithelial cells were noted in this study. The regression and progression patterns, although not well defined, appear to correlate with the natural immunologic and hormonal environment of the pregnant and postpartum patient. The actual percentage of the population infected with anogenital HPVs could be much higher than described in other studies of nonpregnant women. Further studies are needed to elucidate the natural history and true prevalence of anogenital HPV infections.

We thank J. C. Cohn (Pennsylvania Hospital, Department of Orthopedics) and K. Kushner (Pennsylvania Hospital, Department of Pathology) for assistance with data analysis and presentation. We also thank R. Unger (Hospital of the University of Pennsylvania, Department of Obstetrics and Gynecology) for critically reviewing this manuscript, and Microprobe Corp. of Bothell, Wash., for providing HPV-33 and HPV-35 molecular probes.

REFERENCES

1. Centers for Disease Control. *Condylomata acuminata*-United States. MMWR 1983;32:306-8.
2. Meisels A, Fortin R. Condylomatous lesions of the cervix and vagina. I. Acta Cytol 1976;20:505-9.
3. Gissmann L, Schwarz E. Persistence and expression of human papillomavirus DNA in genital cancer. In: Evered D, Clark C, eds. Papillomaviruses. Chichester: Wiley, 1986:190-7.
4. Lorintz AT, Temple GF, Patterson JA, Jenson AB, Kurman RJ, Lancaster WD. Correlation of cellular atypia and human papillomavirus deoxyribonucleic acid sequences in exfoliated cells of the uterine cervix. Obstet Gynecol 1986;68:508-12.
5. De Villiers EM, Schneider A, Miklaw H, et al. Human papillomavirus infections in women with and without abnormal cervical cytology. Lancet 1987;2:703-6.
6. Burk RD, Kadish AS, Calderin S, Romney SL. Human papillomavirus infection of the cervix detected by cervicovaginal lavage and molecular hybridization: correlation with biopsy results and Papanicolaou smear. AM J OBSTET GYNECOL 1986;982-9.
7. Wickenden C, Malcolm ADB, Steele A, et al. Screening for wart virus infection in normal and abnormal cervices by DNA hybridisation of cervical scrapes. Lancet 1987; 1:65-7.
8. Schneider A, Hotz M, Gissmann L. Increased prevalence

of human papillomaviruses in the lower genital tract of pregnant women. Int J Cancer 1987;40:198-201.
9. Sillman FH, Stanek A, Sedlis A, et al. The relationship between human papilloma virus and lower genital intraepithelial neoplasia in immunosuppressed women. Am J Obstet Gynecol 1984;150:300-8.
10. Halpert R, Frichter TG, Sedlis A, et al. Human papillomavirus and lower genital neoplasia in renal transplant patients. Obstet Gynecol 1986;68:251-8.
11. Gary R, Jones R. Relationship between cervical condylomata, pregnancy and subclinical papillomavirus infection. J Reprod Med 1985;30:393-9.
12. World Health Organization. Invasive cervical cancer and combined oral contraceptives. Br Med J 1985;290:961-5.
13. Brinton LA. Current epidemiological studies-emerging hypothesis. In: Peto R, zur Hausen H, eds. Viral etiology of cervical cancer. New York: Cold Spring Harbor Laboratory Press, 1986:17-28.
14. Gloss B, Bernard HU, Seedorf K, Klock G. The upstream regulatory region of the human papillomavirus-16 contains an E2 protein-independent enhancer which is specific for cervical carcinoma cells and regulated by glucocorticoid hormones. EMBO J 1987;6:3735-43.
15. Rando RF, Grodd DE, Chirikjian JG, Lancaster WD. Isolation and characterization of a novel human papillomavirus type 6 DNA from an invasive vulvar carcinoma. J Virol 1986;57:353-6.
16. Maniatis T, Fritsch EF, Sambrook J. Molecular cloning: a laboratory manual. New York: Cold Spring Harbor Laboratory Press, 1982.
17. Guis D, Grossman S, Bedell MA, Laimons LA. Inducible and constitutive enhancer domains in the noncoding region of human papillomavirus type 18. J Virol 1988; 62:665-72.

Mechanism for human papillomavirus transmission at birth

Thomas V. Sedlacek, MD,[a] Steven Lindheim, MD,[a] Catherine Eder, BA,[b] Lisa Hasty, MD,[a] Mark Woodland, MD,[a] Avi Ludomirsky, MD,[a] and Robert F. Rando, PhD[a, b]

Philadephia, Pennsylvania

We attempted to investigate mechanisms, in addition to sexual contact, by which human papillomaviruses associated with anogenital tract lesions could be transmitted. Samples of exfoliated cervical cells were obtained from 45 pregnant women and were assayed by Southern blot hybridization analysis for the presence of human papillomavirus nucleic acids. Twenty-five of the 45 women had cells positive for human papillomavirus deoxyribonucleic acid. A neonatal nasopharyngeal aspirate was obtained at term and analyzed for the presence of human papillomavirus deoxyribonucleic acid. We documented the presence of human papillomavirus deoxyribonucleic acid in the oral pharyngeal cavity of the neonates in 15 of 45 nasopharyngeal samples analyzed. Amniotic fluid was obtained from 13 patients when their membranes were artificially ruptured. These samples were assayed for the presence of human papillomavirus deoxyribonucleic acid; two of the 13 amniotic fluid samples contained human papillomavirus deoxyribonucleic acid. The detection of human papillomavirus deoxyribonucleic acid in the oral cavity of neonates is indicative of a perinatal mechanism of viral transmission. The detection of human papillomavirus deoxyribonucleic acid in the amniotic fluid may suggest an in utero mechanism of transmission. However, problems encountered in collecting the amniotic fluid samples preclude us from definitive interpretation of these data. (Am J Obstet Gynecol 1989;161:55-9.)

Key words: Human papillomavirus (HPV), deoxyribonucleic acid (DNA), uterine cervix, pregnancy, hybridization

Several different human papillomavirus (HPV) types have been identified in benign[1-3] and malignant[4, 5] lesions of the respiratory tract. One of the easiest HPV-induced lesions to identify is laryngeal papillomatosis. Benign laryngeal papillomatosis can lead to hoarseness and progressive enlargement that results in obstruction of the airway.[6] In the majority of cases, papillomatous growths in the respiratory tract can be controlled with a limited number of ablative procedures. However, in some instances the papillomas in the larynx rapidly regrow after apparent complete surgical excision.

In juvenile-onset laryngeal papillomatosis (clinical onset before puberty), 75% of cases are diagnosed before 5 years of age.[6] Several laboratories have established the causative relationship between human papillomaviruses (HPVs) [especially HPV type 6 (HPV-6) and HPV type 11 (HPV-11)] and respiratory papillo-

From the Departments of Obstetrics and Gynecology[a] and Pathology,[b] Pennsylvania Hospital.
Received for publication July 22, 1988; revised October 9, 1988; accepted January 10, 1989.
Reprint requests: Robert F. Rando, PhD, Department of Pathology, Preston Bldg., Rm. 758, Pennsylvania Hospital, 8th and Spruce St., Philadelphia, PA 19107.

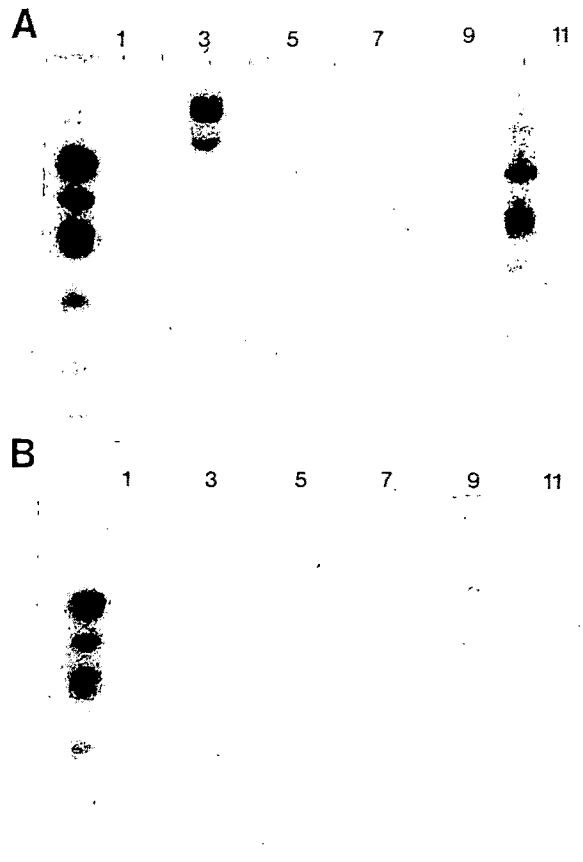


Fig. 1. Detection of HPV-DNA in samples obtained from exfoliated cervical cells, nasopharyngeal aspirates, and amniotic fluid by Southern blot hybridization. DNA was extracted, cleaved with the restriction endonuclease *Pst* I, and transferred onto nylon filter membranes as described in "Material and methods." The filters were hybridized with a mixture of ^{32}P-labeled HPV DNAs. The mixture included HPV types 6,11, 16,18, 31, 33, and 35. Examples of hybridization results obtained for sample DNA (10 to 15 μg) extracted from exfoliated cervical cells (lanes *1* to *5*) and from nasopharyngeal aspirates (lanes *6* to *11*) are shown in the *top panel* **(A).** Lanes *3* and *10* in the *top panel* contain DNA sequences that hybridize with the ^{32}P-labeled molecular probes. The *bottom panel* **(B)** contains total DNA extracted from amniotic fluid (lanes *1* to *11*). Lane *8* in the *bottom panel* contains DNA sequences that hybridize (faintly) with the ^{32}P-labeled molecular probes. The left-hand unmarked lane, in both the top and bottom panels, contains 20 pg of a recombinant HPV-16 genome digested with *Pst* I.

matosis.[7,8] HPV-6 and HPV-11 are also associated with the majority of exophytic genital condylomas.[9] Mounts and Kashima[10] correlated specific subtypes of HPV-6 with extent and severity of disease; HPV-6c was present in all patients with disease extension into the trachea and lungs. It has been reported that HPV genomes can be found in histologically "normal" epithelium adjacent to (in some cases several centimeters away from) the papillomatous lesion.[11,12] This observation may explain the lateral spread of the lesions to previously uninvolved sites, including extension into the trachea and the rapid recurrence in some cases after surgical removal. Factors to help predict a clinical course have yet to be established.

Despite the recent accumulation of information concerning anogenital tract HPV infections, including the prevalence and association with anogenital malignancies.[13] little is known about the natural history of HPV infections. However, alternate mechanisms of transmission may also exist that would explain the fact that the HPV types found in adult genital warts are also found in juvenile-onset laryngeal papillomatosis. The possibility that the HPV infection in patients with juvenile-onset laryngeal papillomatosis is acquired by the newborn during its passage through an HPV-infected birth canal was suggested in 1953 by Webb[14] and again in 1956 by Hajek.[15] A recent study observed that a history of maternal condyloma could be elicited in over 30% of mothers bearing children with these lesions.[16] To date, no prospective study exists to document transmission during the birth process. It is very likely that the virus is transmitted intrapartum as the fetus passes through infected secretions of the birth canal. However, one case of juvenile-onset laryngeal papillomatosis has been documented from a nonruptured elective cesarean section, suggesting that additional mechanisms of transmission may exist.[10]

A prospective study was undertaken at the obstetric clinic of Pennsylvania Hospital to elucidate mechanisms by which neonates can become infected with HPV, and to give further insight into the natural history of this virus.

Material and methods

From April 1987 through August 1987 all new prenatal patients at Pennsylvania Hospital's obstetric clinic were solicited for and subsequently enrolled in this study. The population of women was young (mean age = 22.4 years), sexually active, and from a low socioeconomic group. During their initial first-trimester visit, a sample of exfoliated cervical cells was obtained for cytologic and deoxyribonucleic acid (DNA) hybridization analysis. During the third-trimester visit (between 28 and 34 weeks), a second specimen of exfoliated cervical cells was obtained for DNA hybridization analysis.

Patients with intact membranes who were seen in the labor and delivery suite in active labor had elective artificial rupture of the membranes with an 18-gauge needle. Betadine was used to cleanse the vulva, vagina, and cervix. One patient, who underwent an amniocentesis for fetal maturity at 38 weeks before a repeat cesarean section, had 1 ml of amniotic fluid saved for DNA hybridization analysis.

After delivery nasopharyngeal aspirates from all neo-

Table I. Distribution of HPV types detected in maternal cervical cells

	HPV types			
	6/11	*16/18*	*31/33/35*	*Other*
No. detected (n = 25)	0	12	4	9

nates were obtained with a DeLee suction device. The catheter was placed in the oral pharynx to the level of the nasopharynx and then inserted into the nares. The specimens (3 to 5 ml) obtained were analyzed for the presence of HPV DNA.

Patients were excluded from the study if we were unable to obtain matched samples from the maternal cervical cells and from the neonatal nasopharyngeal aspirate.

Nucleic acid extraction. Exfoliated cervical cells were resuspended in phosphate-buffered saline solution, concentrated by low-speed centrifugation (1500 rpm), and resuspended in 1 ml of cell lysis buffer (10 mmol/L Tris hydrochloride, pH 8.0, 0.6% sodium dodecyl sulfate, and 10 mmol/L ethylenediaminetetraacetic acid). Proteinase K (50 μg/ml) and ribonuclease A (10 μg/ml) were added to the cell lysis buffer and the cell samples were incubated for 2 hours at 37° C. DNA was purified by extracting the samples twice with an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1). The extracted nucleic acids were then precipitated by adjusting the salt concentration to 0.3 mol/L sodium acetate and adding 2.5 volumes of cold absolute alcohol. The nucleic acids were concentrated by centrifugation (11,000 rpm), washed twice with 70% ethanol, dried under vacuum, and resuspended in 1 ml of TE buffer [10 mM Tris hydrochloride (pH 7.5) and 1 mmol/L ethylenediaminetetraacetic acid]. The DNA concentration was estimated by monitoring the absorbance at 260 nm with a Beckman DU-7 spectrophotometer.

DNA was purified from nasopharyngeal aspirates and samples of artificially ruptured membranes by directly extracting these samples with an equal volume of phenol/chloroform/isoamyl alcohol. The DNA was then precipitated in ethanol and resuspended in TE buffer. The amount of DNA obtained from these samples was usually small and the entire sample was used in the hybridization analysis.

Hybridization analysis. DNA (10 to 15 μg) extracted from cervical samples and the total DNA extracted from artificially ruptured membranes and nasopharyngeal specimens was cleaved with the restriction endonuclease *Pst* I as recommended by the manufacturer (New England Biolabs). The resulting DNA fragments were fractionated by electrophoresis in a 0.8% agarose gel and then transferred to a nylon filter membrane (Amersham Hybond-N). DNA hybridization analysis was performed at −20° C with a modification of the Southern blot technique.[17] Molecular probes corresponding to HPV types 6, 11, 16, 18, 31, 33, and 35 were amplified and purified with standard laboratory methods. The HPV genomes were purified from flanking bacterial plasmid DNA and then radiolabeled with α ^{32}P-deoxycytidine triphosphate. Hybridization analysis of DNA extracted from exfoliated cervical cells was performed in triplicate. The molecular probes used in the three hybridization reactions were HPV-16/18, HPV-31/33/35, or HPV-6/11.

Table II. Comparison of nucleic acid hybridization analysis of DNA extracted from maternal cervical cells and neonatal nasopharyngeal aspirates

	*Mother**			
	HPV DNA pos.		*HPV DNA neg.*	
Neonate†	*SVD*	*Cesarean section*	*SVD*	*Cesarean section*
HPV DNA neg.	12	2	14	2
HPV DNA pos.	11	0	4	0
Total samples	23	2	18	2

SVD, Spontaneous vaginal delivery.

*Mothers were considered to have positive results if either their first- or third-trimester sample of cervical cells was positive for HPV DNA.

†Nasopharyngeal samples were all obtained with the DeLee suction device. Samples were obtained after spontaneous vaginal delivery or cesarean section.

Results

Samples of exfoliated cervical cells obtained from 45 pregnant women during their first- and third-trimester examinations were analyzed for the presence of HPV DNA. Comparison of the DNA hybridization results from cervical samples obtained during the patients' first- and third-trimester examinations revealed both a progression (in some patients) and a regression (in other patients) in the detectable levels of HPV DNA (see accompanying article, pp. 50-5). The total number of patients with results positive for HPV DNA in one or both of the cervical samples was 24/45 (55.5%). The cervical DNA samples were hybridized with three different groups of HPV molecular probes. The distribution of viral types found in the cervical samples is shown in Table I.

At the time of delivery, the neonatal nasopharyngeal aspirate was obtained for this study. Forty-one of the 45 aspirate samples were obtained from neonates after spontaneous vaginal delivery and four were obtained from infants who were delivered by cesarean section.

Table III. Analysis of amniotic fluid for presence of HPV DNA

	*Mother**			
	HPV DNA pos.		*HPV DNA neg.*	
Amniotic fluid	*SVD*	*Cesarean section*	*SVD*	*Cesarean section*
HPV DNA neg.	8	1	3	0
HPV DNA pos.	2	0	0	0
Total samples	10	1	3	0

SVD, Spontaneous vaginal delivery.

*Mothers were considered to have positive results if either their first- or third-trimester sample of cervical cells was positive for HPV DNA.

The nucleic acids were extracted from these samples and assayed for the presence of HPV DNA (Fig. 1). The hybridization results have been compared with the hybridization results obtained from the respective mothers (Table II).

HPV DNA was detected in 15 of the 41 (36.5%) nasopharyngeal samples obtained from babies who were delivered vaginally. No viral DNA was detected in the four samples obtained from babies delivered by cesarean section. Four nasopharyngeal samples that were positive for HPV DNA were from babies whose mothers' first- and third-trimester examination results were negative for HPV DNA. One of these mothers had cytologically positive results for HPV (condylomatous atypia) during her first-trimester examination; two mothers' results were positive for HPV DNA in a sample of exfoliating cervical cells obtained during her first postpartum examination (see accompanying article pp. 50-5).

A sample of amniotic fluid from artificially ruptured membranes was obtained from 13 mothers. Total DNA was extracted from these samples and analyzed for the presence of HPV DNA (Table III). One sample of amniotic fluid was obtained from a patient undergoing elective cesarean section. The two samples from artificially ruptured membranes that were positive for HPV DNA were obtained from patients who also had positive results for HPV DNA in their cervical cells.

Comment

We have demonstrated the presence of HPV DNA in the oral cavity in 11 of the 23 (47.8%) children who were delivered vaginally from mothers who had detectable levels of HPV DNA in cervical cells. The presence of viral DNA does not in itself imply the presence of infectious virus, but it does demonstrate a mechanism by which the virus could be transmitted at the time of birth. The commercially available polyclonal antibody which recognizes papillomavirus capsid protein should be used, in complementary experiments, to detect HPV capsid protein in the nasopharyngeal aspirate. In addition, this study did not make use of a matched nonpregnant control group; such a control group should be part of any subsequent follow-up study.

It is difficult to explain the four nasopharyngeal aspirates that were positive for HPV DNA when maternal cervical cells were negative for HPV DNA. However, as noted, three of the four mothers had either cytologic evidence of HPV infection or detection of HPV DNA in cervical samples obtained post partum. The observed fluctuations of detectable HPV DNA in exfoliated cervical cells (see accompanying article pp. 50-5) may explain the last case, in which HPV DNA was detected in the neonate but not in the mother.

The two positive samples from artificially ruptured membranes could have resulted from contamination of the amniotic fluid by vaginal secretions during the sample collection. However, a recent report[18] described HPV DNA associated with foreskins obtained within 3 days of birth from infants who had routine circumcision. The authors of this report argue that the HPV DNA was the result of a viral infection instead of residual HPV DNA from the maternal vaginal secretions at the time of birth. This explanation would suggest that the HPV infection of the foreskin occurred in utero. The presence of HPV DNA in the amniotic fluid also suggests that transmission of the virus can occur in utero. The small number of NAROM samples and the probability of contamination of these samples with cervical or vaginal secretions prevent us from making a definitive interpretation of these data. To alleviate the problem of contamination of these samples, this part of the study should be repeated with amniotic fluid collected transabdominally.

A number of HPV types in addition to HPV-6 and HPV-11 have been found in oral lesions, including HPV-13,[1] HPV-16,[2,3] and HPV-32,[19] and in laryngeal carcinomas, HPV-30.[4] In our study, 12 of the 25 (48%) HPV DNA-positive cervical samples contained HPV types 16 or 18 in their cervical cells; nine of the 25 (36%) were of an undetermined HPV type. Therefore any HPV type that demonstrates a tissue specificity for oral and anogenital mucosa can be transmitted from mother to neonate at birth.

No cervical sample used in this study contained HPV-6 or HPV-11 DNA, and no attempt was made to determine the HPV types associated with the nasopharyngeal samples. No biopsies were performed on vaginal or vulvar epithelial tissue. If the population size was increased, and it included patients with exophytic condyloma of the vulva or vagina (which usually contain HPV types 6 or 11[7]), then the probabilty of detecting these viral types in the nasopharyngeal aspirate would increase. In juvenile oral lesions, HPV-6 and HPV-11

types are associated with juvenile-onset laryngeal papillomatosis, a rare but potentially dangerous disorder. The results from this study define a perinatal transmission of HPVs for the first time. Therefore children born to mothers with overt condyloma may warrant DNA hybridization analysis of their nasopharyngeal aspirates. The information obtained may help in subsequent detection and management of children with juvenile-onset laryngeal papillomatosis.

We thank J. Cohn (Department of Orthopedics, Pennsylvania Hospital) and K. Kushner (Department of Pathology, Pennsylvania Hospital) for expert help in data presentation. We also thank R. Unger (Hospital of the University of Pennsylvania, Department of Obstetrics and Gynecology) for critically reviewing this manuscript, and Microprobe Corp. of Bothell, Wash., for providing HPV-33 and HPV-35 molecular probes.

REFERENCES

1. Pfister H, Hettich I, Runne U, Gissmann L, Chilf GN. Characterization of human papillomavirus 13 from focal epithelial hyperplasia Heck lesions. J Virol 1983;47: 363-6.
2. Loning T, Ikenberg H, Becker J, Gissmann L, Hoepfner I, zur Hausen H. Analysis of oral papillomas, leukoplakias and invasive carcinomas for human papillomavirus type related DNA. J Invest Dermatol 1985;84:417-20.
3. Naghashfar Z, Sawada E, Kutcher MJ, et al. Identification of genital tract papillomaviruses HPV-6 and HPV-16 in warts of the oral cavity. J Med Virol 1985;17:313-24.
4. Kahn T, Schwarz E, zur Hausen H. Molecular cloning and characterization of the DNA of a new human papillomavirus (HPV-30) from a laryngeal carcinoma. Int J Cancer 1986;37:61-5.
5. Byrne JC, Tsao MS, Fraser RS, Howley PM. Human papillomavirus-11 DNA in a patient with chronic laryngotracheobronchial papillomatosis and metastatic squamous-cell carcinoma of the lung. N Engl J Med 1987;317:873-8.
6. Kashima HK, Shah K. Recurrent respiratory papillomatosis: clinical overview and management principles. In: Reid R, ed. Obstetrics and gynecology clinics of North America: human papillomaviruses. Philadelphia: WB Saunders, 1987;14:581-3.
7. Brondbo K, Alberti PW, Crowson N. Adult recurrent multiple laryngeal papilloma: laser management and socioeconomic effects. Acta Otolaryngol (Stockh) 1983;95:37-39.
8. Mounts P, Shah KV, Kashima H. Viral etiology of juvenile and adult-onset squamous papilloma of the larynx. Proc Natl Acad Sci USA 1982;79:5425-9.
9. Gissmann L, Wolnik L, Idenberg H, et al. Human papillomaviruses 6 and 11 DNA sequences in genital and laryngeal papillomas and in some cervical cancers. Proc Natl Acad Sci USA 1983;80:560-3.
10. Mounts P, Kashima H. Association of human papillomavirus subtype and clinical course in respiratory papillomatosis. Laryngoscope 1984;94:28-33.
11. Ferenczy A, Mitao M, Nagai N, Silverstein SJ, Crum CP. Latent papillomavirus and recurring genital warts. N Engl J Med 1985;313:784-8.
12. Steinberg BM, Topp WC, Schneider PS, et al. Laryngeal papillomavirus infection during clinical remission. N Engl J Med 1983;308:1261-4.
13. Gissmann L, Schwarz E. Persistence and expression of human papillomavirus DNA in genital cancer. In: Evered D, Clark C, eds. Papillomaviruses. Chichester: Wiley, 1986:190-7.
14. Webb WW. Papillomata of the larynx. Laryngoscope 1956; 66:871-918.
15. Hajek EF. Contribution to the etiology of laryngeal papilloma in children. J Laryngol Otol 1956;70:166-8.
16. Quick CA, Kryzyzek RA, Watts SL, et al. Relationship between condylomata and laryngeal papillomata. Ann Otol 1980;89:467-71.
17. Rando RF, Groff DE, Chirikjian JG, Lancaster WD. Isolation and characterization of a novel human papillomavirus type 6 DNA from an invasive vulvar carcinoma. J Virol 1986;57:353-6.
18. Roman A, Fife K. Human papillomavirus DNA associated with foreskins of normal newborns. J Infect Dis 1986; 153:855-61.
19. Beaudenon S, Praetorius F, Kremsdorf D, et al. A new type of human papillomavirus associated with oral focal epithelial hyperplasia. J Invest Dermatol 1987;88:130-5.

A ten-year experience treating cervical intraepithelial neoplasia with the CO_2 laser

Michael S. Baggish, MD, James H. Dorsey, MD, and Mark Adelson, MD
Syracuse, New York, and Baltimore, Maryland

Between 1977 and 1987, the CO_2 laser was used to treat 4024 women who were diagnosed by cytology studies, colposcopy, and biopsy examination to have cervical intraepithelial neoplasia. On the basis of specific indications, these women were treated by vaporization, excisional conization, or combination conization procedure. The evolution of methodology for each laser technique was based on specific pathophysiology and long-term observation of results. A total of 3070 patients underwent vaporization, 2881 of whom had no evidence of disease after one laser exposure (93.8%). Of the 954 patients who had laser excisional procedures, 925 (97%) had no evidence of disease. The most common complications observed were intraoperative and postoperative bleeding; however, sutures were required in only 1.34% of the latter. The risks of cervical stenosis (1.1%), incompetent cervix (0%), and pelvic infection (0.05%) were very low. This 10-year study demonstrated that the CO_2 laser was a highly effective method for treating cervical epithelial neoplasia and had the added benefit of preserving reproductive function and anatomic integrity. (Am J Obstet Gynecol 1989;161:60-8.)

Key words: Laser, CO_2 laser, cervical epithelial neoplasia, cervical neoplasia

The early diagnosis and subsequent treatment of preinvasive neoplasia of the cervix result in a greatly diminished risk of invasive disease and preservation of cervical function and anatomy. Numerous radical and conservative therapeutic techniques have been advocated during the past 30 years; however, in recent years the trend has been directed toward outpatient methodology associated with local or no anesthesia. Although tissue-conserving treatment programs have both advocates and detractors, the question of equivalent efficacy remains a point of controversial contention. Townsend et al.[1] showed that inaccurate pretreatment assessment invariably leads to faulty diagnosis, improper selection of therapy, and subsequent discovery of invasive carcinoma.

The evolution of conservative therapy began in the 1950s and 1960s with sharp conization, continued with cryosurgery during the late 1960s and throughout the 1970s, and culminated with the advent of CO_2 laser surgery in the past decade. Initially, the laser appeared to have little advantage over less complex, albeit less precise, methodology. However, as time passed and experience with the new modality was gained, therapeutic results sharply improved.

The purpose of this article is to provide data based on one of the largest case series ever assembled that used the CO_2 laser as the singular therapeutic entity and present follow-up results over a 10-year time period.

From the State University of New York Health Sciences Center, Syracuse, Crouse-Irving Memorial Hospital, and Greater Baltimore Medical Center.
Received for publication September 15, 1988; revised December 15, 1988; accepted January 10, 1989.
Reprint requests: Michael S. Baggish, MD, Department of Obstetrics and Gynecology, Crouse-Irving Memorial Hospital, 736 Irving Ave., Suite 308 West Tower, Syracuse, NY 13210.

Material and methods

Between 1977 and 1987, 3070 women underwent CO_2 laser vaporization and were followed-up for a minimum of 1 year. Every woman had a confirmed histopathologic diagnosis of cervical intraepithelial neoplasia ranging in grade from 1 to 3; 954 patients similarly diagnosed with cervical epithelial neoplasia 1 to 3 had laser excisional or combination conization from 1979 to 1987. The minimum follow-up period was 1 year (321 patients); 1104 women were followed by ≥1 to 5 years and 2599 patients were followed ≥5 to 10 years. All laser procedures were carried out at one of four hospitals: Crouse-Irving Memorial Hospital, Syracuse, N.Y.; Greater Baltimore Medical Center, Baltimore, Md.; Mount Sinai Hospital, Hartford, Conn.; State University of New York Health Science Center—University Hospital, Syracuse, N.Y. Of the 4024 patients, 3098 were white, 845 were black, and 81 were Hispanic or Oriental. Patient ages ranged from 14 to 81 years (mean age, 26.2 years).

Patients were referred either from colposcopy clinics, private offices, or general gynecology clinics because of abnormal cytologic studies or less commonly after a biopsy diagnosis of cervical epithelial neoplasia. Every patient underwent a repeat colposcopic examination and one or more colposcopically directed biopsies. The endocervical canal was investigated by either blind endocervical curettage or endoscopic examination of the

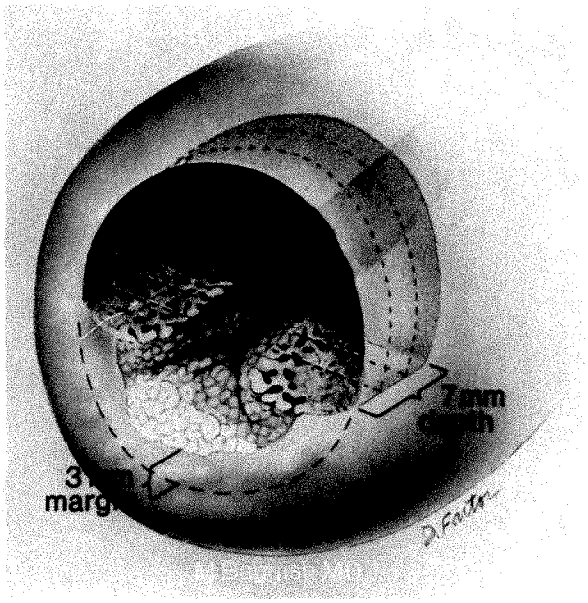


Fig. 1. Three-dimensional view of cervix reveals an abnormal transformation zone (white epithelium and mosaic) at 6-o'clock position. Laser vaporization is performed by cleaving plane on plane of tissue until a depth of 7 mm is reached. Note projection of endocervical clefts after the upper one half of the cervix has been vaporized. The 3 mm margin is designed to take into account possible penetration of neoplasia into the underlying crypts. Projection of the endocervical canal toward the internal os is seen as the shaded area.

canal followed by directed endocervical biopsy. The latter procedure was carried out by means of a small endoscope that was inserted into the cervical canal after swabbing or irrigating the mucosa with 4% acetic acid. Lesions were schematically mapped, and the geographic extent was sketched, with specific notation made of any endocervical or vaginal extension.

Additionally cultures were obtained for the determination of gonorrhea and *Chlamydia* beginning in 1983. Women with positive cultures were treated with a 2 gm injection of spectinomycin or 500 mg of tetracycline (or equivalent) orally administered four times per day for 10 days, respectively.

Forty percent of laser vaporizations were performed in the office, whereas 1873 patients (60%) underwent surgery in the outpatient operation room. Fifty laser conizations have now been done in the office under local anesthesia; the remainder were performed in the outpatient operating room.

Patients undergoing laser vaporization in the office received ibuprofen (Motrin), 400 to 600 mg, 30 minutes before vaporizaton. All patients received suitable informed consent and were previously given an informational booklet describing the details of laser treatment and postoperative routines.

In this series 1520 patients had no anesthesia for vaporization, 1111 patients had local anesthesia, and

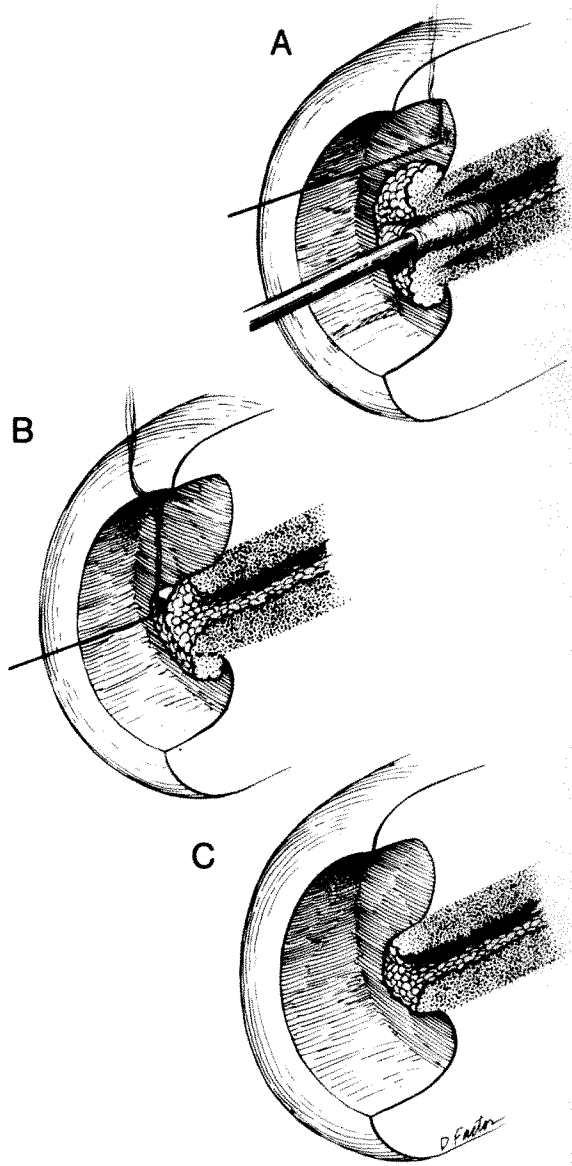


Fig. 2. Cervical vaporization is finished off by setting the location of the new external os. This is accomplished by vaporizing a 2 mm trench just peripheral to the endocervical margin and is facilitated by placing a moistened cotton-tipped applicator into the canal *(A)*. Next, the everted mucosa is vaporized away (clipping) *(B)*, resulting in ablation of addition 2 to 3 mm of tissue *(C)*.

439 patients had general anesthesia. For laser excisional conization 304 patients had local anesthesia and 650 underwent general anesthesia.

Several types of CO_2 laser equipment were used during the years patients were treated, including (1) Coherent 400, (2) Cavitron A0300, (3) Cooper Lasersonics 500Z, (4) Sharplan 733 and 1100, (5) Xanar XA50, and (6) Minnesota laser, Portalase 200. Current laser equipment used for the treatment of cervical intraepithelial neoplasia has a variable spot feature incorporated in the construction of the micromanipulator, a microscope

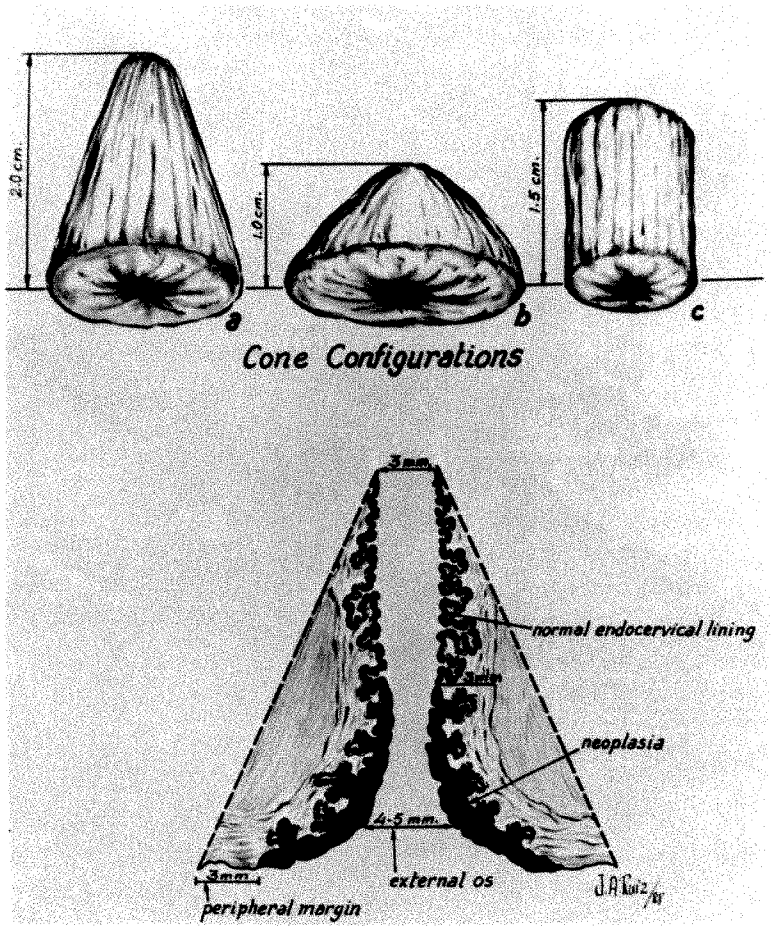


Fig. 3. Geometry of excisional cervical specimens depends on characteristics of geography of neoplasia. *a*, Most common configuration of a laser excisional cone. Base measures about 1.5 to 2 cm and height measures 2 cm. *b*, Broad-based cone measuring 2 to 2.3 cm with a height of approximately 1 to 1.2 cm. *c*, Narrow cylinder made in conjunction with peripheral vaporization (laser combination cone) measures about 1 by 1.5 cm.

Table I. Criteria for performance of laser vaporization and conization

Laser vaporization (all must be satisfied)	*Laser conization (any one)*
Lesion must be ectocervical in location	Lesion extends into endocervical canal
Lesion must be proved by biopsy examination to be intraepithelial	Directed biopsy specimen suggests possible stromal invasion
Endocervical canal must be established to be free of disease by negative endocervical curettage or negative endoscopic examination of canal	Endocervical sampling reveals neoplastic tissue within canal
	Papanicolau smear/biopsy disparity exists*

*A thorough vaginal colposcopic examination has been performed.

with multiple magnification settings, and superpulse capability. Although several micromanipulators permit spot (beam diameter) reduction to 0.2 to 0.3 mm at 10 to 20 W, practically minimum spots delivered at 30 to 40 W of power ranged from 0.4 to 0.5 mm diameter. First-generation lasers produced fixed spots of 1.5 to 2.0 mm diameter. Power capabilities ranged from 1 to 100 W.

Technique for vaporization. Table I lists the criteria for laser vaporization. With a power density of approximately 500 to 800 W/cm^2 (20 W with 1.5 to 2 mm spot), a 3 mm margin was traced along the peripheral extent of the abnormal transformation zone. Laser power was turned up to 30 to 60 W depending on the equipment used, and vaporization was carried out in 1 to 2 mm planes that used a small circular technique by

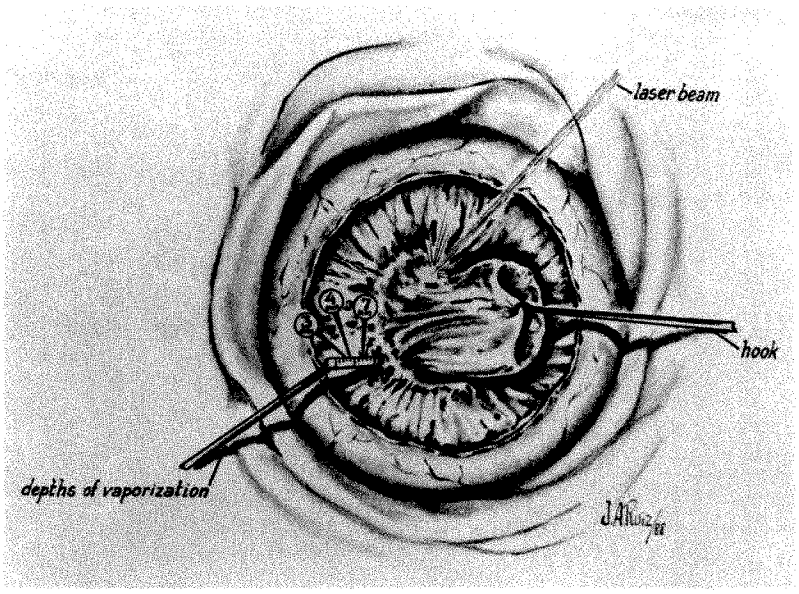


Fig. 4. Easiest method for performing a combination cone is to first vaporize peripheral extent of biopsy that established intraepithelial neoplasia. Next, central core of cervix is excised to a depth of approximately 1.5 to 2.0 cm. Since half the surrounding tissue (7 mm) has already been vaporized, excisional part of operation may be quickly completed.

manipulating the joy stick in a clockwise or counterclockwise motion. Planes were cleaved until a central depth of 7 mm was reached (Fig. 1). Depth was measured by use of a measuring rod device and by relating to the surrounding surface epithelium of the cervix. Char material was swabbed out of the crater by means of large cotton swabs soaked with 4% acetic acid. Next, a small cotton-tipped applicator was inserted into the endocervical canal. Laser power was reduced to 15 to 20 W, and the laser spot was decreased to approximately 1 to 1.5 mm spot diameter. The collagenous stroma in juxtaposition to the endocervix was shallowly vaporized, causing the endocervical mucosa to evert (buttoning); next, 2 to 3 mm more of the endocervix was narrowly vaporized (clipping) (Fig. 2). Thus the margin of endocervix could be placed wherever the operator desired; generally the squamocolumnar junction was located just inside the anatomic external os. The net result was a vaporization cone approximately 9 mm in depth that was narrower at the apex and widest at the ectocervical margin.

Technique for excisonal conization. Cones were always performed by means of the microscope and micromanipulator. The geometric shape of the excision cone specimen was governed by the extent of the abnormal transformation zone. Table I lists the expanded criteria for performing excisional conizations. A thorough colposcopic examination was performed before conization. After the abnormal transformation zone was identified, utilizing power densities of 800 W/cm^2, 1 to 1.5 mm trace spots were placed into the cervix with no anesthesia, allowing a 3 mm margin peripheral to the abnormal tissue. Next, a 1:30 vasopressin local anesthetic (1% lidocaine) mixture was injected directly and shallowly into the substance of the cervix under pressure. No stay sutures whatsoever were placed into the cervix. Fig. 3 shows several cone configurations used during this study. With the help of a specially designed 10-inch titanium laser hook, the tissue was manipulated to achieve the appropriate sculpted effect.

Power was turned up to 30 to 60 W if available on the laser equipment; however, cones could be performed effectively at 20 W. Spots were adjusted to 0.3 to 0.5 mm. An incision was made by moving the micromanipulator slowly connecting the trace spots. This initial incision usually penetrated 5 to 7 mm into the depths of the cervical connective tissue. The endocervical margin was cut off with a sharp scalpel or a superpulsed laser beam.

Technique for combination conization. When cervical intraepithelial neoplasia extended far out onto the portio involving three to four quadrants or spread onto the vaginal mucosa and also involved the endocervical canal, a narrow excisional cone, that is, 1 cm in diameter, was combined with ectocervical vaporization.

Method 1. The ectocervical (vaporization) margin and cone margin were traced with laser impact spots. Power was adjusted to 20 to 60 W, depending on the equipment; the spot increased to 2 mm diameter and vaporization was carried to a depth of 7 mm, banking off to 4 mm on the farther periphery of the ectocervix, then to just under 1 mm as the vagina was approached.

The laser hook was placed into the margin of the

Table II. CO_2 laser vaporization (N = 3070)

CIN	*N*	*NED (%)*	*Persistence (%)*	*Recurrence (%)*	*Progression (%)*
Grade 1	741	675 (91.1)	25 (3.4)	41 (5.5)	0
Grade 2	1048	978 (93.3)	63 (6.0)	6 (0.6)	1 (0.1)
Grade 3	1281	1228 (95.9)	45 (3.5)	7 (0.5)	1 (0.1)

CIN, Cervical intraepithelial neoplasia.
NED, No evidence of disease.

Table III. CO_2 laser excisional and combination cones (954) (follow-up: 12 to 96 months)

CIN	*N*	*Negative margin*	*Positive margin*	*NED (%)*	*Invasive*
Grade 1	48	48	0	48 (100)	0
Grade 2	301	285	16*	290 (96.3)	0
Grade 3	605	548	57†	587 (97)	4‡

CIN, Cervical intraepithelial neoplasia.
NED, No evidence of disease.
*Three patients developed recurrence.
†Eleven patients had persistent or recurrent disease; one patient lost to follow-up returned 3 years later with invasive disease.
‡One patient had prior conization and was lost to follow-up for 3 years; three patients had diagnosis of invasion at time of conization.

cervical remnant to be excised, usually beginning at the 12-o'clock position. Essentially 7 mm of the 1.5 cm portion of the central remanant of the cervix had already been removed (Fig. 4). A small excisional conization was carried out. The coned specimen measured approximately 1 by 1.5 cm. Obviously, the ectocervical margin of this narrow cone will always show cervical epithelial neoplasia.

Method 2. The two series of trace spots were placed into the cervix as described in method 1. In this instance the laser excisional conization was performed by first removing the 1 by 1.5 cm cylindric specimen. Next, the peripheral vaporization was carried out. The operative site was finished off in the same way as previously described (method 1).

Results

Vaporization. Of the 3070 women who underwent a single laser vaporization, 2881 (94%) showed no evidence of disease after at least a 1-year follow-up period. Interestingly, the best results were obtained in the 1281 women diagnosed with grade 3 cervical intraepithelial neoplasia (no evidence of disease, 96%). Table II provides details of these treatment results by grade of disease. Persistence was seen in all grades of cervical intraepithelial neoplasia and totaled 133 women (4.3%); 127 of the 133 patients (95%) with persistent disease had condylomatous disease with or without atypia; only six cases of true cervical epithelial neoplasia were diagnosed. Recurrence was observed almost exclusively in the grade 1 cervical intraepithelial neoplasia group and always took the form of flat condyloma. Progression was identified in only two patients (0.065%).

Excisional conization. Seventy-three women who underwent laser excisional conization had disease that extended to the margin or had an indeterminate margin, and 881 (93.4%) had negative margins (Tables III and IV). Four cases of invasive carcinoma, one case of microinvasive epidermoid, two cases of epidermoid, and one case of adenocarcinoma were diagnosed after section and pathologic evaluation of the cone specimen. The one case of epidermoid carcinoma was diagnosed in a patient who had positive margins (initial diagnosis was grade 3 cervical intraepithelial neoplasia with positive endocervical margin) and did not return for follow-up examination for 3 years. She subsequently appeared at a neighboring clinic because of vaginal bleeding and was referred back to the colposcopy clinic where a directed biopsy specimen showed invasive epidermoid carcinoma. Clinical staging of these invasive cases revealed the last case to be stage IIA; the remaining two epidermoid carcinomas were stages IA and IB; the adenocarcinoma was a very superficially invasive ("microinvasive") lesion with no vascular involvement. Follow-up of the remaining 69 cases with positive margins revealed that 44 had no evidence of disease 3 to 7 years after the initial operation. Twenty-five women had persistent or recurrent abnormal cytologic studies, which prompted subsequent biopsy and repetitive therapy. Thirteen women had repeat conization: four showed no disease on the sectioned specimen, three showed condylomatous atypia; one showed grade 2 cervical intraepithelial neoplasia; and five showed grade 3 cervical intraepithelial neoplasia. One cone again showed indistinct margins, and the patient continues to be followed carefully by colposcopy and

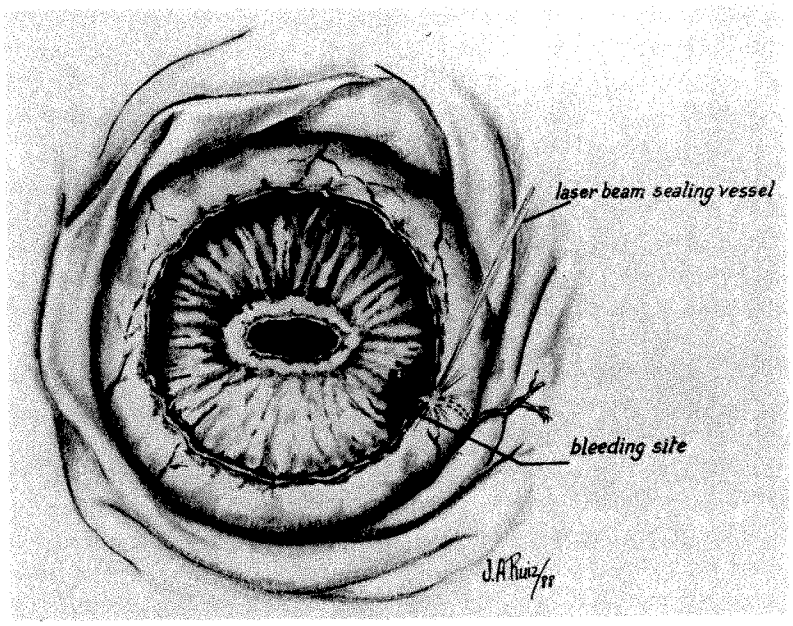


Fig. 5. When intraoperative bleeding occurs during laser surgery of the cervix, the easiest manner of dealing with the problem is to coagulate the vessel before it reaches the crater. The laser beam is aimed peripherally from the bleeding site, laser spot is enlarged (power density reduced), and coagulation of the traversing vessel is accomplished.

cytologic studies. The 12 women who had hysterectomy showed residual cervical intraepithelial neoplasia: grade 1, one case; grade 2, one case; grade 3, eight cases; and no disease in two cases (Table IV).

In the final analysis, 954 women fulfilled the criteria for laser excisional conization, and 925 (97%) had no evidence of persistent or recurrent disease during follow-up examination of up to 8 years' duration (Table V).

Complications. Bleeding was the most common complication observed during vaporization and excisional procedures. The most frequent sites for intraoperative bleeding during vaporization were the two posterior quadrants of the cervix and was most frequently observed in cervices showing histologic evidence of acute cervicitis and neoplasia. Light bleeding was easily controlled by defocusing the laser beam to a >2 mm spot, thus coagulating the vessel peripheral to the crater and sealing it off before its entrance into the crater (Fig. 5). Arterial pumping hemorrhage was more commonly (3.5%) associated with laser conization procedures but also was seen during vaporization (3%). Suturing the vessel under colposcopic guidance with 3-0 Vicryl in a figure-of-eight fashion proved to be the simplest and most direct approach.

Delayed bleeding was most frequently observed after laser vaporization. This problem was usually self-limited and occurred 4 to 10 days after vaporization. In 170 cases this bleeding persisted more than 48 hours (vaporization group). Of the 170 cases, 142 were controlled by the application of Monsel's solution to the bleeding vessel. In 38 cases the placement of one or two sutures was required. Twenty-five patients continued to have light bleeding for 3 to 4 weeks. In this subset healing occurred without interruption, and the bleeding eventually stopped spontaneously.

Table IV. Seventy-three cases of laser conization with positive margins

Invasive disease	4
No further treatment	44
Repeat conization	13
Hysterectomy	12

Postexcisional conization bleeding was seen less frequently than after vaporization. In 31 cases bleeding continued unabated after 7 to 10 days. In 16 cases sutures were required; 15 cases responded to laser coagulation or the application of Monsel's solution. Comparing significant delayed laser bleeding by methodology, the relative risk after vaporization was 1.7 times greater than that after conization.

Two cases of pelvic infection were observed in the entire series. One woman was observed to have a fever of 102° F 48 hours after laser combination cone. On examination she had a purulent cervical discharge and abdominal pain, together with direct and rebound tenderness. The patient was admitted and treated with intravenous antibiotics and discharged home within 4 days after admission. The second patient developed a

general febrile reaction within 2 hours of the laser vaporization. A temperature of 101.8° F was associated with shaking chills. The patient was admitted and treated with intravenous antibiotics. By 8:00 PM on the evening of surgery, she was afebrile. Blood cultures subsequently revealed β-hemolytic streptococcus. She was sent home after 24 hours on oral antibiotics.

Pain was not a significant factor after surgery.

A pink or watery blood-tinged discharge peaked at 72 to 96 hours after laser vaporization. The discharge gradually diminished over the next 7 days and disappeared. Blood-tinged discharge lasted as long as 2 weeks after laser excisional procedures. A small number of patients (≤0.5%) had discharge persisting beyond 3 weeks.

No case of an incompetent cervix was seen.

Cervical stenosis, which was defined as scarring and narrowing of the endocervical canal such that the opening of the canal measured <2 mm in diameter, was observed in 15 cases after the combination conization. In addition, 24 cases were seen after simple excisional conization. Stenosis after vaporization was uncommon and was noted in only six cases.

Comment

The treatment of cervical disease was one of the earliest applications of laser technology in gynecology. Between 1977 and 1987 numerous articles were published in the peer-review literature citing experience with the CO_2 laser as a method for eradicating cervical intraepithelial neoplasia.[2-9] Early treatment programs attempted to focally treat cervical intraepithelial neoplasia, penetrated to depths of only 2 to 3 mm, failed to consider peripheral margins, vaporized disease in the endocervical canal, and used low-power densities. On the basis of the early work of Jordan and Mylotte,[10] Baggish,[11] Masterson et al.,[12] Bellina et al.,[13] and Anderson,[14] the entire transformation zone was first vaporized to a depth of 5 mm and then later to 7 mm. A peripheral margin of 3 mm was established to take into account the deep penetration of neoplastic cells into the endocervical crypts to a depth of 2.92 mm (95% of cases samples).[15] Burke[16] substantively demonstrated that the treatment results improved when endocervical extension of cervical epithelial neoplasia was excluded from treatment by vaporization.

The data presented here represent the pinnacle of 10 years of evolution and the development of CO_2 laser vaporization into a fine art. Few conservative techniques can match the results of laser ablation of cervical epithelial neoplasia, considering that the disease can be treated in the office, the method requires no anesthesia or only local anesthetic injection, and no selection is made for grade of cervical intraepithelial neoplasia or extent of geographic spread. The final product of this precise technique is, in fact, equivalent to a shallow conization procedure.

The buttoning (eversion of endocervical margin) technique was performed between 1979 and 1983 and was originally conceived because of the fear of cervical stenosis. As cases were followed over the years, the risk of stenosis proved to be small; however, many women developed prolapse of the endocervical mucosa.[17] This endocervical protrusion resulted in vaginal discharge and contact bleeding. Since 1983 the everted endocervical mucosa was clipped, that is, precisely vaporized to a further depth of approximately 3 mm. The end result was the creation of a conical crater approaching 1 cm in depth with the subsequent squamocolumnar junction located at the anatomic external os.

Using high-power densities, that is, 1000 W/cm^2, resulted in reduced thermal injury, predictable healing, and rapid attainment of appropriate crater depth.[6] The last item is important because as cellular injury occurs as a result of the thermal action of the laser, water rapidly diffuses into injured tissue and produces swelling. Therefore slow laser vaporization with low-power densities results in the crater "filling in" before completion of treatment. In actuality more tissue is lost and greater peripheral heat damage results if the appropriate specifications are not followed. Compared with other conservative treatment techniques, the laser offers many advantages. Cryosurgery cannot be properly tailored to the geographic extent of the neoplastic lesion. The therapeutic results of this methodology also falter when grade 3 cervical intraepithelial neoplasia is treated. In addition, when one finishes, a cryosurgical procedure, the depth of treatment, and the peripheral margins are inexact. The technique of radical electrocoagulation of cervical intraepithelial neoplasia with needle-and-ball cautery yields results similar to those of the laser but requires general anesthesia, can precipitate a high volume of blood loss, and is clearly the antithesis of controlled, precise, limited tissue trauma. The cold coagulator is less expensive, more effective, and less traumatic than the radical electrocautery; however, to date a small number of cases has been reported at a limited number of centers.

Pesky intraoperative bleeding occurred in approximately 8% to 10% of cases, especially early in the series. The ability to rapidly defocus the laser beam by using the variable spot feature controlled this problem rapidly and effectively. In addition, the technique of coagulating the offending vessel peripherally, that is, before it enters the crater, has proved to be the very best method for controlling this problem.

The presence of chlamydial cervicitis by culture or serologic studies or biopsy evidence of acute cervicitis premonitored an increased chance of immediate and delayed bleeding. Pretreatment of these infections with

tetracycline or other appropriate antimicrobials reduced the risk of bleeding by 50%. The risk of infection after laser surgery of the cervix was predictably small, that is, 0.05%. Theoretically the heat of vaporization is 100° C, and temperatures of 800° C have been measured when incandescence is produced (burning off carbon). Survival of bacteria and viruses is doubtful when exposed to such high temperatures.

The efficacy of CO_2 laser elimination of cervical epithelial neoplasia was documented by the absence of disease in 94% of women who underwent vaporization. Those patients who had persistent or recurrent disease were almost universally (95%) diagnosed to have condylomatous disease of the cervix rather than intraepithelial neoplasia. This finding was not surprising, since one would hardly expect long-established sexual habits to rapidly change. Likewise, the skewed numbers of local recurrence and persistence in the grade 1 cervical intraepithelial neoplasia subset may possibly hinge on the basis that most of these cases were actually condylomatous disease initially. In addition, the human papillomavirus was likely to be present in cells within the endocervical canal above the area of treatment and in the surrounding vagina.[18] The diminished number of persistent and recurrent cases observed in the grade 3 cervical intraepithelial neoplasia group may be explained on the basis that this grade of disease is usually more extensive geographically and the laser surgery more radical and expansive.

For many years the specifications for laser vaporization were erroneously based, that is, the 7 mm depth, toward the goal of ablating the possibly involved endocervical glands. This hypothesis was, of course, inaccurate, since the depth of vaporization was actually determined by trial and error rather than by a preconceived plan. The logic behind sufficient depth of treatment more accurately related to the "gold standard" of conization and the fact that the proximal 1 cm of the canal contained the most likely cell population at risk for progression of cervical epithelial neoplasia.[19] Clearly, the best treatment results in all studies of conservative therapy for cervical epithelial neoplasia were witnessed in women undergoing excision cones. The only difference between this procedure and laser vaporization was the depth to which tissue was removed. Vaporization produced a defect not dissimilar to a small cone. The possible neoplastic involvement of the endocervical clefts was accounted for in the wide peripheral margin. Since the extension of these clefts onto the portio eventuated in a greater peripheral incursion into the stroma than was seen in those clefts emanating from the canal proper, the 3 mm margin of vaporization was more than adequate to provide for their elimination.

It follows that the routine performance of large cylinder–shaped cones to treat intraepithelial neoplasia makes little sense because nothing is accrued toward greater cure rates (Fig. 6); rather, a larger volume of stroma is sacrificed for no useful purpose.

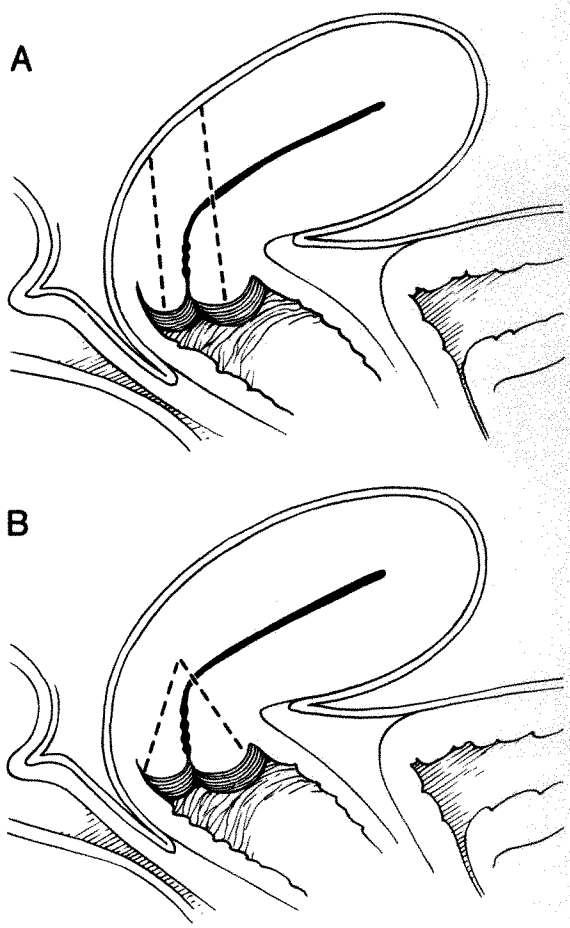


Fig. 6. Larger cylindric cervical excisions performed with a tightly focused laser beam may present a hazard of penetration into abdominal cavity, especially in acutely retroflexed or anteflexed uterus *(A)*. This complication may be avoided by sculpting the specimen in a conical or semi-conical shape *(B)*.

In a previous publication the risk of complications between laser vaporization and laser excisional conization was compared.[20] That study demonstrated very little difference between the two procedures other than the excisional procedure required a few minutes longer to complete. Both operations were done safely in the office under local anesthesia. The mean blood loss for both excisional cone and vaporization was equal. The major weakness of the vaporization technique was that the operation did not produce a specimen for the pathologist, whereas the excisional procedure provided a very ample sample of tissue.

In conclusion, CO_2 laser techniques for vaporizing cervical intraepithelial neoplasia are relatively easy to master; however, the finer points of microsurgical laser finishing and excisional procedures are skill related.

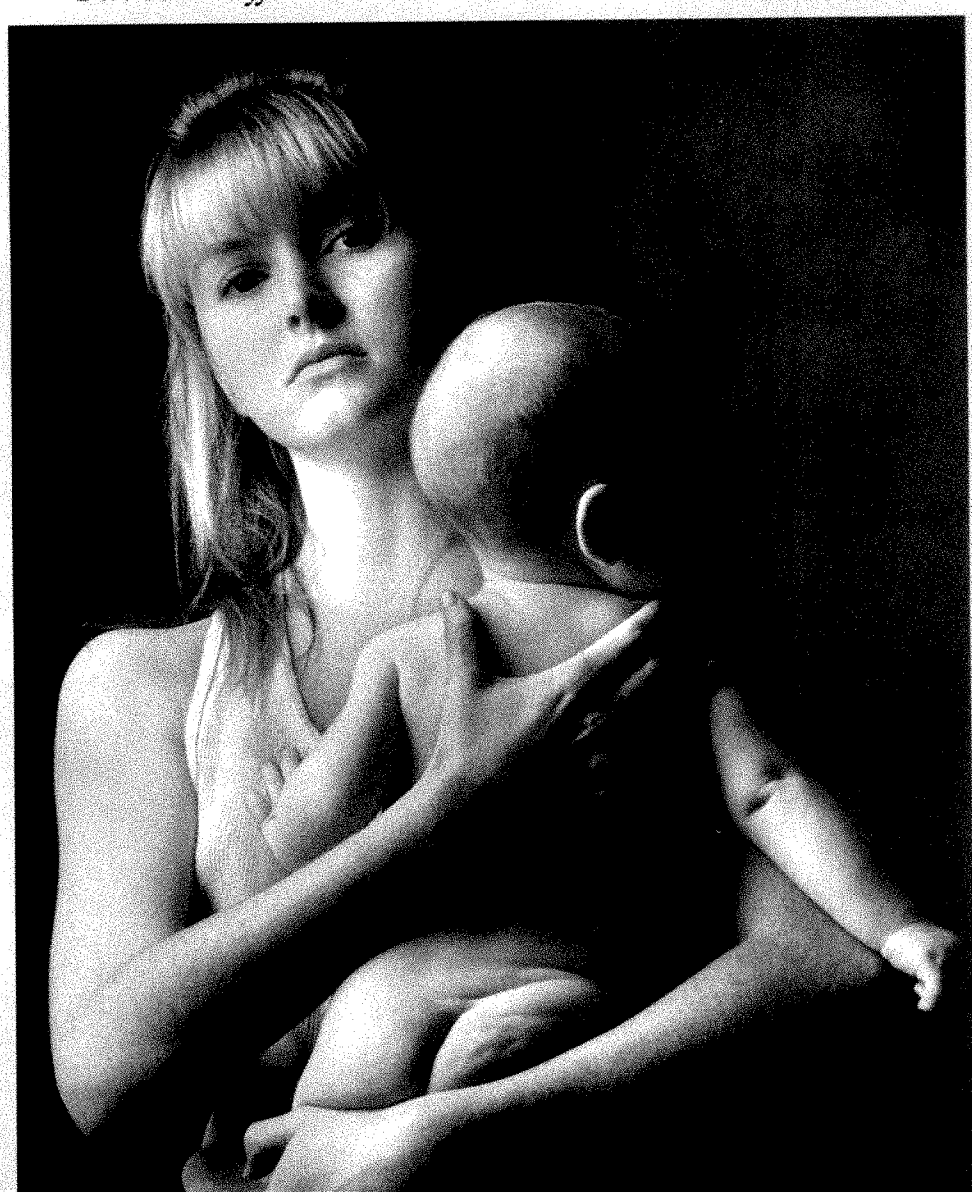
The abilities to appropriately adjust spot size, power density, and vary mode require experience. The final results of laser surgery as mirrored in this report clearly relate to the aforementioned. Individual surgeons must adjust power and more important power density relative to their ability to safely control beam velocity. Overall control must predominate over all other laser delivery parameters.

REFERENCES

1. Townsend DE, Richart RM, Marks E, Nielsen J. Invasive cancer following outpatient evaluation and therapy for cervical disease. Obstet Gynecol 1981;57:145.
2. Baggish MS. Management of cervical intraepithelial neoplasia by carbon dioxide laser. Obstet Gynecol 1982; 60:378.
3. Baggish MS. Laser management of cervical intraepithelial neoplasia. Clinic Obstet Gynecol 1983;26:980.
4. Baggish MS, Dorsey JH. Carbon dioxide laser for combination excisional vaporization conization. AM J OBSTET GYNECOL 1985;151:23.
5. Burke L. The use of the carbon dioxide laser in the treatment of cervical intraepithelial neoplasia. Colpo Gynecol Laser Surg 1986;2:77.
6. Dorsey JH, Diggs ES. Microsurgical conization of the cervix by carbon dioxide laser. Obstet Gynecol 1979;54:565.
7. Evans AS, Monaghan JM. The treatment of cervical intraepithelial neoplasia using the carbon dioxide laser. Br J Obstet Gynaecol 1983;90:553.
8. Larsson G, Gullberg BO, Grundsell H. A comparison of complications of laser and cold knife conization. Obstet Gynecol 1983;62:213.
9. Larsson G, Alm P, Grundsell H. Laser conization versus cold knife conization. Surg Gynecol Obstet 1983;154:59.
10. Jordan JA, Mylotte MJ. Treatment of CIN by destruction—laser. In: Jordan JA, Sharp F, Singer A, eds. Preclinical neoplasia of the cervix. Proceedings of the 9th study group, Royal College of Obstetricians and Gynaecologists. London: Royal College of Obstetricians and Gynaecologists, 1981:205-11.
11. Baggish MS. High-power density carbon dioxide laser therapy for early cervical neoplasia. AM J OBSTET GYNECOL 1980;136:117.
12. Masterson BJ, Krantz KE, Calkins JW, Magrina JF, Carter RP. The carbon dioxide laser in cervical intraepithelial neoplasia: a five year experience in treating 230 patients. AM J OBSTET GYNECOL 1981;139:565.
13. Bellina JH, Wright VC, Voros JI, Riopelle MA, Hohenschutz V. Carbon dioxide laser management of cervical intraepithelial neoplasia. AM J OBSTET GYNECOL 1981; 141:828.
14. Anderson MC. Treatment of cervical intraepithelial neoplasia with the carbon dioxide laser; report of 543 patients. Obstet Gynecol 1982;59:720.
15. Anderson MC, Hartley RB. Cervical crypt involvement by intraepithelial neoplasia. Obstet Gynecol 1980;55:546.
16. Burke L. The use of the carbon dioxide laser in the therapy of cervical intraepithelial neoplasia. Obstet Gynecol 1982;144:337.
18. Reid R. Papillomavirus and cervical neoplasia. Modern implications and future prospects. Colpo Gynecol Laser Surg 1984;1:3.
19. Fluhmann CF. The cervix uteri and its diseases. Philadelphia: WB Saunders, 1961:263-99.
20. Baggish MS. A comparison between laser excisional conization and laser vaporization for the treatment of cervical intraepithelial neoplasia. AM J OBSTET GYNECOL 1986; 155:39.

Fear from breakthrough bleeding can interrupt OC use.

"I was scared...
I didn't know what to do...
So I just said forget it ...
*I'm better off without it..."**

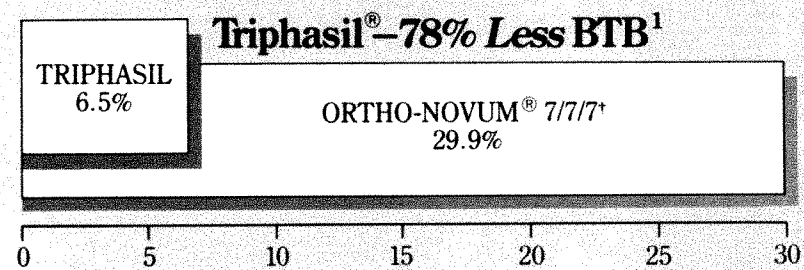


*Direct quote from OC user, data on file.

While the other triphasics have been minimizing the issue of breakthrough bleeding…

Wyeth has been minimizing breakthrough bleeding.*

In Cycle 1...the most critical cycle for compliance



In a separate study of over 4,300 women,[2] in over 70,000 cycles with TRIPHASIL, the total incidence of BTB was only 4.5%

TRIPHASIL –the only OC that needs no backup contraception††...from day 1

Triphasil®

Levonorgestrel and ethinyl estradiol tablets–Triphasic regimen
21- and 28-day regimens

MINIMIZES BTB... FROM DAY 1

* Serious as well as minor adverse reactions have been reported following the use of all oral contraceptives. See prescribing information.

† Norethindrone/ethinyl estradiol triphasic regimen, Ortho Pharmaceutical Corporation.

†† When used as directed.

1. Schilling LH, Bolding OT, Chenault CB, et al: *Am J Obstet Gynecol*, to be published. (Results of a randomized, four-cycle, multicenter U.S. comparison in 313 subjects: Triphasil® vs norethindrone triphasic OCs).
2. Woutersz TB, Korba VD: *Int J Fertil* 1988; 33:406-410.

See brief summary of prescribing information on adjacent page.

© 1989, Wyeth-Ayerst Laboratories.

IN BRIEF:
TRIPHASIL®—6 brown tablets containing 0.050 mg levonorgestrel with 0.030 mg ethinyl estradiol; 5 white tablets containing 0.075 mg levonorgestrel with 0.040 mg ethinyl estradiol; 10 light-yellow tablets containing 0.125 mg levonorgestrel with 0.030 mg ethinyl estradiol (7 light-green tablets containing inert ingredients are included in the 28-day regimen)—Triphasic regimen.

Indications and Usage—TRIPHASIL® is indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OC's) as a method of contraception.

Contraindications—OC's should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5). 8. Benign or malignant liver tumor which developed during use of OC's or other estrogen-containing products.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

. *Thromboembolic Disorders and Other Vascular Problems*—An increased risk of thromboembolic and thrombotic isease associated with use of OC's is well established. Three principal studies in Great Britain and 3 in the U.S. ave demonstrated increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and hrombotic. These studies estimate that users of OC's are 4 to 11 times more likely than nonusers to develop these iseases without evident cause.

EREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with nd without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users ian nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

IYOCARDIAL INFARCTION (MI)—An increased risk of MI associated with the use of OC's has been reported, onfirming a previously suspected association. These studies, conducted in the UK, found, as expected, that the reater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hyper-holesterolemia, obesity, diabetes, history of pre-eclamptic toxemia) the higher the risk of developing MI, regardless f whether the patient was an OC user or not. OC's, however, were found to be a clear additional risk factor. In terms f relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing ondition to MI) are about twice as likely to have a fatal MI as nonusers who do not smoke. OC users who are also mokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke, but about a 10- to 12-old increased risk compared to nonusers who do not smoke. Furthermore, amount of smoking is also an important ictor. In determining importance of these relative risks, however, baseline rates for various age groups must be iven serious consideration. Importance of other predisposing conditions mentioned above in determining relative nd absolute risks has not as yet been quantified; quite likely the same synergistic action exists, but perhaps to a sser extent.

ISK OF DOSE—In an analysis of data derived from several national adverse-reaction reporting systems, British vestigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to dose f estrogen in OC's. Preparations containing 100 mcg or more of estrogen were associated with higher risk of romboembolism than those containing 50-80 mcg. Their analysis did suggest, however, that quantity of estrogen ay not be the sole factor involved. This finding has been confirmed in the U.S.

STIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the K estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and onusers of OC's according to age, smoking habits, and duration of use. Overall excess death rate annually from rculatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/ 0,000; ages 45-49—140/100,000) risk being concentrated in older women, in those with long duration of use d in cigarette smokers. It was not possible, however, to examine interrelationships of age, smoking, and duration use, nor to compare effects of continuous vs. intermittent use. Although the study showed a 10-fold increase death due to circulatory diseases in users for 5 or more years, all these deaths occurred in women 35 or older. ntil larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to sess magnitude of relative risk for this younger group. Available data from a variety of sources have been ana-zed to estimate risk of death associated with various methods of contraception. Estimates of risk of death for each ethod include combined risk of contraceptive method (e.g., thromboembolic and thrombotic disease in the case of C's) plus risk attributable to pregnancy or abortion in event of method failure. This latter risk varies with effective-ss of method. The study concluded that mortality associated with all methods of birth control is low and below at associated with childbirth, with the exception of OC's in women over 40 who smoke. Lowest mortality is sociated with condom or diaphragm backed up by early abortion. Risk of thromboembolic and thrombotic disease sociated with OC's increases with age after about 30 and, for MI, is further increased by hypertension, hypercho-sterolemia, obesity, diabetes, or history of pre-eclamptic toxemia, and especially cigarette smoking. Physician and tient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebi-s, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric rombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A 4- to 6-ld increased risk of postsurgery thromboembolic complications has been reported in OC users. If feasible, OC's ould be discontinued at least 4 weeks before surgery of a type associated with increased risk of thromboembolism prolonged immobilization.

ERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Britain involving cerebro-scular disease and another in the U.S. concerning MI suggest an increased risk of these conditions in users of C's persists after discontinuation of the OC's. In the British study, risk of cerebrovascular disease remained vated in former OC users for at least 6 years after discontinuation. In the U.S. study, increased risk of MI rsisted for at least 9 years in women 40 to 49 years old who had used OC's for 5 or more years. Findings in th studies require confirmation since they are inconsistent with other published information.

Ocular Lesions—There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis sociated with use of OC's. Discontinue OC's if there is unexplained, sudden or gradual, partial or complete loss of sion; onset of proptosis or diplopia; papilledema; or retinal-vascular lesions, and institute appropriate diagnostic d therapeutic measures.

Carcinoma—Long-term continuous administration of either natural or synthetic estrogen in certain animal species creases frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none rrently contained in OC's, have been noted to increase incidence of mammary nodules, benign and malignant, in gs. In humans, 3 case-control studies have reported an increased risk of endometrial carcinoma associated with olonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases bmitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OC's. cases found in women without predisposing risk factors (e.g., irregular bleeding at the time OC's were first given, lycystic ovaries), nearly all occurred in women who had used a sequential OC. These are no longer marketed. No dence has been reported suggesting increased risk of endometrial cancer in users of conventional combination or gestogen-only OC's. Several studies have found no increase in breast cancer in women taking OC's or estrogens. e study, however, while also noting no overall increased risk of breast cancer in women on OC's, found an excess k in subgroups of OC users with documented benign breast disease. Reduced occurrence of benign breast tumors users of OC's has been well documented. In summary, there is at present no confirmed evidence from human idies of increased risk of cancer associated with OC's. Close clinical surveillance of all women on OC's is, never-ldess, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate gnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or h breast nodules, fibrocystic disease, or abnormal mammograms should be monitored with particular care if they ct to use OC's.

Hepatic Tumors—Benign hepatic adenomas have been found to be associated with use of OC's. One study owed that OC's with high hormonal potency were associated with higher risk than lower potency OC's. Although ign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been orted in short-term as well as long-term users. Two studies relate risk with duration of use of OC's, the risk ng much greater after 4 or more years' use. While hepatic adenoma is rare, it should be considered in women senting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma e been reported in women on OC's. Relationship of these drugs to this type of malignancy is not known.

Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring—e of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously nage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, e increased risk of developing in later life a form of vaginal or cervical cancer ordinarily extremely rare. This risk been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence now that OC's ther enhance risk of developing this type of malignancy, such patients should be monitored with particular care if y elect to use OC's. Furthermore, 30 to 90% of such exposed women have been found to have epithelial changes he vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is recursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. ough similar data are not available with use of other estrogens, it cannot be presumed they would not induce ilar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been orted with use of sex hormones, including OC's, in pregnancy. One case-control study estimated a 4.7-fold ease in risk of limb-reduction defects in infants exposed in utero to sex hormones (OC's, hormonal withdrawal s for pregnancy, or attempted treatment for threatened abortion). Some exposures involved only a few days. a suggest that risk of limb-reduction defects in exposed fetuses is somewhat less than 1 in 1,000 live births. In past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual rtion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OC's. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OC's is unknown. It is recommended that, for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing OC's. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at time of first missed period, and further use of OC's should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and advisability of continuation of the pregnancy should be discussed. It is also recommended that women who discontinue OC's with intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this. The administration of progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease*—Studies report increased risk of surgically confirmed gallbladder disease in users of OC's and estrogens. In one study, increased risk appeared after 2 years' use and doubled after 4 or 5 years' use. In one of the other studies, increased risk was apparent between 6 and 12 months' use.

7. *Carbohydrate and Lipid Metabolic Effects*—Decrease in glucose tolerance has been observed in a significant percentage of patients on OC's. For this reason, prediabetic and diabetic patients should be carefully observed while on OC's. Increases in triglycerides and total phospholipids have been observed in patients on OC's. Three studies were performed with Triphasil and no significant alterations in lipid metabolism were noted except for a slight increase in triglyceride levels in 1 study. Clinical significance of these findings remains to be defined.

8. *Elevated Blood Pressure*—Increase in blood pressure has been reported in patients on OC's. In some women, hypertension may occur within a few months of beginning OC's. In the 1st year of use, prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. Prevalence in users increases, however, with longer exposure, and in the 5th year of use is 2½ to 3 times the reported prevalence in the 1st year. Age is also strongly correlated with development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure on OC's. Hypertension that develops as a result of taking OC's usually returns to normal after discontinuing the drug.

9. *Headache*—Onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of OC's and evaluation of the cause.

10. *Bleeding Irregularities*—Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing OC's. In breakthrough bleeding, as in all cases of irregular vaginal bleeding, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another OC may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase risk of thromboembolic disease. Women with past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuing OC's. Women with these preexisting problems should be advised of this possibility and encouraged to use other methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. *Ectopic Pregnancy*—Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. *Breast-feeding*—OC's given in the postpartum period may interfere with lactation and decrease quantity and quality of breast milk. Furthermore, a small fraction of the hormones in OC's has been identified in the milk of mothers on OC's; effects, if any, on the breast-fed child have not been determined. If feasible, defer OC's until infant has been weaned.

Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OC's. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Pap smear and relevant laboratory tests. As a general rule OC's should not be prescribed for longer than 1 year without another physical examination and Pap smear.

2. Under influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size.

3. Patients with history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while on OC's should stop OC's and use an alternate method to try to determine whether the symptom is drug-related.

4. OC's may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency.

5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while on OC's. If jaundice develops, OC's should be discontinued.

6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution.

7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. Clinical significance is undetermined.

8. Serum folate levels may be depressed by OC's. Since the pregnant woman is predisposed to development of folate deficiency and incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OC's, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency.

9. The pathologist should be advised of OC therapy when relevant specimens are submitted.

10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OC's.

a. Increased sulfobromophthalein retention.

b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.

c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered.

d. Decreased pregnanediol excretion.

e. Reduced response to metyrapone test.

Information for the Patient—See Patient Package Labeling.

Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Carcinogenesis—See Warnings section for information on carcinogenesis.

Pregnancy—Category X. See Contraindications, Warnings.

Nursing Mothers—See Warnings.

Adverse Reactions—An increased risk of these serious adverse reactions has been associated with use of OC's (see Warnings): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies. There is evidence of an association between the following conditions and use of OC's although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients on OC's and are believed to be drug-related. Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea, amenorrhea during and after treatment, temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement, and secretion; change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening), intolerance to contact lenses. The following adverse reactions have been reported in users of OC's, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, hemolytic uremic syndrome.

Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OC's by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Dosage and Administration—For maximum contraceptive effectiveness, Triphasil must be taken exactly as directed and at intervals not over 24 hours. (If Triphasil is first taken later than first day of first menstrual cycle of medication or postpartum, contraceptive reliance should not be placed on it until after the first 7 consecutive days of use. Possibility of ovulation and conception prior to initiation of medication should be considered.)

Any time patient misses 1 or 2 brown, white or light-yellow tablets, she should also use another contraceptive method until she has taken a tablet daily for 7 consecutive days.

For full details on dosage and administration see prescribing information in package insert.

Triphasil®

Levonorgestrel and ethinyl estradiol tablets–Triphasic regimen
21- and 28-day regimens

MINIMIZES BTB... FROM DAY 1

WYETH AYERST™

3421-3 12/10/8

©1989, Wyeth-Ayerst Laboratories

Anal submucosal injection: A new route for drug administration in pelvic malignancies

IV. Submucosal anal injection in the treatment of cancer of uterine cervix—Preliminary study

Ahmed Shafik, MD

Cairo, Egypt

Seven patients with advanced cancer of the uterine cervix were treated by anal submucosal injection of methotrexate. Each course consists of five injections, one every 5 days; each injection contains 100 mg of methotrexate. The course was repeated at 3-week intervals and was given on an outpatient basis. Investigations were performed before, during, and after treatment. Methotrexate blood level was measured 4 and 24 hours after administration. Complete response occurred in all seven patients. Relapse occurred in four but could be controlled by further methotrexate administration. Average duration of survival is 46½ months. No side effects were encountered as a result of a low methotrexate serum level that was recorded 4 hours after administration, 0.82 μmol after anal injection compared with 2.8 μmol for the intravenous route. The beneficial effect seems to be the result of the high methotrexate concentration in uterine and tumor tissue. The route adopted by the drug to reach the uterus from the anal submucosa is through the hemorrhoidogenital communicating veins. This preliminary study demonstrates that anal injection of methotrexate is effective in the treatment of advanced cervical cancer. It is safe, well tolerated, and can be used as an outpatient treatment. (Am J Obstet Gynecol 1989:161:69-72.)

Key words: Uterine cervix, cancer, treatment, methotrexate, uterine cervix cancer

Patients with advanced cancer of uterine cervix have a poor prognosis, with a 5-year survival rate of 30% in International Federation of Gynecology and Obstetrics stage III and only 10% to 15% in International Federation of Gynecology and Obstetrics stage IV.[1, 2] Surgical procedures for these advanced stages are mainly palliative. Likewise, radiotherapy is much less effective in the treatment of stage III and IV disease than stages I and II.

The role of chemotherapy in the treatment of advanced cancer of the uterine cervix is increasing, and there is continued interest in the development of more effective cytotoxic therapy. There are many reports on the effectiveness of chemotherapy in squamous cell carcinoma of the uterine cervix in patients with advanced or recurrent disease who cannot be helped by surgery or radiotherapy.[3-5]

Efforts continue to improve the efficacy of cytotoxic therapy by means of altered drug doses, combination chemotherapy, and different routes of administration.

Herein are presented seven cases of advanced cancer of the uterine cervix treated with methotrexate administered by the submucosal anal route.

From the Department of Surgery, Faculty of Medicine, Cairo University.

Received for publication March 22, 1988; revised November 2, 1988; accepted January 11, 1989.

Reprint requests: Ahmed Shafik, MD, 2, Talaat Harb Street, Cairo, Egypt.

Physioanatomic considerations

A recent study[6] has shown that the hemorrhoidal venous plexus communicates with the urogenital plexus by two to six communicating veins called "hemorrhoidogenital veins." The latter are unidirectional, transmitting the blood from the hemorrhoidal to the urogenital plexus, but not in the reverse direction.

Injection of a radiopaque dye into the submucosa of the anal canal led to visualization of the urinary bladder, uterus, and vagina.[6] Thus anal cystography[7] and anal cystovaginohysterography[8] could be achieved by this route. Further studies have demonstrated that the urinary bladder after injection of misonidazole (a radiation sensitizer) into the anal submucosa showed a concentration of the drug as high as eight times the serum level,[9] whereas the uterus showed 10 times concentration (Shafik A, El-Desouky G. Unpublished observations).

The submucosal anal route is being used to administer drugs for pelvic malignancies and other pathologic conditions. It has been used for the treatment of advanced bladder cancer with encouraging results.[10] It is also used in the treatment of chronic relapsing prostatitis (Shafik A. Unpublished observations).

These findings prompted me to use the submucosal

July 1989
Am J Obstet Gynecol

Table I. Data on seven patients with advanced carcinoma of uterine cervix treated by submucosal anal injection of methotrexate

Patient No.	Age (yr)	Stage of disease	Tumor (cm^3)	Previous treatment		No. of courses	Serum MXT after		Side effects	Remission status	Relapse		Survival (mo)
				RT (rads)	CT		4 hr	24 hr			Mo after trt	No. of additional courses	
1	63	III	45	6000	Cisplatin	6	0.75	0.00	Nausea, vomiting	CR	—	—	66
2	69	III	36	6000	—	6	0.65	0.00	—	CR	8	3	56
3	56	III	54	6000	Cycloph + methotrexate + mitomycin C	6	1.00	0.10	—	CR	30	4	55
4	68	III	30	—	Cisplatin	6	1.20	0.30	—	CR	—	—	46
5	48	III	36	5000	—	6	0.90	0.00	—	CR	10	3	41
6	72	III	60	6000	—	6	0.65	0.00	—	CR	14	3	33
7	52	III	24	4500	—	6	0.58	0.00	—	CR	—	—	29

RT, Radiotherapy; *Cycloph*, cyclophosphamide; *trt*, treatment, *CT*, chemotherapy; *MXT*, methotrexate; *CR*, complete response.

anal route for methotrexate administration in the treatment of advanced cancer of the uterine cervix. This procedure aims at inducing a high methotrexate concentration in the uterine cervix with a minimum serum level, thus attaining a high therapeutic result and minimal side effects. The results of treatment of seven patients via this route are herein presented.

Case reports

Case 1. A 63-year-old patient had advanced cervical carcinoma (stage IIIB). She had received radiation therapy to the pelvis for a total dose of 6000 rads and cisplatin with no response. Examination of the cervix showed that a large mass, 5 by 3 by 3 cm, replaced the cervix and encroached on the upper 2 cm of the vagina. Biopsy examination showed that the tumor was a squamous cell carcinoma of the large cell and nonkeratizing type. Sonography revealed enlarged iliac and obturator glands. Hematologic reserve and renal and hepatic functions were adequate.

Six courses of methotrexate were given by the submucosal anal route starting in June 1982. Each course consisted of five injections of methotrexate. One injection was given every 5 days; each injection contains 100 mg of methotrexate. The course was completed in 25 days and was followed by a 3-week interval of rest before the next course was started. Routine hematologic assessment was performed before each injection. Serum methotrexate level was assessed 4 and 24 hours after administration. The patient received the treatment on an outpatient basis.

Follow-up examination was comprised of pelvic sonography and cervical tumor biopsy studies performed every 3 months for the first year, every 6 months for the second year, and annually thereafter or on a report of vaginal bleeding. Computed tomography scan of the abdomen and chest x-ray studies were done every 6 months for 1 year and every year thereafter.

During the third course, the patient had nausea and vomiting that were controlled by medical measures without discontinuation of treatment. Hematologic reserve and renal and liver functions remained almost unchanged throughout the period of treatment. Methotrexate blood levels 4 and 24 hours after injection recorded 0.75 and 0.00 μmol, respectively. After the fourth course, the tumor disappeared completely, and cervical biopsy findings were negative. Sonography showed disappearance of iliac and obturator nodes. The patient has survived now 5½ years with no evidence of recurrence.

Case 2. A 69-year-old woman had stage III cervical cancer. Examination revealed an exophytic mass 4 by 3 by 3 cm, which occupied the uterine cervix and involved the two lateral vaginal fornices. Pelvic examination revealed induration in the right parametrial region proved by computed tomography scan to have the size of 3.5 by 2 by 1.5 cm. Biopsy studies of the cervical mass showed small cell, nonkeratizing, squamous cell carcinoma.

When the patient showed no response to radiation therapy to the pelvis (total dose, 6000 rads), she was given six courses of methotrexate injected per anus in the aforementioned dose. The first course was given in March 1983. The patient was assessed and followed up by pelvic sonography, cervical tumor biopsy studies, and computed tomography scan. Methotrexate serum level was measured 4 and 24 hours after injection.

No side effects from the drug were encountered. Methotrexate serum levels were 4 and 24 hours after injection 0.65 and 0.00 μmol, respectively. After the third course of methotrexate treatment, the cervical mass and parametrial induration disappeared. At the end of the fifth course, cervical biopsy findings were negative and computed tomography scan recorded disappearance of the parametrial mass. Cervical tumor relapse occurred 8 months after termination of the sixth course. The patient received three additional

courses of methotrexate by the submucosal anal route. She has now survived for 56 months and is free of malignancy.

Case 3. A 56-year-old woman had a huge 6 by 3 by 3 cm mass that replaced the entire uterine cervix and encroached on the upper half of the vagina. The mass was fixed to the urinary bladder anteriorly and the anal canal posteriorly. Bilateral parametrial induration was confirmed by computed-tomography scan. Biopsy studies showed that the tumor was poorly differentiated squamous cell carcinoma.

Cystogram revealed that the tumor was encroaching on the urinary bladder. One year before presentation, the patient received 6000 rads to the pelvis with no response. Combination chemotherapy of cyclophosphamide, methotrexate, and mytomycin C also proved to be ineffective.

The first course of methotrexate, which was administered per anus, was given in April 1983. There were no side effects. Methotrexate serum level 4 and 24 hours after injection was 1.0 and 0.10 μmol, respectively. The patient completed six courses. By the end of the fourth course, the cervical mass and parametrial induration disappeared. The results of the cervical biopsy examination were negative after termination of the sixth course.

In July 1986, cervical recurrence occurred as shown by biopsy studies. A negative biopsy finding was obtained after four courses of methotrexate, and the patient is alive and tumor free since then.

Cases 4 to 7 (Table I). Four female patients, ranging in age from 48 to 72 years, had stage III squamous cell carcinoma of the uterine cervix. Their data are shown in Table I. They were managed in the same way as the aforementioned cases. All had negative cervical biopsy results by the end of the sixth course of methotrexate injection. Recurrence occurred in two patients 10 and 14 months after termination of the six courses. Three additional methotrexate courses were given, of which the cervical biopsy findings of the two patients at the end of treatment were again negative. The four patients have now survived 46, 41, 33, and 29 months since the start of treatment and are tumor free.

Technique of anal submucosal injection. The 100 mg methotrexate vial is diluted with 10 ml of saline solution. The usual 10 ml capacity syringe is used for injection. Anesthesia is not required, since the procedure is painless. With the patient in the lithotomy or left lateral position, a proctoscope is introduced, the anal canal is inspected, and the pectinate line is identified. Injection is done in the submucosa of the anterior wall of the anal canal above this line. The mucosa at the proposed site of injection is swabbed clear with a pledget of cotton wool on forceps. The needle is then inserted obliquely through the mucosa.

A small amount of methotrexate solution is injected. If the mucosa balloons up in an edematous wheal with obvious vessels crossing it, this indicates that the fluid is spreading in the submucous plane as desired. If no wheal is produced, the needle is too deep and should be gradually withdrawn with simultaneous, repeated injections of minimal amounts of the fluid until the effect described is achieved. A dead white mucosa at the injection site signifies that the fluid is being injected into the mucosa itself. When the proper submucosal plane is reached, the rest of the solution is then injected.

Methotrexate assay. Blood methotrexate was estimated by an enzyme immunoassay kit obtained from Syva, (California). Blood samples were withdrawn 4 and 24 hours after methotrexate injection. They were centrifuged at 1500 *g*, and sera were subjected immediately to the assay. Methotrexate concentrations were expressed in micromoles.

Comment

The present results show that complete response occurred in all seven patients treated with methotrexate injected by the submucosal anal route. However, relapse occurred in four of seven patients 8 to 30 months after termination of treatment (average, 15½ months). It could be controlled by the administration of further courses of methotrexate (average, three). The duration of survival from the start of treatment varied from 29 to 66 months, with an average of 46½.

Investigators reported objective responses in 10% to 25% of patients with cervical cancer for 4 to 6 months when single chemotherapeutic agents were used.[11, 12] More recently the Gynecologic Oncology Group reported a response rate of 38% for cisplatin in squamous carcinoma of the cervix[13] and 31% for bleomycin.[14] Good response rates have been reported for several combination chemotherapy regimens. A response rate of 89% was obtained with a combination of cisplatin, bleomycin, and methotrexate,[15] and 77% was obtained with a combination of cisplatin, bleomycin, and mitomycin C.[16] However, the responses were of short duration and drug toxicity was significant.[15, 16]

No side effects were encountered in these seven patients. Only one patient had nausea and vomiting which could be controlled without discontinuation of treatment. The absence of toxic manifestations is the result of the low methotrexate serum level obtained by the submucosal anal route compared with the intravenous route, the average after 4 hours was 0.82 μmol for the former and 2.8 μmol for the latter.[10] The difference is highly significant. Furthermore, the serum levels obtained 24 hours after methotrexate injection (mean, 0.66 μmol) indicate that there is no cumulative effect of methotrexate in the circulation.

The beneficial effects of anal injection of methotrexate seem to be the result of the high drug concentration in uterine and tumor tissue. A recent experimental study has shown that carbon 14–labeled misonidazole concentration in uterine tissue after anal injection was 10 times that of serum 15 minutes after injection (Shafik

A, El-Desouky G. Unpublished observations). Measurement of methotrexate level in cervical tumor tissue after various routes of administration is being performed and will be reported in a separate communication.

The route adopted by methotrexate to reach the uterine cervix from the anal submucosa needs to be discussed. Recent studies have shown that when a radiopaque dye was injected in the anal submucosa, it was absorbed by hemorrhoidogenital lymphatics and veins and reached the uterovaginal plexus.[6, 8] Anal injection of methotrexate seems to follow the same route and reaches the uterus in concentrations high enough to effect tumor regression.

In conclusion, the present study demonstrates that methotrexate anal injection is effective in the treatment of cervical cancer. It is safe, well tolerated, and used on an outpatient basis. A further advantage of this route is that it allows for the administration of much greater drug doses than the intravenous route without the threat of toxicity. The maximal drug concentration is in the tumor rather than in the blood.

Admittedly, the number of patients in this communication is small, but the results warrant publication as a preliminary study.

The valuable assistance of Mrs. Margot Yehia in revising the manuscript is highly appreciated.

REFERENCES

1. Chism SE, Park RC, Keys HM. Prospects for paraaortic irradiation in treatment of cancer of the cervix. Cancer 1975;35:1505-609.
2. Jampolis S, Andras EJ, Fletche GH. Analysis of sites and causes of failures of irradiation in invasive squamous cell carcinoma of the intact uterine cervix. Radiology 1975; 115:681-5.
3. Llorens AS. Chemotherapy of squamous cell carcinoma of the cervix. Obstet Gynecol 1980;55:373-5.
4. Thippen T, Shingleton H, Homesley H, Lagasse L, Blessing J. Cisplatinum in treatment of advanced or recurrent squamous cell carcinoma of the cervix. A phase II study of the Gynecologic Oncology Group. Cancer 1981;48: 899-903.
5. Chan WK, Aroney RS, Levi JA, Tattersall MHN, Fox RM, Woods RL. Four-drug combination chemotherapy for advanced cervical carcinoma. Cancer 1982;49:2437-2400.
6. Shafik A, Mohi A. A new concept of the anatomy of the anal sphincter mechanism and the physiology of defecation. XXIV. Hemorrhoidal venous plexuses: anatomy and role in hemorrhoids. Coloproctology 1985;7:291-6.
7. Shafik A. Anal cystography. New technique of cystography. Urology1984;23:313-6.
8. Shafik A, Mohi M. Pelvic organ venous communications. Anatomy and role in urogenital diseases. A new technique of cysto-vagino-hysterography. Am J Obstet Gynecol 1988;159:347-51.
9. Shafik A, El-Merzabani MM, El-Aaser AA, El-Desouky GHI. Anal submucosal injection. A new route for drug administration in pelvic malignancies. I. Experimental study of misonidazole distribution in serum and tissues, with special reference to urinary bladder. Invest Radiol 1986;21:278-81.
10. Shafik A, Haddad S, Elwan F, El-Metnawi W, Olfat E. Anal submucosal injection. A new route for drug administration in pelvic malignancies. II. Methotrexate anal injection in the treatment of advanced bladder cancer. Preliminary study. J Urol 1988;140:501-5.
11. De Vita VT, Wasserman TH, Young RC, Carter SK. Perspectives on research in gynecologic oncology. Cancer 1976;18:509-25.
12. Wasserman TH, Carter SK. The integration of chemotherapy into combined modality treatment of solid tumors. VII. Cervical cancer. Cancer Treat Rev 1977;4:25-46.
13. Thigpen J, Vauce RB, Balducci L, Blessing J. Chemotherapy in the management of advanced or recurrent cervical and endometrial carcinoma. Cancer 1981;48:658-65.
14. Kradoff IH, Cvitkovic E, Currie V, Yeh S, La Monte C. Clinical pharmacologic and therapeutic studies of bleomycin given by continuous infusion. Cancer 1977;40: 2027-37.
15. Vogl SE, Mouktar M, Kaplan BH. Chemotherapy for advanced cervical cancer with methotrexate, bleomycin and cisplatin. Cancer Treat Rep 1979;63:1005-6.
16. Vogl SE, Mouktar M, Kaplan BH, Coanog A. Effective combination chemotherapy of advanced cervical cancer with cisplatin, bleomycin, vincristine and mitomycin C. Proc ASCO-AASR 1980;21:189.

Aggressive angiomyxoma first diagnosed as levator hernia

Gregory P. Sutton, MD,[a] Robert E. Rogers, MD,[a] Lawrence M. Roth, MD,[b] and Clarence E. Ehrlich, MD[a]

Indianapolis, Indiana

When a 32-year-old woman was first seen, physical findings suggested she had a large levator hernia, but at the time of surgical resection an aggressive angiomyxoma was found. (AM J OBSTET GYNECOL 1989;161:73-5.)

Key words: Angiomyxoma, levator hernia

Aggressive angiomyxoma is a benign but locally aggressive neoplasm seen almost exclusively in the pelvis or perineum of premenopausal women.[1, 2] The tumor in this case was initially diagnosed as a levator sling hernia. A pelvic computerized axial tomogram helped to clarify the diagnosis and to direct surgical excision.

Case report

I. W., a 32-year-old white woman (para 2-0-0-2) was referred to Indiana University Medical Center because of a large, soft mass of the left vulva and buttock. The patient noticed asymmetry of her left buttock shortly after she was uneventfully delivered of her second child in November 1981. On standing, the mass protruded into the left buttock and was accompanied by a progressive traction sensation in the lower abdomen. She had no associated bowel complaints but did have progressive urinary frequency. She was thought to have a levator or perineal hernia, and was referred for evaluation in June 1987.

The patient was a healthy-appearing postal worker whose physical examination results were normal. On pelvic examination a 6 × 8 cm protuberant soft mass was identified arising from the anterior left buttock and labium majus (Fig. 1). The mass could be easily reduced through a palpable 7 cm defect in the left midlevator plate and gave the impression of a hernia. The uterus was retrodisplaced, and there was a mass sensation on bimanual examination.

A computerized tomogram showed an 11 × 8 cm well-demarcated mass that extended from the pelvis to the left buttock. There was a central, cleft-like hypodense area, but no fat density was observed (Fig. 2). An intravenous pyelogram demonstrated moderate hydronephrosis on the right side, marked displacement of the bladder to the right, and deviation of the left ureter across the midline of the pelvis (Fig. 3).



Fig. 1. Appearance of mass distorting left labium majus and buttock.

A biopsy specimen obtained through the left side of the vagina showed myxomatous and vascular components diagnostic of aggressive angiomyxoma, and the next day the large mass was completely resected using a combined abdominoperineal approach. The reproductive organs were preserved. The patient did well and had no evidence of disease 1 year postoperatively.

Estrogen and progesterone receptor content were determined with a dextran-coated charcoal assay. The estrogen receptor level was 29.4 femtomoles/mg of protein and the progesterone receptor level was 5.2 femtomoles/mg of protein; both were intermediate levels.

Pathology

Gross and microscopic findings. On gross examination the specimen was soft, easily compressible, and somewhat fleshy in consistency. The cut surface of the tumor was circumscribed, soft, gray-white, homogeneous, gelatinous, and moist (Fig. 4).

Histologically, the tumor was circumscribed and was

From the Departments of Obstetrics and Gynecology[a] and Pathology,[b] Indiana University School of Medicine.

Supported in part by the American Cancer Society Career Development Award 86-74.

Received for publication September 7, 1988; revised December 13, 1988; accepted January 11, 1989.

Reprint requests: Gregory P. Sutton, MD, Associate Professor and Chief, Gynecologic Oncology, Department of Obstetrics and Gynecology, Indiana University School of Medicine, 926 W. Michigan St., Indianapolis, IN 46223.

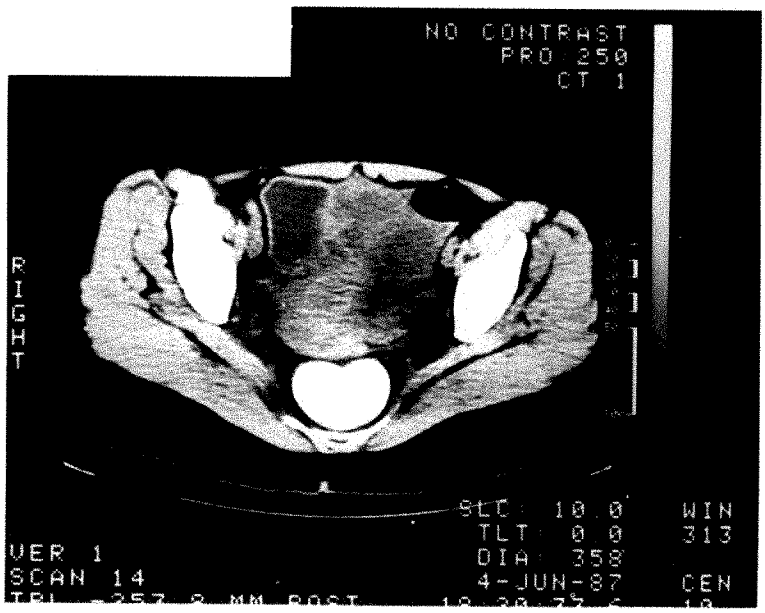


Fig. 2. Computerized tomography shows rightward displacement of bladder by heterogenous mass.

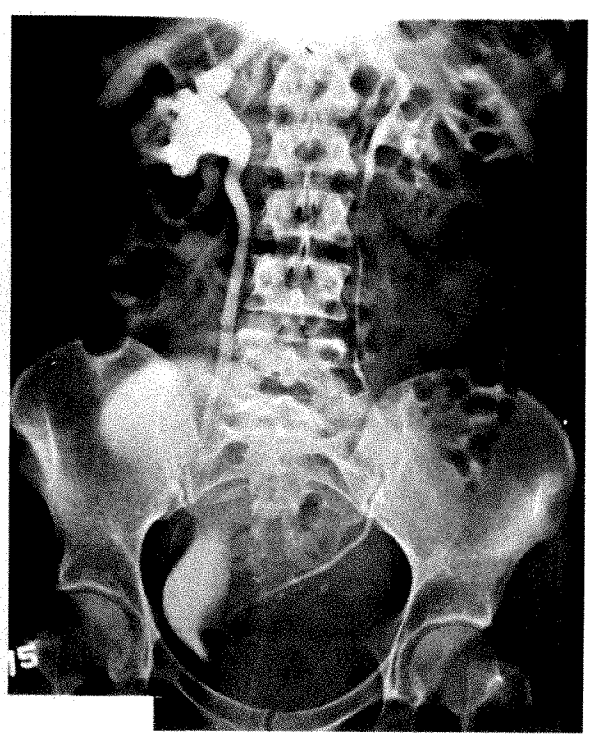


Fig. 3. Intravenous pyelogram demonstrates marked displacement of bladder and left ureter and right hydronephrosis.

confined by a condensation of eosinophilic stromal material and tumor cells. Two distinct components were seen. The first, a loosely textured myxomatous component, contained bipolar to multipolar mesenchymal cells with small uniform vesicular nuclei with an occasional small inconspicuous nucleolus, but lacking mitoses. Interspersed throughout the myxomatous matrix were small to medium-sized vessels, some of which were thin-walled and resembled capillaries, whereas others had thick muscular walls. In some areas around medium-sized muscular blood vessels, slips of smooth muscle were observed within the myxomatous stroma. No small nerves were noted within the tumor.

Comment

This case was, for the most part, a typical aggressive angiomyxoma, although it was better circumscribed than many of the reported cases. Although multiple small nerves have been found within the myxoid stroma of several of the tumors, this case is the first in which fascicles of smooth muscle cells were present focally within the stroma.

The ultrastructural features of the neoplasm were similar to those of the previously studied cases. Steeper and Rosai[1] coined the term "aggressive angiomyxoma" in 1983 to describe nine cases of a benign but locally infiltrative neoplasm that arose in the pelvis or perineum of women who ranged from 21 to 38 years of age. The next year Begin and associates[2] reported nine additional cases including two tumors that arose in male patients and one in a 63-year-old woman. These masses were alternatively diagnosed as Bartholin cysts or neoplasms, vaginal prolapse, or hernias of the pelvic floor or obturator fossa. The history and physical findings in the current case were strongly suggestive of a levator hernia. Computed tomographic findings, however, suggested the diagnosis of aggressive angiomyxoma. These

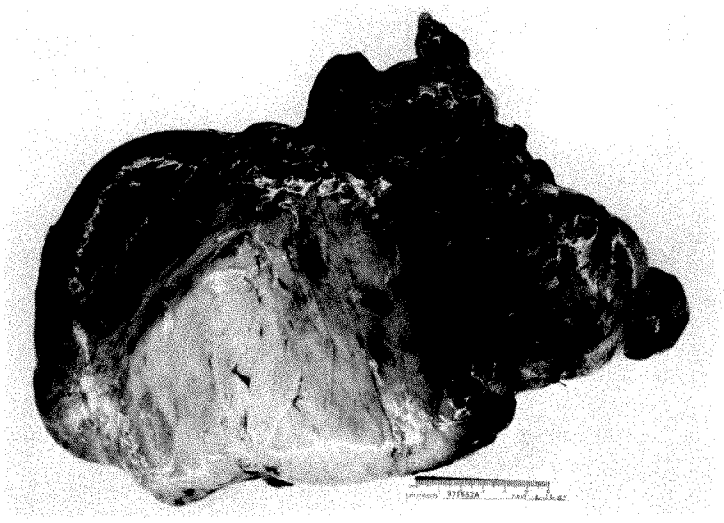


Fig. 4. Cut surface of neoplasm was homogeneous, moist, and gelatinous.

findings underscore the importance of radiographic evaluation before operation for suspected perineal or levator hernias.

Local infiltration of these tumors is characteristic, although they may be clearly demarcated in some areas. Infiltration into skeletal muscle and fat has been observed. Local recurrences at intervals from 9 to 180 months were reported in 10 of the 18 cases reported by Steeper et al.[1] and Begin et al.[2] All were thought to be a result of an inadequate primary excision. No metastases or deaths have been described.

Aggressive angiomyxoma must be distinguished from a variety of similar neoplasms including myxoid lipoma and liposarcoma, soft tissue myxoma, nerve sheath tumor, myxoid neurofibroma, embryonal rhabdomyosarcoma, malignant fibrous histiocytoma, and fibromatosis with focal myxoid areas.

Vulvar, pelvic, or perineal masses that are soft and slow growing must be evaluated with the diagnosis of aggressive angiomyxoma in mind so that adequate extirpative surgery may be performed and recurrences obviated. The presence of estrogen and progesterone receptor protein suggests a possible role for hormonal therapy in those cases that cannot be completely removed.

REFERENCES

1. Steeper TA, Rosai J. Aggressive angiomyxoma of the female pelvis and perineum. Am J Surg Pathol 1983;7:463-75.
2. Begin LR, Clement PB, Kirk ME, et al. Aggressive angiomyxoma of pelvic soft parts: a clinicopathologic study of nine cases. Hum Pathol 1985;16:621-8.

Asymptomatic thrombocytopenia associated with chorioangioma of placenta

Nirmala S. Limaye, MD, and Jean-Gilles Tchabo, MD
Arlington, Virginia

Chorioangioma is a common primary tumor of the placenta. Most tumors are small and are only detected by careful sectioning of the placenta. Large tumors can be diagnosed antenatally by ultrasonography and may result in maternal and fetal complications. (AM J OBSTET GYNECOL 1989;161:76-7.)

Key words: Chorioangioma, maternal complication

A case of chorioangioma, a common primary tumor of the placenta, is reported. A healthy male infant was delivered by cesarean section.

Case report

R. B., a 21-year-old woman, gravida 2, para 1, aborta 0, whose last menstrual period was May 1, 1987, was admitted in December 1987 for mild pelvic pain and urinary frequency. The patient had an unexplained stillbirth at 38 weeks' gestation on June 8, 1986. Autopsy revealed a normal fetus and placenta.

The current pregnancy was uneventful until December 7 when results of a routine blood count indicated platelets at 117,000/mm^3. The coagulation profile showed fibrin split products >40 and <80, but normal fibrinogen, partial thromboplastin time and prothrombin time levels. An ultrasonogram revealed a male fetus of 33 weeks' gestation with no congenital anomalies (Fig. 1). The placenta was anterior and a 5.5 cm sonolucent subchorionic necrotic lesion was noted with a normal amount of amniotic fluid.

A nonstress test on admission was reactive. Repeat ultrasonography placed the fetus at 35 to 36 weeks' gestation with estimated fetal weight of 6 pounds; the mass was unchanged.

The platelet count was 107,000/mm^3 and the hematocrit was 34. Fibrin split products were >80 but <160; fibrinogen, partial thromboplastin time, and prothrombin time levels were normal.

On December 29 the platelet level dropped to 102,000/mm^3 and hemoglobin to 10 gm; the urine culture grew streptococci.

From the Department of Obstetrics and Gynecology, Arlington Hospital.
Received for publication October 22, 1988; revised December 21, 1988; accepted February 17, 1989.
Reprint requests: Nirmala S. Limaye, MD, Department of Obstetrics and Gynecology, Arlington Hospital, 1701 N. George Mason Dr., Arlington, VA 22205.

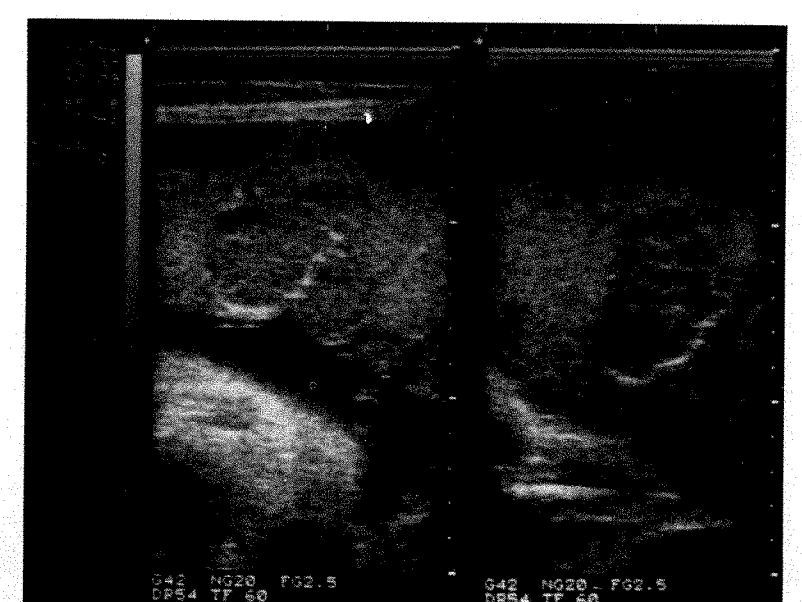


Fig. 1. Sonogram showing male fetus at 33 weeks' gestation with no congenital anomalies.

A healthy male infant with 1- and 5-minute Apgar scores of 8 and 9, respectively, and a birth weight of 5 pounds 11½ ounces was delivered by cesarean section with normal cord pH. The placenta was delivered with a 5 cm loose homogeneous mass.

The patient did well after operation except for a low-grade fever that was treated with intravenous ampicillin. The infant had mild respiratory distress with pneumonitis and was treated with intravenous antibiotics.

Histologic examinations of the placenta showed normal villous pattern. Sections through the mass revealed benign chorioangioma.

Comment

Chorioangioma, the most common tumor of the placenta, was described by John Clarke in 1798. The incidence of small tumors is between 1:70 and 1:100, but large chorioangiomas are reported in only 1:9000 deliveries. These tumors appear as homogeneous, firm, rubbery masses. Histologically they appear as well-differentiated capillary patterns; whether this represents a true neoplasm or hamartoma is uncertain.

Larger chorioangiomas cause maternal and fetal complications such as toxemia, abruptio placentae, premature labor, hydrops, fetal malformations, fetal thrombocytopenia, and angiomas of the skin. Easy diagnosis by ultrasonography has been reported.[1,2]

Maternal asymptomatic thrombocytopenia with mild coagulopathy was noted in our case. Both were probably triggered by necrosis and infarction of the chorioangioma.

REFERENCES

1. Forehlich LA, Fujikura T, Fisher P. Chorioangiomas and their clinical implications. Obstet Gynecol 1971; 37:51.
2. Sprit BA, Gordon LN, Cohen W, Yambao T. Antenatal diagnosis of chorioangioma of the placenta. Am J Radiol 1980;135:12173-5.

Bound volumes available to subscribers

Bound volumes of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY are available to subscribers (only) for the 1989 issues from the Publisher, at a cost of $56.00 ($79.00 international) for Vol. 160 (January-June) and Vol. 161 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue in the volume. The binding is durable buckram with the JOURNAL name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact The C.V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318, USA; phone (800) 325-4177, ext. 351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular JOURNAL subscription.

First-trimester and early second-trimester diagnosis of nuchal cystic hygroma by transvaginal sonography: Diverse prognosis of the septated from the nonseptated lesion

M. Bronshtein, MD, S. Rottem, MD, N. Yoffe, MD, and Z. Blumenfeld, MD
Haifa, Israel

Fetal cystic hygroma is a congenital malformation of the lymphatic system appearing as a single or multiloculated fluid-filled cavity, most often in the nuchal region. The malformation is believed to arise from failure of the lymphatic system to communicate with the venous nuchal system. Sometimes the lesion progresses to fetal hydrops, causing fetal death. To further delineate the cause and natural history of this disorder, we have prospectively studied eight cases of cystic hygroma of the neck, detected at gestational ages of 9 to 15 weeks by transvaginal sonography. Three of the eight fetuses survived (37.5%) and were normal at birth. Either hydrops fetalis or intrauterine fetal death occurred in the other five fetuses. In one of these five, therapeutic abortion was induced because of trisomy 21. In another fetus of these five, trisomy 18 was diagnosed by amniocentesis. This pregnancy ended in intrauterine fetal death. The ultrasonic evaluation of the cystic hygromas revealed that those that were reabsorbed in the three ultimately normal viable fetuses were nonseptated cysts, whereas all the four cystic hygromas ending in fetal death or associated with aneuploidy were septated, multilocular hygromas. In another fetus with nonseptated hygroma, nonimmune hydrops developed, which resulted in premature delivery and early neonatal death. (AM J OBSTET GYNECOL 1989;161:78-82.)

Key words: Fetal cystic hygroma, nuchal hygroma, transvaginal sonography, first-trimester diagnosis

Cystic hygroma is a congenital malformation of the lymphatic system, which can be generalized or localized most often in the nuchal area.[1-5] This malformation is often associated with chromosomal aneuploidies, especially Turner's syndrome, in which case it has a poor prognosis.[1-3, 6] Smith[7] and Graham et al.[8] have proposed a mechanism of lymphatic maldevelopment that explains both cystic hygromas and hydrops in fetuses and neck webbing and peripheral edema in adults with Turner's syndrome.[2] Substantiation of their hypothesis, termed the *jugular-lymphatic obstruction sequence*, and further karyotype studies have been limited because only abortuses could be evaluated previously.[2] The ability to diagnose fetal malformations by first-trimester transvaginal sonography allows for the early recognition of cystic hygromas and follow-up of their development or reabsorption and for detection of generalized hydrops and/or lymphedema[9, 10] associated with nuchal cystic hygroma.

From the Department of Obstetrics and Gynecology "A," Rambam Medical Center and Faculty of Medicine, Technion—Israel Institute of Technology.
Received for publication September 14, 1988; revised December 21, 1988; accepted December 31, 1988.
Reprint requests: Z. Blumenfeld, MD, Dept. of Obstetrics and Gynecology "A," Rambam Medical Center and Faculty of Medicine, Technion—Israel Institute of Technology, Haifa, Israel, 31096.

We report eight cases of nuchal cystic hygromas, five of which ended in fetal or early neonatal death, whereas in the other three the hygromas disappeared and the gestations generated normal term fetuses.

Material and methods

During the past year, from September 1987 to August 1988, the diagnosis of cystic hygroma of the neck was made by first-trimester or early midtrimester transvaginal sonography in eight fetuses. These eight cases of cystic hygroma were diagnosed among 800 patients routinely evaluated at 9 to 15 weeks of gestation for detection of fetal malformations. Equipment used in the study included the Elscint ESI-1000 ultrasound imaging system (Elscint Inc., Boston) with a 6.5 MHz vaginal probe (ESI ED-65 TV), which consisted of a mechanical sector scanner with an oscillating transducer, and a 3.5 MHz abdominal probe (ESI ED-35). Gestational age was determined by crown-rump length, biparietal diameter, head circumference, abdominal circumference, and femur length measurements, in conjunction with menstrual or ovulation dating.[9] A careful sonographic search for other malformations or anatomic abnormalities was conducted in each case.[9, 10] If sufficient amniotic fluid was present, amniocentesis was performed, in each case of cystic hygroma, to determine the fetal karyotype. The α-fetoprotein concen-

Table I. Clinical follow-up and outcome data in the eight cases of cervical cystic hygroma

Case No.	*Patient's age (yr)*	*Gravidity and parity*	*Gestational age at diagnosis (wk)*	*Type of hygroma*	*Karyotype*	*Outcome*
1	32	G2P1	9.5	Septated	—	Fetal death (11 wk)
2	40	G1P0	13	Septated	—	Nonimmune hydrops at 15 wks, fetal death (16 weeks)
3	33	G3P2	11	Septated	Trisomy 21, (47,XX)	Elective abortion (24 wk)
4	41	G1P0	15	Septated	Trisomy 18	Nonimmune hydrops at 15 wk; fetal death (17 wk), multiple malformations
5	22	G1P0	14	Nonseptated	46,XY	Normal term neonate, 3800 gm male
6	27	G1P0	14	Nonseptated	46,XY	Normal term neonate, 3000 gm male
7	30	G2P1	15	Nonseptated	46,XX	Normal term neonate, 3100 gm female
8	33	G2P1	12	Nonseptated	46,XY	Nonimmune hydrops, premature delivery at 30 wk; early neonatal death

tration in the amniotic fluid was measured by radioimmunoassay, and karyotypes were obtained from amniocytes or from peripheral lymphocytes obtained from cord blood at delivery or after therapeutic abortion (cases No. 1 and 5 through 8).

Results

The eight cases of cystic hygroma of the neck were diagnosed out of about 800 gestations carefully evaluated at 9 to 15 weeks of pregnancy for detection of malformations (1%) (Table I). Cervical cystic hygroma was the second most common malformation detected in this series, the central nervous system malformations being the most prevalent (10 of 800 cases, 1.25%). Gestational age at the time of diagnosis ranged from 9 to 15 weeks (from last menstrual period). In four of the eight cases (50%), the cervical cystic hygroma was septated and multiloculated. In two of them (cases No. 1 and 2), early fetal death occurred at 11 and 16 weeks, respectively, within 2 weeks of diagnosis; hydrops fetalis developed in the latter before fetal death. In the third case, diagnosed at 11 weeks of gestation in a 33-year-old white woman, gravida 3, para 2, amniocentesis was performed at 16 weeks and again, because of a lack of growth in the amniocytes, at 20 weeks. Trisomy 21 was diagnosed and therapeutic abortion was done at 24 weeks' gestation. The female abortus showed the phenotype of trisomy 21 (this was reestablished by karyotyping of fetal lymphocytes).

The fourth case of septated cystic hygroma was diagnosed in a 40-year-old white woman. The pregnancy was induced by human menopausal gonadotropin–human chorionic gonadotropin and multiple malformations including hydrops fetalis, short fetal bones, pleural effusion, triangulocephaly, clubfoot, and septated cervical cystic hygroma were diagnosed by transvaginal sonography at 15 weeks since the last menstrual period. At this time fetal biparietal diameter, femur length, abdominal circumference, and head circumference measurements were consistent with those of a 12-week-old fetus. The results of transvaginal sonographic scanning were suggestive of aneuploidy, and trisomy 18 was diagnosed by amniocentesis. Hydrops fetalis developed at 15 weeks and fetal death occurred 2 weeks later.

In the other four cases, nonseptated posterior cervical cystic hygromas were detected at 12 to 15 weeks since last menstrual period. Amniocentesis in all these four cases (cases No. 5 to 8) revealed normal karyotypes and normal levels of α-fetoprotein. Sonographic follow-up revealed absorption of the cystic hygromas, with apparent communication of the cystic lesion with the lumen of the internal jugular vein (Figs. 1 and 2). The cystic hygromas developed into webbed neck (Fig. 3) and gradually disappeared. The karyotype in three of the fetuses was 46,XY and in one was 46,XX without mosaicism. Three pregnancies resulted in normal term neonates, two boys and one girl (without cervical hygroma or webbed neck). The other pregnancy (case No. 8) resulted in a fetus with nonimmune hydrops who was delivered at 30 weeks and died 2 days later at another hospital. No other malformations were described.

Comment

Cystic hygromas are believed to develop from a defect in the formation of lymphatic vessels.[1,2] The fetal lymphatic vessels normally drain into two large sacs lateral to the jugular veins.[2] These jugular lymph sacs

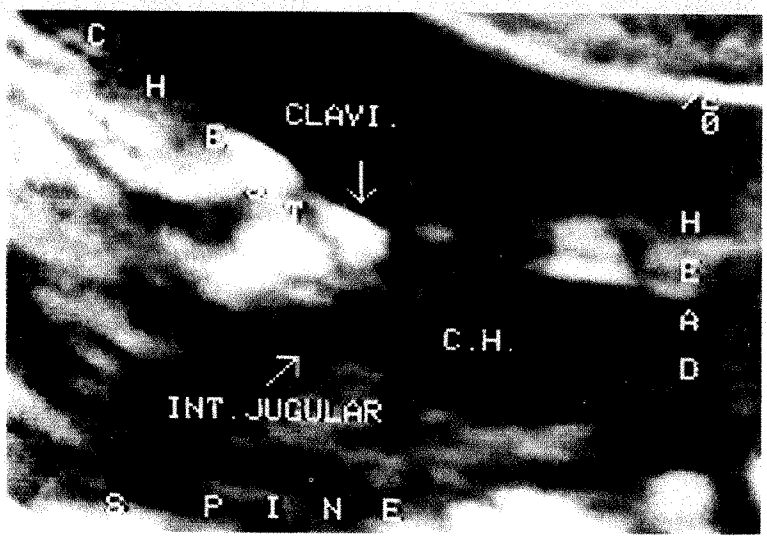


Fig. 1. Cystic hygroma of 14-week-old male fetus (case No. 5), ultimately born normal. Note communication between cystic hygroma *(C.H.)* and lumen of internal jugular vein *(INT. JUGULAR)*. Fetal head, spine, chest, and clavicle *(CLAVI)* are marked.

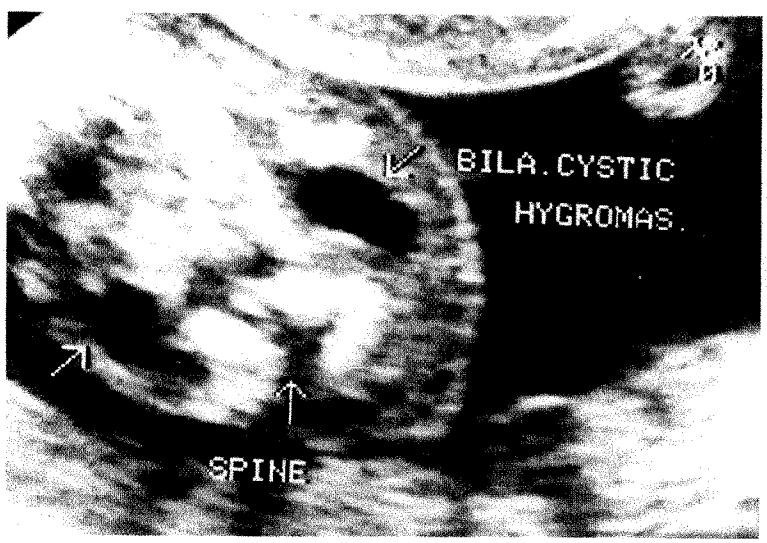


Fig. 2. Transverse scan of fetal neck obviating bilateral nonseptated cystic hygromas at 14 weeks of pregnancy (case No. 6). The nonseptated hygromas were resolved later in pregnancy and a normal male fetus was born at term.

eventually form communications with the venous system and become the terminal portions of the right lymphatic duct and the thoracic duct (Fig. 1). If lymphatic and venous structures fail to communicate, the jugular lymph sacs enlarge and lymph accumulates in tissues.[2] Cystic hygromas of the posterior triangles of the neck (Fig. 2) and hydrops result.[2, 10, 11] If the jugular lymph sacs and the jugular veins then connect or an alternative route of lymphatic drainage develops before intrauterine death, the cystic hygromas regress and peripheral edema may resolve.[2] We believe this sequence of events occurred in three of the cases in our small series (cases No. 5 through 7), and the birth of normal fetuses resulted. The above sequence of embryologic events comprises the jugular-lymphatic obstruction sequence described by Smith[7] and Graham et al.[8]

Two observations corroborate these ideas. Lymphangiography performed on adults with Turner's syndrome demonstrates hypoplasia of peripheral lymphatics in this disorder[12] and raises the possibility that lymph may find an alternative direct route to the venous system while still in the extremities.[2, 13] In addition,



Fig. 3. Transverse scan of fetal neck at 16.5 weeks by transvaginal sonography delineating very thick (9.2 mm) nuchal fold in case No. 5.

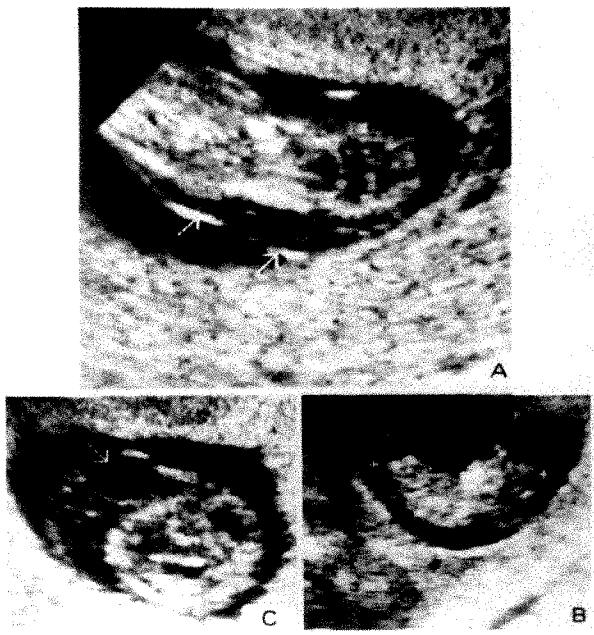


Fig. 4. Septated cystic hygroma in a 9.5-week-old fetus (case no. 1) where the lesion is clearly visualized by transvaginal sonography on longitudinal section **(A)** and on transverse section **(C)** but not detected by transabdominal scan **(B)**. *Arrows* point to the lesion on transvaginal sonography.

Shaub et al.[14] have described a fetus with a unilateral left cystic hygroma and edema of all body parts except the right arm.[2] This pattern suggests that the jugular lymph sac and the thoracic duct that empties into it were obstructed, whereas there was adequate drainage of the right lymph sac and its tributaries.[2] Besides Turner's syndrome, several other congenital malformation syndromes have been reported in association with fetal cystic hygromas, neck webbing (Fig. 3), or redundant posterior neck skin after birth.[1,2] These include Noonan's syndrome, fetal alcohol syndrome, distichiasis-lymphedema syndrome, familial pterygium colli, and several chromosomal aneuploidies: trisomy 18, trisomy 21, 13q-, and 18p-, partial 11q/22q trisomy, trisomy 22 mosaicism, 45,X/46,XX mosaicism, and 47,XXY.[1,2] In our small series 50% of the fetuses had normal karyotype.

The incidence of this malformation at the end of the first trimester to the early midtrimester is around 1% (8 in 800). This disparity is understandable when one takes into consideration the high rate of intrauterine death that occurs in those cases in which hydrops fetalis develops on one side and the spontaneous absorption of those hygromas in eukaryotic fetuses, in whom lymphatic-venous communications develop, on the other side. Previous studies accentuated the grave prognosis in the cases in which the cystic hygroma is associated with hydrops but claimed that only scant information existed about the prognosis in the absence of hydrops.[1,2,15] This statement was probably correct before transvaginal sonography was introduced. When transvaginal sonographic scanning was introduced at the end of the first trimester to the beginning of the midtrimester, it enabled the detection of 30 fetal malformations as early as 9 weeks after the last menstrual period. This relatively new technology enabled us to detect eight cases of fetal cystic hygroma among 800 gestations (1%) routinely scanned for the early detection of fetal malformations. We believe that about 37% of the early detected cystic hygromas, especially the nonseptated ones, bear a good prognosis, according to our small series. Moreover, one of the cases in which cystic hygroma of the neck was diagnosed had undergone abdominal sonographic evaluation without detection of the malformation (case No. 1, Fig. 4).

All the cases in our series of septated cystic hygroma ended in fetal death and/or aneuploidy, whereas 75% (3 of 4) of the cases with a nonseptated lesion resulted in delivery of normal neonates. This may suggest two possible explanations to the diverse prognosis:

1. The septated hygromas, which are also the nuchal ones, may consist of a different category from the nonseptated lesions, which are the more lateral ones and bear a better prognosis; or

2. The septated lesions are associated with complete obstruction of the cervical lymphatic sacs from the communication with the jugular venous system, and the accumulating lymphatic fluid dissects between the cervical tissues creating multilocular cysts. Later, the further accumulating lymph may create subaxillary cystic lesions (observed in one of our cases) and ultimately generalized fetal hydrops (as in cases No. 1, 2, 4, and 8 in our series) (Fig. 5). The nonseptated hygromas may represent temporary accumulation of lymph and

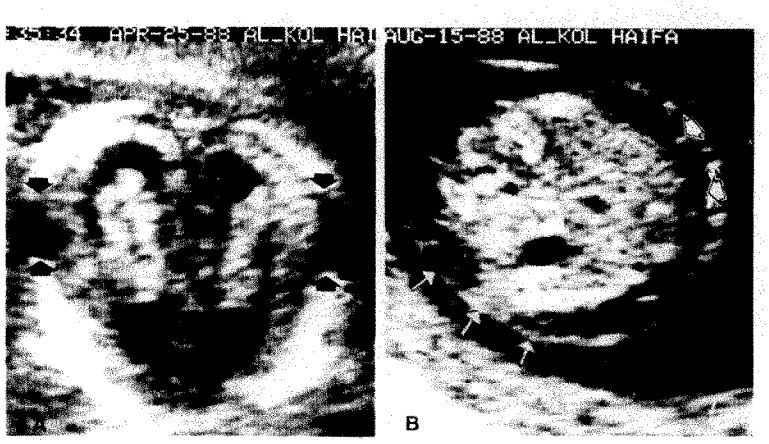


Fig. 5. Fetal hydrops. Transvaginal sonographic scan of fetal skull at 15 weeks (fetus in case No. 2). **A,** Brain edema is diagnosed by relatively big "windows" at lateral sutures of skull *(arrows)* and hyperechogenicity of brain. Choroid plexus appears normal (transverse section). **B,** Fetal hydrops and subcutaneous lymphedema by transvaginal sonographic scan of upper abdominal area. *Arrows* delineate subcutaneous septations caused by generalized lymphedema.

therefore enlargement of the lymphatic sacs lateral to the jugular veins. The increased pressure resulting from this temporary lymph accumulation may overcome an incomplete obstruction between these lymphatic sacs and the jugular veins and result in gradual disappearance of these enlarged sacs. One may speculate that whereas the complete obstruction is associated with chromosomal aneuploidy and the incomplete obstruction is associated with eukaryotic fetuses, the later may be a variant of normal physiologic embryogenesis of the lymphatic jugular venous communications (Fig. 1). In previous reports the diagnosis of cervial hygroma has been made at gestational ages older than 9 to 15 weeks, and mainly the hygromas associated with complete obstruction (septated, multiloculated) were diagnosed, which therefore resulted in a poor prognosis being given to these lesions. Since the availability of transvaginal sonographic technology, the early scanning practiced at 9 to 15 weeks of gestation has enabled the diagnosis of both types of hygromas, and enabled the distinction of "septated lesion, bad outcome" on the one hand versus "nonseptated lesion, good outcome" on the other to be made.

Until more data are collected to support the relatively good prognosis of those cases not associated with hydrops, we recommend amniocentesis for karyotyping and α-fetoprotein measurement in each case of cervical cystic hygroma.

REFERENCES

1. Benacerraf BR, Frigoletto FD Jr. Prenatal sonographic diagnosis of isolated congenital cystic hygroma, unassociated with lymphedema or other morphologic abnormality. J Ultrasound Med 1987;6:63-6.
2. Chervenak FA, Isaacson G, Blakemore KJ, et al. Fetal cystic hygroma: cause and natural history. N Engl J Med 1983;309:822-5.
3. Knurr G. Das cystiche lymphangion des halses. Virchows Arch 1951;319:347-69.
4. Goetsch E. Hygroma colli cysticum and hygroma axillare: pathologic and clinical study and report of twelve cases. Arch Surg 1938;36:394-479.
5. Potter EL, Craig JM. Pathology of the fetus and the infant. 3rd ed. Chicago: Year Book Medical, 1975.
6. Rodis JF, Vintzileos AM, Campbell WA, Nochimson DJ. Spontaneous resolution of fetal cystic hygroma in Down's syndrome. Obstet Gynecol 1988;71:976-7.
7. Smith DW. Recognizable patterns of human malformation: genetic, embryologic and clinical aspects. 3rd ed. Philadelphia: WB Saunders, 1982:472-3.
8. Graham JM, Stephen TD, Shepard TM. Nuchal cystic hygroma in a fetus with presumed Robert's syndrome. Am J Med Genet 1983;15:163-7.
9. Blumenfeld Z, Rottem S, Elgali S, Timor-Tritsch IE. Transvaginal assessment of early embryologic development. In: Timor-Tritsch IE, Rottem S, eds. Transvaginal sonography. New-York: Elsevier Science, 1988:87-108.
10. Timor-Tritsch IE, Rottem S, Blumenfeld Z. Pathology of the early intrauterine pregnancy. In: Timor-Tritsch IE, Rottem S, eds. Transvaginal sonography. New-York: Elsevier Science, 1988:109-24.
11. Van der Putte SCJ. The development of the lymphatic system in man. Adv Anat Embryol Cell Biol 1975;51:3-60.
12. Van der Putte SCJ. Lymphatic malformation in human fetuses: a study of fetuses with Turner's syndrome or status Bonnevie-Ullrich. Virchows Arch [A] 1977;376:233-46.
13. Alvin A, Diehl J, Lindsten J, Lodin A. Lymph vessel hypoplasia and chromosome aberrations in six patients with Turner's syndrome. Acta Derm Venereol (Stockh) 1967;47:25-33.
14. Shaub M, Wilson R, Collea J. Fetal cystic lymphangioma (cystic hygroma): prepartum ultrasonic findings. Radiology 1976;121:449-50.
15. Garden AS, Benzie RJ, Miskin M, Gardner HA. Fetal cystic hygroma colli: Antenatal diagnosis, significance, and management. Am J Obstet Gynecol 1986;154:221-5.

Enlarged cisterna magna in trisomy 18: Prenatal ultrasonographic diagnosis

Amy S. Thurmond, MD[a, b] David W. Nelson, MD,[a] Richard I. Lowensohn, MD,[b] William P. Young, MD,[b] and Lowell Davis, MD[b]

Portland, Oregon

Ultrasonographic visualization of an enlarged cisterna magna was the initial clue that led to the prenatal diagnosis of trisomy 18 in five patients. Prenatal diagnosis of trisomy 18, a lethal defect, is important for proper patient counseling and management. (AM J OBSTET GYNECOL 1989;161:83-5.)

Key words: Ultrasonography, trisomy 18, cerebellum, cisterna magna

Trisomy 18 is the second most common trisomy syndrome, occurring in 0.3/1000 live births.[1,1] The prenatal diagnosis of trisomy 18 currently depends on the recognition of a constellation of subtle findings: fetal structural anomalies detected by ultrasonography, intrauterine growth retardation (IUGR), and abnormal amniotic fluid volume.[1-3] The diagnosis is then confirmed by chromosome analysis of either amniotic fluid or fetal blood.

We report the study of five fetuses in whom prenatal identification of an enlarged cisterna magna (presumably a result of cerebellar hypoplasia) caused us to search for more subtle structural fetal abnormalities, and led to the diagnosis of trisomy 18. Our results suggest that an enlarged cisterna magna may be a more specific ultrasonographic finding for diagnosis of trisomy 18.

From the Departments of Diagnostic Radiology[a] and Obstetrics and Gynecology,[b] Oregon Health Sciences University.
Received for publication August 19, 1988; revised January 6, 1989; accepted February 8, 1989.
Reprint requests: Amy S. Thurmond, MD, Oregon Health Sciences University, 3181 S.W. Sam Jackson Park Rd., Portland, OR 97201.

Methods

All patients referred to our institution for ultrasonography to exclude fetal anomalies have a sector real-time ultrasound examination (Acuson, Mountainview, Calif.; ATL, Bothell, Wash.) that includes a transaxial view of the head angled 15 degrees caudal to the canthomeatal line. Measurements are taken of the transverse cerebellar diameter according to the method of McLeary et al.[4] and of the anteroposterior cisterna magna diameter according to Mahoney et al.[5] (Fig. 1).



Fig. 1 Ultrasonogram of fetus with trisomy 18 (patient No. 3) at 36½ weeks' gestation. Transducer is angled 15 degrees caudal to BPD plane. Transverse cerebellar diameter *(long arrows)* is small, and cisterna magna depth *(short arrows)* is large.

Table I. Summary of data in five fetuses with trisomy 18

	Clinical history			
Patient no.	*Maternal age (yr)*	*Gestation by dates (wk)*	*Ultrasound indication*	*Fetal monitoring*
1	32	34	PROM	Variable decelerations
2	25	36.5	IUGR(?)	
3	28	36.5	IUGR(?)	Variable decelerations
4	25	32	PROM	Variable decelerations
5	19	36.5	Fetal bradycardia	Reactive, sinus bradycardia

CI, Cephalic index = biparietal diameter/occipitofrontal diameter; *PROM*, premature rupture of membranes; *VSD*, ventricular septal defect.

Five fetuses in whom the measurements were abnormal had more detailed ultrasonographic examination, which included visualization of the hands and feet, and echocardiography. These tests are not usually performed as part of our ultrasonographic screening for fetal anomalies. Except for one patient whose labor could not be arrested, subsequent chromosome analysis of amniotic fluid or fetal cord blood was performed. All patients were delivered of their infants in our hospital, and clinical and autopsy findings were reviewed.

The five patients were seen in a 1-year period from March 1987 to March 1988. Retrospective review of all prenatal or neonatal genetic studies, and review of all deliveries for this same time period did not reveal any additional patients with trisomy 18.

Results

Results are summarized in Table I and include the gestational age of the fetus at initial examination, the indication for ultrasonography, prospective ultrasonographic findings, and the predicted biparietal diameter (BPD) determined from the measured cerebellar width.

All patients were examined in the third trimester. One patient (No. 2) was also examined at 20 weeks for confirmation of dates, at which time only mild bilateral fetal hydronephrosis was noted. Indications for ultrasonographic examination were premature rupture of the membranes (patients 1 and 4), suspicion of IUGR (patients 2 and 3), and fetal bradycardia (patient 5).

All five fetuses had small cerebella. This was concluded because the predicted BPD, which was based on the measured transverse width of the cerebellum, was smaller than the measured BPD.[4] (The BPD was thought to be reliable in all fetuses since measurements were consistent with gestational age by menstrual history and because the cephalic index was within normal limits.[6] The small cerebella were presumed not to be caused by IUGR[7] (present in fetuses 2, 3, and 4), because in fetuses without chromosomal abnormalities the cerebellar size is unaffected by IUGR.[8] All five fetuses had enlarged cisterna magnas that measured 10 to 23 mm (normal, 5 ± 3 mm[5, 9]).

The posterior fossa findings prompted a more detailed search for other abnormalities and revealed a ventricular septal defect (patients 1 and 5), rocker-bottom feet (patients 2 and 4), and cleft lip (patient 2). The constellation of ultrasonographic findings suggested a trisomy syndrome. Chromosome analysis of amniotic fluid or fetal cord blood confirmed the diagnosis in patients 2, 3, 4, and 5. These fetuses were delivered vaginally without monitoring and either were stillborn or died shortly after birth. Patient 1 presented in active labor and was counseled regarding the high likelihood of a lethal chromosomal abnormality in her fetus. However, because fetal distress developed before chromosome analysis could be performed, the fetus was delivered by cesarean section and died 7 days later.

Newborn No. 1 had a postpartum computed tomography scan of the head that revealed cerebellar and vermian hypoplasia with partially fused lateral ventricles. Fetus No. 2 had an autopsy that indicated cerebellar hypoplasia. Fetuses 3, 4, and 5 did not undergo postmortem examination.

Additional findings that were not identified by prenatal ultrasonography were single umbilical artery, short sternum, finger flexions, and left diaphragmatic eventration (fetus No. 1); small placenta and low-set ears (fetus No. 2); clenched fists and low-set ears (fetus No. 3); and hypotelorism, clinodactyly, and low-set ears (fetus No. 4).

Comment

The more common findings in trisomy 18 are quite subtle: polyhydramnios, small placenta, single umbilical artery, growth deficiency, prominent occiput, narrow palatal arch, low-set ears, clenched or overlapping fingers, short sternum, inguinal and umbilical hernias,

Prospective ultrasonographic findings						
Amniotic fluid	*CI (%)*	*Fetal anomalies*	*Cerebellar width (cm)*	*Predicted BPD (cm)*	*Actual BPD (cm)*	*Cisterna magna (cm)*
None	82.8	VSD, hypotelorism	3.2	6.6-7.4	8.0	1.6
Slightly decreased	79.4	IUGR, bilateral hydronephrosis, cleft lip, rocker-bottom foot	3.7	7.3-8.1	8.8	1.7
Increased	85.8	IUGR	4.1	7.8-8.5	8.6	2.3
Increased	81.9	IUGR, rocker-bottom foot, hypotelorism	3.3	6.8-7.5	8.3	2.1
Decreased	81.5	VSD	4.5	8.2-8.9	9.1	1.0

ventricular and atrial septal defects, and patent ductus arteriosus.[1] Cerebellar hypoplasia in trisomy 18 is well described but is thought to occur in <10% of affected persons.[1, 10] Our experience suggests this abnormality may be present more often. A recent study utilizing detailed computed tomography and postmortem morphometric analysis of the cerebellum in nine newborns with trisomy 18 supports this concept.[11] All of the patients had cerebellar hypoplasia as a dominant finding, including three who also had hypoplasia of the posterior cranial fossa. This latter observation may explain our patient No. 5, who had a small cerebellum but only a mildly enlarged cisterna magna.

The importance of a large cisterna magna–small cerebellum for prenatal diagnosis of trisomy 18 has been suggested previously based on examination of two fetuses in the third trimester.[8] However, recent reviews of the ultrasonographic findings in trisomy 18 did not include large cisterna magna or small cerebellum as a finding.[2, 3] Cerebellar hypoplasia in infants with open neural tube defects has been described.[12] The incidence of large cisterna magna–small cerebellum by prenatal ultrasonography in trisomy 18 and in other conditions is not known; therefore the predictive value of a normal or abnormal measurement cannot be determined. In a 1-year period at our institution, all five patients diagnosed with trisomy 18 had a large cisterna magna and small cerebellum.

It is also not known how early this finding is present. In the patient initially evaluated at 20 weeks for confirmation of dates, large cisterna magna–small cerebellum was not visualized. We do not know whether in fact it was not present, or was present but not recognized.

The importance of any ultrasonographic finding relates to its impact on clinical management. Except for the patient who presented in active labor, all patients were spared unnecessary monitoring during labor and cesarean sections for fetal distress. Each patient, including the patient in active labor, benefited from counseling before delivery.

Measurements of the cerebellum and cisterna magna are easily obtained by angling the transducer 15 degrees caudal to the standard axial plane used for determining BPD. It is hoped that determination of these parameters in a large number of patients will reveal the true incidence of large cisterna magna–small cerebellum in trisomy 18 and other conditions. Enhanced attention to this finding may also result in its visualization in the first or second trimesters, leading to even earlier diagnosis of trisomy 18.

REFERENCES

1. Smith DW. Recognizable patterns of human malformation. 3d ed. Philadelphia: WB Saunders, 1982:14-5.
2. Bundy AL, Saltzman DH, Pober B, Fine C, Emerson D, Doubilet PM. Antenatal sonographic findings in trisomy 18. J Ultrasound Med 1986;5:361-4.
3. Benacerraf BR, Miller WA, Frigoletto FD. Sonographic detection of fetuses with trisomies 13 and 18: accuracy and limitations. Am J Obstet Gynecol 1988;158:404-9.
4. McLeary RD, Kuhns LR, Barr M. Ultrasonography of the fetal cerebellum. Radiology 1984;151:439-42.
5. Mahoney BS, Callen PW, Filly RA, Hoddick WK. The fetal cisterna magna. Radiology 1984;153:773-6.
6. Jeanty P, Romero R. Obstetrical ultrasound. New York: McGraw-Hill, 1984:91.
7. Sanders RC, James AE. Principles and practice of ultrasonography in obstetrics and gynecology. Norwalk, Connecticut: Appleton-Century-Crofts, 1985:163-7.
8. Reece EA, Goldstein I, Pilu G, Hobbins JC. Fetal cerebellar growth unaffected by intrauterine growth retardation: a new parameter for prenatal diagnosis. Am J Obstet Gynecol 1987;157:632-8.
9. Comstock CH, Boal DB. Enlarged fetal cisterna magna: appearance and significance. Obstet Gynecol 1985;66: 25S-27S.
10. Bergsma D. Birth defects compendium. 2nd ed. New York: Liss, 1979:201-2.
11. Nakamura Y, Hashimoto T, Sasaguri Y, et al. Brain anomalies found in 18 trisomy: CT scanning, morphologic and morphometric study. Clin Neuropathol 1986;5:47-52.
12. Pilu G, Roberto R, Reece EA, Goldstein I, Hobbins JC, Bovicelli L. Subnormal cerebellum in fetuses with spina bifida. Am J Obstet Gynecol 1988;158:1052-6.

Neonatal mortality in normal birth weight babies: Does the level of hospital care make a difference?

Cynthia J. Berg, MD,[a] Charlotte M. Druschel, MD, MPH,[a] Brian J. McCarthy, MD,[a] Michael LaVoie,[b] and R. Louise Floyd, RN, MSN[c]

Atlanta, Georgia

Although neonatal intensive care for low birth weight infants has been extensively studied, few researchers have looked at the impact of the level of care at the delivery hospital for infants weighing >2500 gm. Using linked birth-death records from Georgia for 1979 to 1982, we examined the effect that the level of care available at the hospital of delivery had on neonatal mortality in infants weighing 2500 gm and above. We used a risk scoring system designed for use with vital records to determine prepartum risk and the presence or absence of a complication of labor as indicated on the birth certificate to determine intrapartum risk. We found that women with a high prepartum risk score had increased neonatal mortality at level 1 hospitals. Women who developed a complication of labor, regardless of their prepartum risk status, had the highest neonatal mortality rates when they delivered at level 1 hospitals. We suggest prenatal risk assessment for all women and referral of high risk women to level 2 or 3 hospitals for delivery even at term. (AM J OBSTET GYNECOL 1989;161:86-91.)

Key words: Neonatal mortality, regionalization, high-risk pregnancy, hospital level

One of the 1990 *Objectives for the Nation*[1] is to reduce infant mortality to 9/1000 livebirths. Two thirds of U.S. infant deaths occur during the neonatal period (within the first 28 days of life). Among the more successful intervention strategies developed in an attempt to lower neonatal mortality is the establishment of neonatal intensive care units—special hospital facilities for the care of critically ill newborns.

Because birth weight is the strongest predictor of neonatal mortality, we tend to believe that neonatal deaths occur predominantly in low birth weight infants. However, 93% of all infants born in Georgia from 1979 to 1982 weighed 2500 gm or more, and these non–low birth weight infants contributed 26% of the neonatal deaths in the state in that time period. In the Closing the Gap conference in 1985, sponsored by the Carter Center, researchers from the World Health Organization Collaborating Center in Perinatal Care reported that the United States could, with the use of current technologies, reduce infant mortality by 40%.[2] Of the achievable reduction in neonatal mortality, 95% was among infants weighing ≥2500 gm.

Numerous investigators have looked at the effect of the hospital of birth on neonatal mortality rates in low birth weight and very–low birth weight infants, and have found that care in a neonatal intensive care unit improved the survival of these babies.[3-5] However, few investigators have examined the effect of neonatal care facilities in non–low birth weight infants on a population basis.[6, 7]

We examined the impact that the level of neonatal care available at the hospital of delivery had on neonatal mortality in infants with birth weights of 2500 gm and greater (which, for the sake of convenience, we will refer to as normal birth weight babies[7]). We used the presence of prenatal and intrapartum risk factors to help predict neonatal mortality and assess the appropriate level of care for babies in this birth weight group. We used a population-based data set for the years 1979 to 1982, a time period during which neonatal intensive care was widely available.

Methods

Data for this study were provided by the Office of Vital Records, Georgia Department of Human Resources. All birth certificates of infants born to Georgia residents are entered into a computer data base. Each reported death of an infant under 1 year of age who

From the Division of Birth Defects and Developmental Disabilities, Center for Environmental Health and Injury Control, Centers for Disease Control, Public Health Service, United States Department of Health and Human Services,[a] and the Office of Vital Records[b] and Family Health Services,[c] Georgia Department of Human Resources.

A preliminary, partial version of these findings was presented at the 113th Annual Meeting of the American Public Health Association, Washington, DC, November 18, 1985, and summarized in Fam Plann Perspect 1985;17:229.

Received for publication August 29, 1988; revised January 19, 1989; accepted January 23, 1989.

Reprint requests: Cynthia J. Berg, MD, Division of Birth Defects and Developmental Disabilities, Center for Environmental Health, Centers for Disease Control, 1600 Clifton Road, Atlanta, GA 30333.

Table I. Maternal prepartum risk score calculation[14]

Variable	*Coding*	*Weight*
Race	White = 0 Black = 1	5
Maternal age (yr)	≥20 = 0 <20 = 1	6
Prior fetal loss	No = 0 Yes = 1	6
Prior low birth weight delivery	No = 0 Yes = 1	11
Complication unrelated to pregnancy*	No = 0 Yes = 1	8
Complications unrelated to pregnancy†	No = 0 Yes = 1	26
Multiple gestation	No = 0 Yes = 1	38

Risk group based on risk score

Total score	Risk group
0-5	Low
6-15	Moderate
16-100	High

*Hypertension, diabetes, drug addiction, cardiac or renal disease, pyelonephritis, syphilis, rubella.

†Toxemia, bleeding, infection, anemia, urinary tract infection, Rh sensitization.

was born in Georgia is manually linked to its birth certificate. The birth and infant death data bases are merged, providing linked birth-death records by birth year cohort. Check boxes for complications of pregnancy, complications not related to pregnancy, and complications of labor and delivery, as well as other data from the birth certificate, were used to calculate prepartum and intrapartum risk.

All 315,830 singleton, liveborn infants born to Georgia residents between Jan. 1, 1979, and Dec. 31, 1982, with birth weights 2500 gm and above, made up the study population. Neonatal mortality was defined as deaths of liveborn infants at less than 28 days of age per 1000 livebirths.

To assess maternal prepartum risk, we used a neonatal mortality risk scoring system designed specifically for use in studies using vital record data.[8] This system, developed with discriminant analysis, predicts the risk of neonatal death by using information that is available before the onset of labor and that is on the Georgia birth certificate. Seven variables are weighed and summed, yielding a risk score. Scores have been combined into three risk groups—low, moderate, and high (Table I).

As a measure of risk during the intrapartum period, we used the presence of a complication of labor as recorded on the birth certificate. These complications included abruptio placenta, placenta praevia, prolapsed cord, dystocia, maternal hypotension, chorioamnionitis, and breech presentation.

Table II. Distribution (percent) of births and risk-specific neonatal mortality rates* by prepartum risk status in normal birth weight infants: Georgia, 1979 to 1982

Prepartum risk status	*% of births*	*NMR*
Low	55	1.7
Moderate	34	2.3
High	12	4.1
RR of neonatal death 2.5 (95% CI: 2.1, 3.0) (High- vs low-risk group)		

NMR, Neonatal mortality rates; *RR*, relative risk; *CI*, confidence interval.

*Per 1000 livebirths.

Hospitals were classified as having level 1, 2, or 3 neonatal care capacity by the Georgia Maternal and Infant Council in accordance with the joint American Academy of Pediatrics/American College of Obstetricians and Gynecologists *Guidelines for Perinatal Care*. Assignment of neonatal care level was based on on-site visits by members of the Maternal and Infant Council under the direction of the each of the five regional perinatal care directors. Level 1 facilities provide care for normal, healthy newborns with the capacity to stabilize sick infants for transport. Level 2 facilities have the capacity for an intermediate level of care of more complicated cases, including the administration of intravenous fluids, continuous positive airway pressure and short-term ventilation, and treatment of pneumothoraces. A level 3 facility contains a neonatal intensive care unit capable of complete care of the high-risk fetus, neonate, and mother, has a full-time neonatologist on staff, and can provide specialized neonatal surgery. Information on maternal or prepartum transfer between hospitals was not available. Thus the term "hospital of delivery" refers to the hospital in which the actual delivery occurred.

Statistical analysis was done by means of the Statistical Analysis System (SAS) software package. Rates were compared with the χ^2 technique. Confidence intervals for relative risks were calculated by the Taylor series method.[9]

Results

Neonatal mortality rate by year. The crude neonatal mortality rate for singleton infants weighing 2500 gm and above born in Georgia was 2.3/1000 livebirths in 1979, 2.4 in 1980, 2.1 in 1981, and 1.8 in 1982, with an overall rate for the 4-year period of 2.1/1000 livebirths.

Prepartum risk score. The distribution of births by prepartum risk score group is shown in Table II. More than half of the women delivering normal birth weight infants fell into the low-risk category, whereas 12%

Table III. Distribution (percent) of births by prepartum risk status and hospital level in normal birth weight infants: Georgia, 1979 to 1982

Prepartum risk status	*Hospital level*			
	1	*2*	*3*	*%*
Low	57	23	20	100
Moderate	56	21	22	100
High	55	19	25	100
Overall	57	22	21	100

Table IV. Neonatal mortality by prepartum risk status and hospital level, normal birth weight infants: Georgia, 1979 to 1982

Prepartum risk status	*Hospital level*			*RR of neonatal death at level 1 vs level 3 hospital (95% CI)*
	1	*2*	*3*	
Low	1.7	1.4	1.8	1.0
Moderate	2.4	2.1	2.1	1.2
High	5.0	3.1	3.1	1.6 (1.1, 2.4)

RR, Relative risk; *CI*, confidence interval.

were classified as high risk. The risk of a neonatal death was 2.5 times greater for a high-risk woman compared with a low-risk woman.

Level of care at hospital of delivery. More than half of the women with normal birth weight infants delivered at level 1 hospitals, regardless of their risk status (Table III). The remainder of the deliveries were almost equally divided between level 2 and 3 hospitals, although slightly more high-risk women delivered at level 3 hospitals than at level 2 hospitals.

The effect of the interaction between the prepartum risk group of women and the level of care at the birth hospital on neonatal mortality is shown in Table IV. For low- and moderate-risk women, there was no statistically significant difference in the neonatal mortality rate at level 1, 2, and 3 hospitals. However, for high-risk women, the risk of having a neonatal death was 1.6 times greater if the infant was delivered at a level 1 hospital, compared with a level 2 or 3 hospital.

Intrapartum complications of labor. Complications of labor were reported more frequently in pregnancies among women in the high prepartum risk group—23.7% of such cases compared with 13.8% of low prepartum-risk women (Table V). The rate of these complications was uniformly elevated, from 1.5- to 1.9-fold, among high-risk women compared with low-risk women in all level hospitals. In addition, we found an increase in the reported frequency of complications of labor among low- and moderate-risk women who delivered at level 2 and 3 hospitals.

The presence of an intrapartum complication increased the neonatal mortality rate in all the prepartum risk groups and at all hospital levels, with the neonatal mortality rate as much as five times greater if a complication of labor was present (Table VI). For women who experienced a complication of labor, the risk of a neonatal death was greatest at a level 1 hospital, whatever the prepartum risk status of the mother. For moderate- and high-risk women with a complication of labor, the risk of a neonatal death was least at a level 3 hospital and highest at a level 1 hospital. For low-risk women with a complication of labor, the risk of a neonatal death was essentially the same at level 2 and 3 hospitals.

To evaluate whether the level of hospital or the size (number of deliveries) of the hospital was the factor responsible for this increased risk of neonatal death in high-risk women delivering at level 1 hospitals, we divided level 1 hospitals into those with 1000 or more deliveries and those with under 1000 deliveries per year. This placed 25% of the level 1 deliveries in the high-volume level 1 hospitals and 75% of the level 1 deliveries in the lower volume level 1 hospitals. We found no difference in the neonatal mortality rates in the high-volume versus lower-volume level 1 hospitals among women with an intrapartum complication of labor, regardless of their prepartum risk status (data available on request).

Comment

With few dissenting voices, the concept of regionalization of perinatal services is well accepted today. Regionalization implies providing a level of care for the fetus, mother, and infant that matches the level of risk of the maternal-fetal unit. Regionalization requires (1) an assessment of risk status and (2) provision of the appropriate level of services to the patients, including referral and transport if necessary. This idea has been better explored and accepted for low birth weight and, especially, very low birth weight deliveries than it has for normal birth weight deliveries. Looking at linked birth-death records from four states for 1978 and 1979, Gortmaker et al.[5] found significantly lower mortality rates in infants weighing 750 to 1500 gm who were delivered at level 3 hospitals. Paneth et al.[4] found similar results in 501 to 2250 gm infants born in New York City between 1976 and 1978. Other studies have shown an increasing percentage of deliveries of very low birth weight infants occurring at level 3 centers in the past 15 years.[10] This increased delivery of low birth weight and especially very low birth weight infants at level 3 centers has been found to be associated with a decrease in birth weight–specific neonatal mortality rates.[4, 10]

However, few investigators have looked at the effect

Table V. Reported intrapartum complications (percent) by prepartum risk status and hospital level in normal birth weight infants: Georgia, 1979 to 1982

Prepartum risk status	*Hospital level*			
	1	*2*	*3*	*Overall*
Low	11.7	16.7	13.8	13.8
Moderate	11.2	15.6	15.2	13.1
High	22.4	26.2	24.7	23.7
RR of intrapartum complication 1.7 (95% CI, 1.7, 1.8) (high- vs low-risk women)				

RR, Relative risk; *CI*, confidence interval.

Table VI. Neonatal mortality by prepartum and intrapartum risk status and hospital level in normal birth weight infants: Georgia, 1979 to 1982

Prepartum risk status	*Intrapartum complication*	*Hospital level*			
		1	*2*	*3*	*Overall*
Low	No	1.1	1.1	1.3	1.1
	Yes	5.4	3.3	3.6	4.4
Moderate	No	1.8	1.7	1.9	1.8
	Yes	5.8	4.5	2.7	4.7
High	No	3.0	1.4	1.9	2.4
	Yes	11.5	7.6	5.6	9.1

of the level of care in normal birth weight infants. In an analysis of regional perinatal mortality in Norway, Bakketeig et al.[11] found lower fetal and early neonatal mortality rates in those areas that had higher levels of perinatal medical services, an effect that was most pronounced in infants weighing >3500 gm. In South Carolina, perinatal mortality rates were lower in a program that required delivery at a level 2 or 3 hospital for all participants.[12]

Our results from Georgia seem to indicate several things. Even among women delivering normal birth weight babies, women with a high prepartum risk score had an increased chance of experiencing a neonatal death. However, this risk was lower if the mother delivered at a hospital having level 2 or 3 neonatal care facilities. When looking at prepartum level of risk alone, we found no difference in the risk-specific neonatal mortality rates for normal birth weight infants born in level 2 versus level 3 hospitals.

Complications during labor, however, can develop in any pregnancy. Women who were high risk before the onset of labor had a risk of developing a complication during labor and delivery that was 70% greater than that of low-risk women. This increase in intrapartum complications in high prepartum risk women was present at all hospital levels. Overall, almost one quarter of all women who were high risk in the prepartum period developed some problem in the intrapartum period.

As would be expected, a complication of labor increased the risk of neonatal death in all the risk groups and at all the hospital levels. The chance of neonatal death was from 1.4 to 5.4 times greater in women with an intrapartum complication compared with women of the same prepartum risk group delivering at the same level of hospital who did not develop a complication of labor.

When we stratified by the presence or absence of an intrapartum complication, we found an interactive effect in the relationship between prepartum level of risk, hospital level of care, and risk of neonatal death. In women *without* a complication of labor, those with low and moderate prepartum risk had a risk of a neonatal death that was essentially the same for all levels of hospital care. However, for high prepartum risk women without a complication of labor, we found a 58% increased risk of a neonatal death if the woman delivered at a level 1 compared with a level 3 hospital.

However, for women *with* an intrapartum complication, we found that within *each* risk group, there was a gradient of decreasing neonatal mortality with increasing level of hospital care. For each risk group, women who developed a complication of labor had the greatest risk of neonatal death if they delivered at a level 1 hospital. This was not a function of the volume of deliveries at the level 1 hospitals but seemed to be a characteristic of the level 1 status of the facility.

Despite the lower risk of neonatal death among women in the high prepartum risk group, as well as all women developing a complication of labor if they delivered at a higher level hospital, there was little evidence of referral of such women to these higher level facilities. In its recent evaluation of the cost and effectiveness of neonatal intensive care in low birth weight infants,[13] the U.S. Office of Technology Assessment found that some very low birth weight infants were not delivered at level 3 hospitals despite the fact that improved outcomes, both in mortality and long-term development, were found with referral to neonatal intensive care units. The Office of Technology Assessment found three main reasons for the lack of use of this technology: (1) most important, a lack of awareness on the part of physicians of the improvement in outcome with delivery at an appropriate level facility; (2) the desire of physicians or lower level hospitals to be viewed as capable of providing a complete range of services, and (3) financial inability of the woman to pay for the high cost of neonatal intensive care.

We suspect that the factors cited by the Office of Technology Assessment may play an even greater role in the lack of referral in the case of normal birth weight

infants. The knowledge that normal birth weight babies can and do suffer excess mortality despite their overall low neonatal mortality rate is not widespread and thus may not be fully appreciated. Thus the advisability of referring a high-risk woman who has reached term is frequently not considered. Because of the public perception that all normal birth weight infants survive, hospitals and physicians may be especially reluctant to refer women near term, feeling that it reflects adversely on their abilities. In addition, the prepartum diagnosis of low birth weight and very low birth weight is relatively straightforward, especially in patients for whom gestational age is known. To assess prepartum risk on the basis of criteria other than birth weight, a formal prepartum risk assessment needs to be performed. Although this concept has been discussed for many years, especially in the public health sector, it is not currently the norm in all obstetric practices. The risk scoring method that we used for this study was designed specifically for use with vital record data. However, many excellent clinically based risk systems are available[14, 15] that can incorporate those factors found in prenatal care and provide the caregiver with a more detailed picture of the woman's risk status.

Because we performed a study using linked vital records, we encountered those limitations common to all studies in which vital records are used. (1) The precision of our risk assessment, diagnosis of complications of labor, and inability to look at maternal and neonatal transport were limited by our data source. However, vital records offer the advantage of large numbers of births and deaths and readily available data. We feel that it is an appropriate first approach to take when looking at an event, such as neonatal death in normal birth weight infants, that occurs at a relatively low rate (2.1/100 livebirths in our population). (2) Information on risk factors and complications of labor were recorded retrospectively, with the possibility of recall bias. However, the risk factors are obtained from the maternal medical record and are prepartum events. Thus neonatal outcome should not bias the calculation of the risk score. (3) The potential for incomplete ascertainment exists whenever vital records are used. Since 1974, Georgia birth certificates have contained check boxes for complications of pregnancy, complications not related to pregnancy, and complications of labor and delivery, a method shown to capture more complications than open-ended items. In 1979 to 1982, 95% of Georgia birth certificates were complete for these complications, which leads us to believe that overall ascertainment is satisfactory. In addition, the fact that there was an increased rate of complications of labor in high-risk women at all hospitals indicates that there was not a substantial difference in reporting at the different levels of hospitals.

On the basis of our findings, we suggest that neonatal mortality in normal birth weight infants might be lowered by performing an appropriate clinical risk assessment on all pregnant women and planning to deliver those women with a high prepartum risk score at a hospital with level 2 or 3 neonatal care facilities. Those women who develop a complication of labor, even at term, should be considered for maternal transport if this can be safely accomplished. For this to occur, perinatal care providers and the public need to be educated about the importance of risk assessment and delivery at an appropriate level facility for all babies, regardless of birth weight. Further research should be conducted to corroborate the findings of this study, which used clinical risk assessment instruments and evaluated the safety of intrapartum transport.

If we are to meet our 1990 objectives for neonatal mortality, obstetricians and public health planners must identify those groups at increased risk of mortality and develop specific intervention strategies targeted at specific groups.[16] To achieve this in this era of emphasis on decreasing medical care costs, it is important to use medical technology and health care resources in the most cost-effective manner while still striving for the lowest mortality rate. This implies that the level of care provided directly corresponds to the risk of experiencing an adverse outcome. Our challenge must be to use our current technology in the most effective manner to improve the outcome for all infants, including the 95% that are not low birth weight.

REFERENCES

1. U.S. Public Health Service. Promoting health, preventing disease—objectives for the nation. Washington, DC: U.S. Department of Health and Human Services, 1980.
2. WHO Collaborating Center in Perinatal Care and Health Service Research in Maternal and Child Health. Unintended pregnancies and infant mortality and morbidity: strategies and interventions for closing the gap. Presented at the Closing the Gap National Health Policy Consultation at the Carter Center of Emory University, Atlanta, Ga., Nov. 26-28, 1984.
3. Cordero O, Backes CR, Zuspan FP. Very low–birth weight infants. 1. Influence of place of birth on survival. Am J Obstet Gynecol 1982;143:533-7.
4. Paneth N, Kiely JL, Wallenstein S, et al. Newborn intensive care and neonatal mortality in low-birth weight infants. N Engl J Med 1982;307:149-55.
5. Gortmaker S, Sobol A, Clark C, et al. The survival of very low–birth weight infants by level of hospital of birth: a population study of perinatal systems in four states. Am J Obstet Gynecol 1985;152:517-24.
6. Philips JB, Dickman AM, Resnick MB, et al. Characteristics, mortality and outcome of higher-birth weight infants who require intensive care. Am J Obstet Gynecol 1984;149:875-9.
7. Paneth N, Wallenstein S, Kiely JL, et al. Medical care and preterm infants of normal birth weight. Pediatrics 1986; 77:158-65.
8. McCarthy BJ, Schultz K. Identifying neonatal risk factors and predicting neonatal deaths in Georgia. Am J Obstet Gynecol 1982;142:557-62.

9. Kleinbaum DG, Kupper LL, Morgenstern H. Epidemiologic research: Principles and quantitative methods. Belmont, Calif.: Lifetime Learning Publications, 1982.
10. McCormick MC, Shapiro S, Starfield MD, et al. The regionalization of perinatal services. JAMA 1985;253:799-804.
11. Bakketeig LS, Hoffman HJ, Sternthal PM. Obstetric service and perinatal mortality in Norway. Acta Obstet Gynecol Scand 1978;77(suppl):1-19.
12. Heins HC, Miller JM, Sear A, et al. Benefits of a statewide high-risk perinatal program. Obstet Gynecol 1983;62: 294-6.
13. U.S. Office of Technology Assessment. Neonatal intensive care for low birthweight infants: costs and effectiveness. Health technology case study 38. Washington, DC: Office of Technology Assessment, 1987.
14. Hobel CJ, Hyvarinen MA, Okada DM, Oh W. Prenatal and intrapartum high-risk screening 1. Prediction of the high-risk neonate. AM J OBSTET GYNECOL 1973;117:1-9.
15. Sokol RJ, Rosen MG, Stojkov J, Chik L. Clinical application of high-risk scoring on an obstetrical service. AM J OBSTET GYNECOL 1977;128:652-61.
16. Friede AM, Rochat RW. Maternal and perinatal mortality: An epidemiologic perspective. In: Sachs BP, Acker D, eds. Clinical obstetrics: a public health perspective. Littleton, Mass.: PSG Publishing Co, 1986:1-34.

Postdate fetal surveillance: Is 41 weeks too early?

Denise A. Guidetti, MD, Michael Y. Divon, MD, and Oded Langer, MD
Bronx, New York

During an 18-month period, 293 patients had a nonstress test and ultrasonographic evaluation of amniotic fluid volume twice weekly beginning at 41 weeks' gestation. Patients were admitted for induction of labor for either an abnormal nonstress test result or oligohydramnios. A control population consisted of 59 low-risk patients who were delivered between 39 and 41 weeks' gestation and had antepartum testing within 4 days of delivery. Study patients who were delivered between 41 and 42 weeks' gestation had a significantly increased incidence of abnormal nonstress tests, oligohydramnios, cesarean sections for fetal distress, and admissions to the neonatal intensive care unit compared with control patients. The abnormal fetal testing and adverse perinatal outcome associated with pregnancies over 42 weeks were also found in pregnancies between 41 and 42 weeks' gestation. These data support the concept that postdate fetal testing should begin at 41 weeks of gestation. (AM J OBSTET GYNECOL 1989;161:91-3.)

Key words: Postdate pregnancy, antepartum fetal testing

More than 25 years ago, McClure-Browne[1] showed that perinatal morbidity and mortality are increased after 42 weeks' gestation. Eighty percent of these deaths occurred before or during labor.[2] The presumed pathogenesis of the complications associated with the postdate pregnancy relates to progressive uteroplacental insufficiency. As gestational age advances, uteroplacental insufficiency leads to fetal asphyxia, oligohydramnios, meconium aspiration, decreased subcutaneous tissue, and in severe cases fetal central nervous system damage and death.[3]

In recent years improved outcome has been realized through advances in fetal testing. Electronic fetal monitoring and ultrasonography have helped identify the postdate fetus who would benefit from delivery. As a result, current trends favor selective intervention instead of routine induction. A variety of testing protocols have been designed and usually incorporate some combination of the nonstress test, contraction stress test, biophysical profile, or amniotic fluid volume assessment, either weekly or twice weekly. These testing schemes are usually initiated when the pregnancy had exceeded 42 weeks from the onset of the last menstrual period. However, the changes ascribed to the uteroplacental insufficiency of postdate pregnancies probably represent a continuum that may in fact begin before 42 weeks. Several recent studies suggest that perinatal morbidity and mortality actually begin after 40 weeks' gestation.[4, 5]

The purpose of this study was to assess the value of antepartum fetal testing in the 41- to 42-week period.

Material and methods

The study group consisted of 293 patients who had fetal testing at the Hospital of the Albert Einstein College of Medicine between July 1986 and January 1988. To assure accurate assignment of gestational age, study

Department of Obstetrics and Gynecology, Albert Einstein College of Medicine of Yeshiva University.
Received for publication July 20, 1988; revised December 1, 1988; accepted January 10, 1989.
Reprint requests: Michael Y. Divon, MD, Department of Obstetrics and Gynecology, Hospital of the Albert Einstein College of Medicine, Rm. 709, 1825 Eastchester Rd., Bronx, NY 10461.

Table I. Perinatal outcome of control subjects and study patients delivered at 41 to 42 weeks

	Control subjects	*Delivery at 41-42 wk*	*p*
No. of patients	59	139	
Abnormal nonstress test	4	34	<0.01
Oligohydramnios	0	19	<0.02
Cesarean section for fetal distress	1	14	<0.05
Apgar score ≤6 at 5 min	0	0	NS
NICU admissions	0	9	<0.05
Perinatal deaths	0	0	NS

NICU, Neonatal intensive care unit.

Table II. Perinatal outcome of study patients delivered at 41 to 42 weeks and <42 weeks

	Delivery at 41-42 weeks	*Delivery after 42 weeks*	*p*
No. of patients	139	154	
Abnormal nonstress test	34	22	<0.05
Oligohydramnios	19	16	NS
Cesarean section for fetal distress	14	14	NS
Apgar score ≤6 at 5 min	0	1	NS
NICU admissions	9	6	NS
Perinatal deaths	0	0	NS

NICU, Neonatal intensive care unit.

patients were required to meet all of the following criteria: (1) last menstrual period known with certainty, (2) regular menses, (3) first-trimester clinical examination that confirmed dates, and (4) an ultrasonographic examination before 20 weeks' gestation consistent with dates.

These patients had no medical or obstetric problems. Antepartum fetal testing began at 41 weeks' gestation and consisted of a nonstress test and an ultrasonographic evaluation of amniotic fluid volume. The nonstress test was considered abnormal in the presence of recurrent variable-late decelerations or 120 minutes of a nonreactive tracing. Oligohydramnios was defined by ultrasonogram as the largest pocket of amniotic fluid <2 cm in vertical depth. Patients were admitted for labor induction for either an abnormal nonstress test result or oligohydramnios. Patients with normal antepartum fetal testing were followed up twice weekly until spontaneous labor occurred.

The control population consisted of patients who were delivered between 39 and 41 weeks' gestation. This was a group of low-risk patients who were referred for routine testing at term. There were 59 such patients during the study period. These patients had their last antepartum assessment within 4 days of delivery. Patients with an abnormal nonstress test result or oligohydramnios were referred for induction of labor. All others labored spontaneously.

Obstetric and neonatal records were reviewed for study cases and controls. Perinatal outcome was evaluated by the following criteria: cesarean section for fetal distress (scalp pH <7.2 or recurrent severe variable-late decelerations occurring in early labor when a scalp pH was technically not obtainable), Apgar scores of ≤6 at 5 minutes, admissions to the neonatal intensive care unit, and perinatal deaths. Statistical analysis was performed by means of the χ^2 test.

Results

Of the 293 patients in the study group, 139 patients were delivered between 41 and 42 weeks' gestation and 154 were delivered after 42 weeks. Perinatal outcome for controls and study patients between 41 and 42 weeks is shown in Table I. The group of women who were delivered between 41 and 42 weeks had a significantly increased incidence of abnormal nonstress tests, oligohydramnios, cesarean sections for fetal distress, and admissions to the neonatal intensive care unit when compared with the control group who were delivered between 39 and 41 weeks. There was no significant difference in Apgar scores.

When the study group who were delivered between 41 and 42 weeks was compared to the women who were delivered after 42 weeks, there was no significant difference in any of the outcome parameters except abnormal nonstress tests. Surprisingly, there were more abnormal nonstress tests in the 41- to 42-week group than in the over 42-week group. There were no perinatal deaths in any of the groups evaluated.

Comment

The results of this study indicate that the abnormal fetal testing and adverse perinatal outcome associated with pregnancies >42 weeks' gestation are also found in pregnancies between 41 and 42 weeks' gestation.

Recent studies that evaluated intrapartum and neonatal parameters suggest that significant morbidity and mortality exist before the period classically used to define the postdate pregnancy (294 days). Grausz and Heimler[5] found the mean gestational age of neonates with unexpected asphyxia to be 288.8 days. Those infants who died or had neurologic sequelae had a mean gestational age of 291 days. Arias[4] showed that complications related to macrosomia, fetal distress, meconium aspiration, and postmaturity significantly increased from 5.6% at 38 to 40 weeks to 24.7% between 41 and 42 weeks. In a recent review on antepartum testing in the postdate pregnancy, Benedetti and Easterling[6] stated that "the traditional use of 42 weeks, or 294 days, to identify the fetus at risk in postterm pregnancy should be reconsidered."

The purpose of this study was to evaluate the role

of antepartum testing in the 41- to 42-week period. When conservative management of the postdate fetus is elected, antenatal testing is used to identify the fetus that needs to be delivered. Abnormal fetal heart rate patterns and oligohydramnios are well-accepted indicators for intervention in pregnancies over 42 weeks.[6] In our study, both of these indicators were found with increased frequencies in pregnancies between 41 and 42 weeks (Table II). This may be explained in part by the fact that patients with abnormal testing between 41 and 42 weeks were referred for induction and were therefore excluded from the over 42-week group. Twenty-five percent of patients who were delivered between 41 and 42 weeks had abnormal nonstress tests and 14% had oligohydramnios. In contrast, a very low frequency of abnormal tests was found in pregnancies between 39 and 41 weeks.

A presumed cause of the morbidity and mortality associated with postdate pregnancies is progressive uteroplacental insufficiency. It is postulated that when the aging placenta cannot keep pace with the demands of the fetus, both chronic and acute compromise become evident in the form of decreased growth, oligohydramnios, and fetal hypoxia.[2] Additionally, Leveno et al.[7] have shown that fetal distress in postdate patients can be explained by oligohydramnios and cord compression.

Several studies have shown increased perinatal mortality after 42 weeks' gestation.[1, 2, 8, 9] The purpose of antepartum fetal testing is to recognize changes in the fetal-placental unit that occur before the onset of permanent fetal damage. Therefore, it is not surprising that fetal testing would detect pathologic conditions before 42 weeks' gestation. In addition, the exact pathophysiologic features of uteroplacental insufficiency are still unclear.[10]

Morphometric studies have attempted to correlate fetal postmaturity with a wide variety of placental lesions. Other investigators have attempted to explain these changes on the basis of biochemical alterations in the postdate fetus.[2] None have been uniformly successful. Additional studies are needed before the uteroplacental insufficiency associated with postdate pregnancies is clearly understood. However, there is increasing evidence to suggest that these changes do not begin in all pregnancies at precisely 294 days.

In summary, this study supports the concept that the traditional use of 42 weeks to identify the fetus at risk does, in fact, need to be reconsidered. These results indicate that postdate fetal testing should begin at 41 weeks of gestation.

REFERENCES

1. McClure-Browne JC. Postmaturity. Am J Obstet Gynecol 1963;85:573.
2. Vorherr H. Placental insufficiency in relation to postterm pregnancy and fetal postmaturity. Am J Obstet Gynecol 1975;123:67.
3. Dyson DC. Fetal surveillance vs. labor induction at 42 weeks in postterm gestation. J Reprod Med 1988;33:262.
4. Arias F. Predictability of complications associated with prolongation of pregnancy. Obstet Gynecol 1987;70:101.
5. Grausz JP, Heimler R. Asphyxia and gestational age. Obstet Gynecol 1983;62:175.
6. Benedetti TJ, Easterling T. Antepartum testing in postterm pregnancy. J Reprod Med 1988;33:252.
7. Leveno KJ, Quizk JG, Cunningham FG, et al. Prolonged pregnancy. I. Observations concerning the causes of fetal distress. Am J Obstet Gynecol 1984;150:465.
8. Eden RD, Seifert L, Winegar A. Perinatal characteristics of uncomplicated postdate pregnancies. Obstet Gynecol 1987;69:296.
9. Callenbach JC, Hall RT. Morbidity and mortality of advanced gestational age: post-term or postmature. Obstet Gynecol 1979;53:721.
10. Guidetti DA, Divon MY, Cavalieri RL, Langer O, Merkatz IR. Fetal umbilical artery flow velocimetry in postdate pregnancies. Am J Obstet Gynecol 1987;157:1521.

Sarcoma associated with pregnancy

Yutaka Kyodo, MD, Kenji Inatomi, MD, and Tadayuki Abe, MD
Shizuoka, Japan

A case is reported of sarcoma associated with pregnancy in which the patient underwent a cesarean section with myomectomy and was delivered of a living infant. A pathologic examination indicated sarcoma. (AM J OBSTET GYNECOL 1989;161:94-6.)

Key words: Sarcoma, pregnancy

Sarcoma is rarely associated with pregnancy. This case report describes a patient with sarcoma in pregnancy. The pathologic examination revealed well-differentiated leiomyosarcoma. The patient was delivered of a healthy infant.

Case report

A 29-year-old, gravida 0, unmarried woman with her marriage near at hand requested the examination of antibody titer for rubella on December 23, 1982. On B-scan ultrasonography there were unclear small myomatous nuclei in the uterus (Fig. 1). Her medical history was noteworthy in that she had infantile rheumatism at 7 years of age and was hospitalized 3 months. On November 25, 1983, at 8 weeks' gestation, on B-scan ultrasonography a tumor three times as large as an adult thumb was detected on the right anterior side of the uterus (Fig. 1). Blood and biochemical test results were normal.

On May 26, 1984, at 34 weeks' gestation, she had abdominal pain, which was eliminated by analgesics. There seemed to be a partial necrosis in the subserous myoma on B-scan ultrasonography (Fig. 1). On June 20, at 37 weeks' gestation, she underwent a cesarean section with myomectomy because of nonreactivity in a nonstress test and a low estriol level. She was delivered of a living 2640 gm infant with an Apgar score of 9. The placenta was intact and it weighed 700 gm. The membrane was normal. The subserous myoma was the size of a goose egg (80 × 55 mm) and it was soft on the right anterior side of the uterus. There were several holes on the surface of the uterus. Fluid-like pus oozed from the holes. The uterus had several subserous myomas, but they were left as they were. The subserous myoma of goose egg size was diagnosed as well-differentiated leiomyosarcoma (Fig. 2).

On July 6, 1984, she underwent a total abdominal hysterectomy. Bilateral ovaries were intact so they were left alone (Fig. 3). After the surgical procedure she received treatment with Picibanil. α-Fetoprotein, carcinoembryonic antigen, ferritin, and CA 125 levels were normal before and after the two operations. On August 6, pyelitis was diagnosed. Picibanil was stopped, and she was discharged from the hospital August 19. We have closely watched the course of the disease.

Comment

Malignant tumor (i.e., malignant ovarian cyst) in pregnancy is rare; it was reported in 1 of 25,000 pregnancies.[1] Sarcoma associated with pregnancy is rarer than malignant ovarian cyst associated with pregnancy. There are many statistical and pathologic reports with regard to sarcoma, but there are few practical case reports that pertain to sarcoma in pregnancy. The sarcoma destructively invaded neighboring tissue and degeneration and necrosis in the tumor center resulted. Sarcoma is well known for its metastasis in the circulation of the blood. The purpose of this case report is to describe the resistance to sarcoma cells during pregnancy.

When we first saw the patient she was not pregnant but had subserous myoma in the uterine cavity. After she was pregnant the subserous myoma gradually interfered with the developing fetus and was pressed into the anterior segment of the uterus. The sarcoma, which we originally thought to be a subserous myoma, was scattered in the pelvic cavity (Fig. 4).

Why couldn't the sarcoma invade the gestational sac? Why couldn't the sarcoma cell in this case pass through the placenta?

Sarcomas are very destructive. But the fetal membrane was neither damaged nor stained yellow by the sarcoma. It was intact. The gestational sac is made up of three layers: amnion, chorion, and decidua (Fig. 4). Chorion consists of five layers: superficial cells, type IV collagen, type V collagen, type III collagen, and type I collagen. In premature rupture of the membranes, the break in the chorion is thought to be made at the layer of type III collagen by esterase of leukocytes.[2] But it also has been reported that cancer cells with strong metastatic power secrete large amounts of type IV col-

From the Department of Obstetrics and Gynecology, Shizuoka Kosei Hospital.
Received for publication February 23, 1989; accepted March 3, 1989.
Reprint requests: Yutaka Kyodo, MD, National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Hertfordshire, UK EN6 3QG.

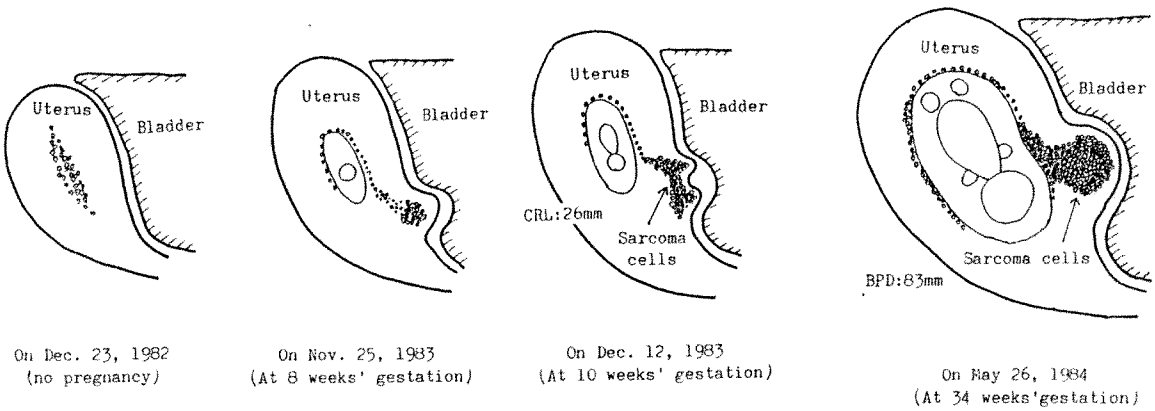


Fig. 1. Growth and location of sarcoma as pregnancy progresses according to B-scan ultrasonography.

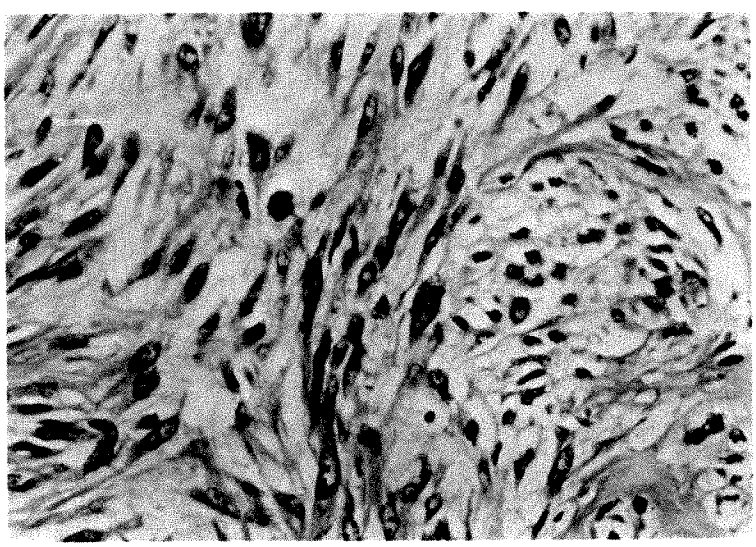


Fig. 2. Subserous myoma of goose egg size was diagnosed as well-differentiated leiomyosarcoma. There are large and small fibrous atypical cells after several mitoses.

lagenase.[3] The patient's placenta was normal; it had no defect and weighed 700 gm.

Chorion and decidua are part of the endometrium. Decidual cells are said to change the fibroblast cells of endometrium. Fibroblast cells are concerned with fibronectin. It is said that fibronectin "catches" carcinoma cells, which enables both leukocytes and macrophages to "kill" them. Fibronectin has an opsonic function against carcinoma cells. There are fibrinoids in the cellular space of decidual cells. We believe the role of fibrinoids is to bind both villi and decidua on myometrium during pregnancy. Deep in the decidua there is decidual spongiosa, which is tough and has a low response level so sarcoma cells cannot invade the gestational sac.

Amnion, chorion, and decidua provide a strong defense against foreign invaders; however, they have a low tolerance to inner invaders such as destructive mole

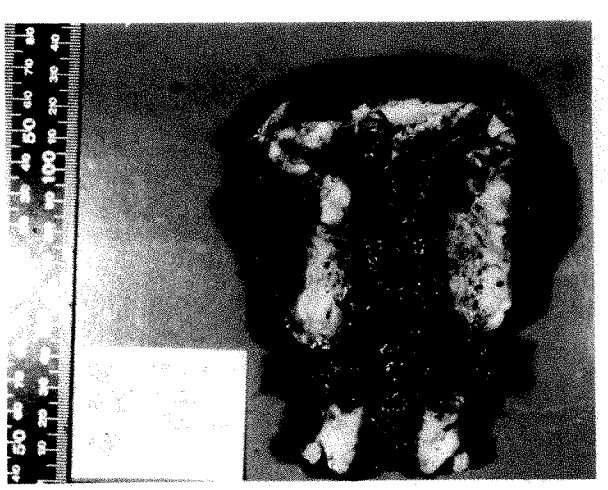


Fig. 3. Total abdominal hysterectomy was performed on day 16 after cesarean section. Specimen has numerous tumors in uterine cavity.

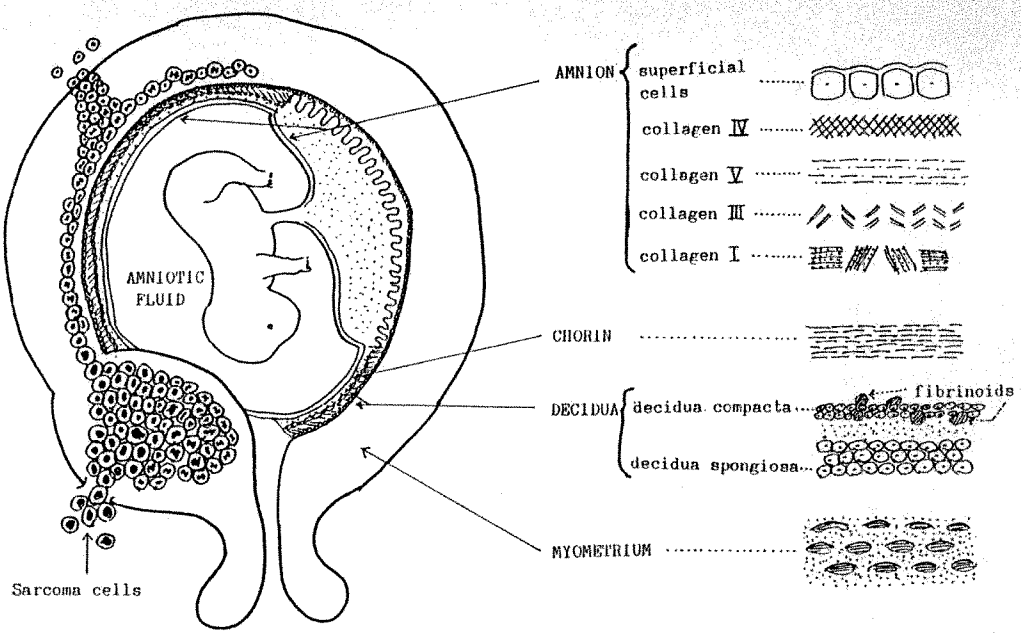


Fig. 4. Sarcoma cells in relationship to structural layers of uterus during pregnancy.

and choriocarcinoma. It is natural for villi to invade the myometrium. The human body is helpless with regard to internal reproductive invaders. From this case it is evident that both homeostasis and a strong instinct to protect the body from the foreign invader exist in the human body.

REFERENCES

1. Terashima Y, Ochiai K. Malignant ovarian tumors associated with pregnancy. Acta Obstet Gynecol Jpn 1987; 39:145.
2. Kanayama N, Terao T, Kawashima Y, Horiuchi K, Fugimoto D. Collagen types in normal and prematurely ruptured amniotic membranes. AM J OBSTET GYNECOL 1985;153:899.
3. Liotta LA. Tumor invasion and metastasis: role of the basement membrane. Am J Pathol 1984;117:339.

Primary stress urinary incontinence and pelvic relaxation: Prospective randomized comparison of three different operations

Arieh Bergman, MD, Paul P. Koonings, MD, and Charles A. Ballard, MD
Los Angeles, California

There were 289 women with clinical and urodynamic diagnosis of primary stress urinary incontinence, stable bladder, and pelvic relaxation who underwent a single-stage surgical procedure because of incontinence and pelvic relaxation. Patients underwent one of three surgical procedures because of stress incontinence—anterior colporrhaphy, revised Pereyra procedure, or Burch retropubic urethropexy. Decisions with regard to the type of bladder neck suspension and the surgeon were made randomly with a randomization table. Each patient had a complete clinical and urodynamic evaluation before surgery and at 3 and 12 months after surgery. Cure rate as defined by strict clinical and urodynamic criteria was not significantly different among the three groups at the 3-month postsurgical evaluations; however, at the 12-month postsurgical evaluations, the cure rate among women who underwent Burch urethropexy (n = 101) was significantly higher than that of either Pereyra or anterior colporrhaphy (cure rates were 87%, 70%, and 69%, respectively; $p < 0.01$). The Burch urethropexy was more effective than the Pereyra procedure or anterior colporrhaphy in the stabilization of the bladder base and resulted in a significantly better cure rate in women with primary stress urinary incontinence and pelvic relaxation (Am J Obstet Gynecol 1989;161:97-101.)

Key words: Stress incontinence, pelvic relaxation

Genuine stress urinary incontinence is a pathologic condition usually accompanied by poor support to the pelvic organs and excessive mobility of the bladder base on stress.[1, 2] Anatomically, the pelvic organs are supported by the pelvic diaphragm (levator ani muscle and fascia) and the pubovesicocervical ligaments.[2-4] Failure of support to the bladder frequently is part of a picture of generally defective pelvic support, and pelvic relaxation results in prolapse of both bladder and uterus.[5] Most women with primary stress urinary incontinence and pelvic relaxation will show various degrees of uterine prolapse, cystocele, and rectocele. Surgical procedures to lift and stabilize the bladder and urethrovesical junction frequently necessitate vaginal hysterectomy and posterior colporrhaphy to correct the other components of pelvic relaxation.

Numerous surgical approaches to stabilize the bladder base have been designed. The three most popular surgical approaches for primary stress urinary incontinence are anterior colporrhaphy,[6] vaginal retropubic procedure (Pereyra-Stamey type[4, 7]) and abdominal retropubic procedures (Burch and Marshall Marchetti-Krantz[8, 9]). Comparative studies of the surgical results of these procedures are difficult to interpret because all but one are retrospective studies of various groups of patients with different pathologies and degrees of pelvic relaxation.[10-13] One prospective study was done without author randomization and with the degree of pelvic relaxation uncontrolled.[14] The present work was undertaken to study prospectively and in a randomized comparative manner the results of three different operations for stress urinary incontinence (anterior colporrhaphy, revised Pereyra procedure, and Burch retropubic urethropexy) in women with genuine stress incontinence and pelvic relaxation with the use of objective clinical and urodynamic criteria.

Material and methods

A total of 342 women with clinical and urodynamic diagnosis of primary genuine stress urinary incontinence and pelvic relaxation were evaluated in the Gynecology-Urology Service at Women's Hospital, University of Southern California School of Medicine, between January 1986 and June 1987 and were found to be eligible for this study. Women with previous anti-incontinence surgical procedures or findings of combined stress incontinence with detrusor instability were excluded from the study. Women with gynecologic conditions that required laparotomy were not included. All women had various degrees of pelvic relaxation neces-

From the University of Southern California School of Medicine, Women's Hospital.
Received for publication July 11, 1988; revised November 21, 1988; accepted January 11, 1989.
Reprint requests: Arieh Bergman, MD, 1240 N. Mission Road, Los Angeles, CA 90033.

Table I. Results of anti-incontinence procedures in 298 patients undergoing operation because of stress urinary incontinence

Procedure	Failure rate	
	3 Months after surgery	*12 Months after surgery*
Burch $N = 101$	11 (11%)	13 (13%)
Pereyra $N = 98$	19 (19%)*	29† (30%)
Anterior colporrhaphy $N = 99$	20 (20%)*	31‡ (31%)

*No change of statistical significance compared with Burch procedure by χ^2 test.

†$p < 0.02$ by χ^2 test compared with Burch procedure.

‡$p < 0.01$ by χ^2 test compared with Burch procedure.

sitating vaginal correction (cystorectocele repair with or without vaginal hysterectomy). Patients' mean age was 57 years (range, 31 to 80) and mean parity was 3 (range, 1 to 13). There were 191 patients who were postmenopausal and 151 patients who were premenopausal.

Evaluation included history and physical examination with neurologic screening tests of the S_2 to S_4 lower micturition center, urine culture, Q-tip test, dynamic water urethrocystoscopy, standing provocative urethrocystometry (at a filling rate of 60 ml/min), and urethral pressure profiles at rest and stress (repeat coughing) in the sitting position at maximal cystometric capacity. Pressures in the abdomen (approximated by vaginal recording), bladder, and urethra were measured with two small (4 French) catheters with microtip pressure transducers (Models 20K 60 and 20K 62, Dantek Electronics) and recorded on a six-channel electrophysiologic recorder (Model 2100, Dantek Electronics, N.J.). All postmenopausal women received Premarin vaginal cream (1 gram daily) for a minimum of 2 weeks before urodynamic evaluation and 4 additional weeks before surgery. These patients were encouraged to continue estrogen replacement therapy indefinitely.

Nineteen of the 342 women had had a previous hysterectomy because of benign conditions and were scheduled to undergo vaginal cystorectocele correction in addition to the anti-incontinence operation. The other 323 women were scheduled for vaginal hysterectomy and cystorectocele repair in addition to their anti-incontinence procedure. All women in this series had pelvic relaxation that necessitated vaginal surgery. When hysterectomy was not indicated, women with genuine stress incontinence and minimal pelvic relaxation or with a well-supported uterus had an anti-incontinence procedure only and were entered into a different protocol that was reported separately.[15] Vaginal hysterectomy was abandoned because of technical difficulties in three women (two had endometriosis and one had previous pelvic inflammatory disease). These three patients underwent abdominal hysterectomy and were not included in the final analysis.

After clinical and urodynamic evaluation, patients were randomly assigned (with the use of a randomization list[16]) to one of three anti-incontinence surgical procedures. Randomization included the type of anti-incontinence procedure as well as the surgeon (one of the three authors). All surgical procedures were done by house staff and one of the three authors. Results of the urodynamic studies or the severity of stress incontinence were not considered when the type of anti-incontinence operation was selected. Women in group 1 had Burch urethropexy after the vaginal surgery. Women in group 2 had a modified Pereyra procedure after surgery for pelvic relaxation, and women in group 3 had anterior colporrhaphy as their anti-incontinence procedure (in addition to cystocele and rectocele repair with or without vaginal hysterectomy). Anterior colporrhaphy was performed by mobilization of the bladder with upward displacement and buttressing of the bladder neck with the use of Kelly plication sutures, which included the pubocervical fascia with a delayed absorption suture (#0 Vicryl[6]). The modified Pereyra procedure included all of the modifications of the original technique with special emphasis on inclusion of the pubourethral ligaments and endopelvic fascia at the level of the proximal urethera and bladder neck with use of delayed absorbable sutures (#0 Vicryl) under endoscopic guidance.[4] The Burch urethropexy included the basic principles of the Tanagho modification[8]: (1) minimal dissections within 2 cm of the urethrovesical junction and urethra; (2) placement of sutures through the full thickness of the paravaginal fascia; (3) use of two delayed absorbable sutures (#0 Vicryl) on each side, one opposite of the urethrovesical junction and another at the level of the midurethra; (4) removal of fatty tissue lateral to the sutures to stimulate fibrosis; and (5) tying of the sutures to the Coopers ligament with the use of intravaginal fingers to elevate the anterior vaginal wall. After surgery the bladder was drained by suprapubic catheter (5 F Bonnano catheter). The catheter was removed once; postvoid residual volume was $\leq$50 ml on two consecutive occasions.[17]

Clinical and urodynamic evaluations were repeated at 3 months and 12 months after surgery. A total of 339 patients were eligible for this study (342 minus the 3 who started as vaginal hysterectomy patients and became abdominal hysterectomy patients). There were 298 patients available for 1-year follow-up and they are the subject of this report. The other 41 patients were lost to follow-up.

All terminology conforms to that proposed by the

'able II. Urodynamic findings of 298 patients with stress urinary incontinence

Procedure	*Urethral closure pressure (cm water)*		*Urethral function length (cm)*		*Abdominal pressure transmission ratio to the %**	
	Before surgery	*1 Year after surgery*	*Before surgery*	*1 Year after surgery*	*Before surgery*	*1 Year after surgery*
Burch						
$N = 101$						
Success	43 ± 21	47 ± 26†	3.3 ± 1.7	3.5 ± 1.9†	87 ± 20	114 ± 19‡
$n = 88$						
Failure	41 ± 1.4	43 ± 17†	3.2 ± 1.2	3.3 ± 1.1†	85 ± 22	89 ± 14†
$n = 13$						
Pereyra						
$N = 98$						
Success	46 ± 25	48 ± 24†	3.4 ± 1.9	3.7 ± 2.1†	86 ± 29	108 ± 2.3‡
$n = 69$						
Failure	44 ± 27	45 ± 46†	3.3 ± 1.6	3.8 ± 2†	83 ± 24	86 ± 21†
$n = 29$						
Anterior colporrhaphy						
$N = 99$						
Success	45 ± 24	46 ± 27†	3.5 ± 1.4	3.7 ± 1.9†	88 ± 19	109 ± 22‡
$n = 68$						
Failure	47 ± 28	44 ± 23†	3.4 ± 1.6	3.7 ± 1.4†	84 ± 17	85 ± 20†
$n = 31$						

Values are mean ± SD.

*Pressure transmission ratio = abdominal pressure transmission to urethra/abdominal pressure transmission to bladder × 100 (measured at mid urethra).

†No significant change by two-tailed paired t test.

‡$p < 0.05$ by two-tailed paired t test.

International Continence Society[18] except where specifically mentioned. Cure from stress incontinence is defined as no history of urinary incontinence after surgery, no loss of urine at any postsurgical evaluation, and negative cough profile on postsurgical urodynamic testing. Few women with presurgical findings of a stable bladder had postsurgical cystometric findings of detrusor instability. They were considered to be "cured" if the detrusor instability was only a urodynamic finding with no subjective or objective evidence of urinary incontinence. The two-tailed paired t test and χ^2 tests were used for statistical anaylsis of the results.

Results

Of the 298 women in this study, 101 had Burch retropubic urethropexy as their anti-incontinence procedure (group 1), 98 women had a Pereyra procedure (group 2), and 99 had anterior colporrhaphy as their anti-incontinence procedure (group 3). The three groups were comparable with regard to mean age, parity, and menopausal status. As shown in Table I, the failure rates 3 months after surgery were higher with the Pereyra procedure and anterior colporrhaphy, although this was not statistically significant. At the 1-year postsurgical evaluation, the failure rate of Burch retropubic urethropexy was significantly lower than that of either the Pereyra procedure or anterior colporrhaphy ($p < 0.01$, Table I). The failure rate of the Burch procedure was similar at the 3-month and 12-month postsurgical evaluations, whereas many failures of the other two procedures (Pereyra and anterior colporrhaphy) occurred between the 3-month and 1-year postsurgical evaluations (Table I).

None of the three procedures resulted in significant changes in urethral resting parameters of functional length or closure pressure (Table II). All three procedures resulted in significant increase in abdominal pressure transmission to the urethra with cough only when the procedure was successful (Table II).

Known risk factors such as presurgical low urethral pressure,[19] presurgical negative Q-tip test results,[20] and previous hysterectomies[21] were equally distributed among the three groups. Successful Burch procedures resulted in a well-supported urethrovesical junction at the 1-year postsurgical evaluation, as judged by negative Q-tip test results (Q-tip change of ≤30° on straining). Four of the 12 Burch failures had positive postsurgical Q-tip test results (change of Q-tip angle ≥35° on straining). Of the 69 women cured by a Pereyra procedure, 36 had negative postsurgical Q-tip test results after 1 year, whereas 18 of the 29 procedural failures had positive postsurgical Q-tip test results. Of the 65 women cured by anterior colporrhaphy, 36 had 1-year postsurgical negative Q-tip test results, whereas 29 of the 34 procedural failures had positive postsurgical Q-tip test results.

Table III. Resumption of spontaneous postsurgical voiding in 298 patients with stress urinary incontinence

	Procedure		
	Burch	*Pereyra*	*Anterior colporrhaphy*
Days on catheter	4 ± 2.7	4.4 ± 2.3	4.3 ± 2.5

Values are mean ± SD.

None of the procedures resulted in significant postsurgical voiding difficulties, and most women were able to void spontaneously with adequate (≤50 ml) residual urine volume by the fifth day after surgery (Table III).

Comment

Results of this prospective randomized study with the use of strict clinical and urodynamic criteria demonstrate that Burch retropubic urethropexy resulted in a better 1-year cure rate than either the Pereyra procedure or anterior colporrhaphy in women with primary stress urinary incontinence and pelvic relaxation. At the 3-month postsurgical follow-up evaluations, cure rates of the three procedures were not significantly different; however, at the 1-year postsurgical evaluation the differences in cure rates among the three procedures were significant in this study. The cure rates of the Burch procedure were similar throughout the study period, whereas the vaginal techniques did not hold their early good cure rates over time. Numerous clinical studies have retrospectively compared results of vaginal versus abdominal retropubic surgical approaches for stress urinary incontinence.[10-13] The majority of these retrospective comparative studies demonstrate that long-term results of the vaginal procedures are not as good as the abdominal ones.[11-13] All of these studies have the weakness of being retrospetcive series with different surgical approaches performed on different populations. Stanton et al.[14] compared vaginal versus abdominal retropubic procedures in a prospective manner, but patients and surgeons in that study were not randomized. The results of Stanton's series show that the vaginal operation is not as good as the abdominal retropubic procedure with long-term objective follow-up.[14] The present series confirms the previous retrospective observations as well as Stanton's findings, in a prospective randomized manner.

Only two criteria were used in this study to assess surgical results—"cure" or "failure." No patients were described as "improved" because such a diagnosis leaves room for subjective interpretations and personal bias. More than half of the procedures considered as failures by strict objective criteria were not considered to be failures by subjective criteria of the patients. The differences among subjective and objective criteria were similar in the three groups.

Primary stress urinary incontinence is usually accompanied by various degrees of pelvic relaxation. Pelvic relaxation often requires vaginal correction in addition to anti-incontinence bladder neck suspension. A surgeon who performs a vaginal operation is tempted to do a vaginal bladder neck suspension because the patient is already positioned for vaginal surgery. To change the patient's position to "frog leg" and to redrape her for abdominal incisions adds about 10 minutes to surgical time and requires additional nursing work to reposition the patient. Improved long-term surgical results, as reported in this series, justify this extra time and effort.

The three techniques of bladder neck suspension are those practiced in the community, although they have undergone some modifications. The Pereyra procedure we described has few of the elements of the Stamey bladder neck suspension.[7] Pereyra et al.[4] in their last report used nonabsorbable sutures. We have used delayed absorbable sutures as described earlier,[13] and used similar sutures in all three procedures.

Known risk factors such as preoperative low urethral pressure,[19] negative Q-tip test results,[20] or previous operations[21] were equally distributed among the three groups and did not apparently affect differences in final cure rate. All three procedures increased abdominal pressure transmission to the urethra when successful, and all three procedures had an insignificant effect on urethral closure pressure or urethral functional length. All procedures resulted in adequate resumption of spontaneous postsurgical voiding. The primary difference among the three procedures was their ability to stabilize the bladder base, as evaluated by postsurgical Q-tip test results. The Burch procedure stabilized the bladder base and urethrovesical junction better than did the two vaginal procedures. Many women who were clinically cured of their stress incontinence after their vaginal procedure still had increased bladder base mobility as judged by positive postsurgical Q-tip test results. This finding indicates that other factors may have cured these women other than the stabilization of their bladder base. Women may be continent during stress despite increased bladder base mobility by a kinking effect on the urethra as suggested by Beck[22] and Bump,[23] or by increased abdominal pressure transmission to the urethra through the rectum as suggested by Hertogs and Stanton.[24] The main difference in clinical response among the three groups was in long-term cure rate. Positive postsurgical Q-tip test results and increased bladder base mobility after the vaginal procedures may represent a less effective bladder base support, which may result in further clin-

ical failures. Longer follow-up of 3 to 5 years is necessary to deterimne whether this is true.

Results of this series demonstrate that the Burch retropubic urethropexy was more effective in treating genuine stress incontinence in our study than either the Pereyra procedure or the anterior colporrhaphy. Anterior colporrhaphy and the Pereyra procedures are associated with a 70% objective cure rate, which makes them reasonable options for patients with primary genuine stress urinary incontinence, if a procedure has to be terminated quickly because of a patient's poor general condition, or if the patient refuses abdominal procedure.

REFERENCES

1. Hodgkinson CP. Stress incontinence in the female. Surg Gynecol Obstet 1965;102:595.
2. Zacharin RF. Abdominoperineal urethral suspension in the management of recurrent stress incontinence of urine: a 15-year experience. Obstet Gynecol 1983;62:644-54.
3. Oelrich TM. The strated urogenital sphincter muscle in the female. Anat Rec 1983;205:223-32.
4. Pereyra AJ, Lebherz TB, Growdon WA, Powers JA. Pubourethral support in perspective: modified Pereyra procedure for urinary incontinence. Obstet Gynecol 1982; 59:643-8.
5. Milley PS, Nichols DH. The relationship between the pubourethral ligaments and the urogenital diaphragm in the human female. Anat Rec 1971;170:281-93.
6. Beck PR, McCormick S. Treatment of urinary stress incontinence with anterior colporrhaphy. Obstet Gynecol 1982;59:269-75.
7. Stamey TA. Endoscopic suspension of the vesical neck for urinary incontinence. Surg Gynecol Obstet 1973;136:547-56.
8. Tanagho EA. Colpocystourethropexy: the way we do it. J Urol 1976;116:751-4.
9. Marshall V, Vaughan ED, Parnell JP. Suprapubic vesicourethral suspension (Marshall Marchetti-Krantz) for stress incontinence. In: Walsh PC, Gittes RF, Perlmutter AD, Stamey TA, eds. Campbell's Urology. 5th ed. Philadelphia: WB Saunders, 1986:2711-7.
10. Park GS, Miller EJ. Surgical treatment of stress urinary incontinence: a comparison of the Kelly plication, Marshall Marchetti-Krantz and Pereyra procedures. Obstet Gynecol 1988;71:575-9.
11. Riggs JA. Retropubic cystourethropexy: a review of two operative procedures with long term follow-up. Obstet Gynecol 1986;68:98-105.
12. Green DF, McGuire EJ, Lytton B. A comparison of endoscopic suspension of the vesical neck versus anterior urethropexy for the treatment of stress urinary incontinence. J Urol 1986;136:1205-7.
13. Bhatia NN, Bergman A. Modified Burch versus Pereyra retropubic urethropexy for stress urinary incontinence. Obstet Gynecol 1985;66:225-61.
14. Stanton SL, Cardozo LD. A comparison of vaginal and suprapubic surgery in the correction of incontinence due to urethral sphincter incompetence. Brit J Urol 1979; 51:497-9.
15. Bergman A, Ballard CA, Koonings PP. Comparison of 3 different surgical procedures for geniune stress incontinence: prospective randomized study. Am J Obstet Gynecol 1989;160:1102-6.
16. Diem K. Random numbers. In: Scientific tables. Ardley, New York: Geigy Pharmaceuticals 1962:131.
17. Bergman A, Matthews L, Ballard CA, Roy S. Suprapubic versus transurethral bladder drainage after surgery for stress urinary incontinence. Obstet Gynecol 1987;69: 546-9.
18. Bates P, Bradley WE, Glen E, et al. The standardization of terminology of lower urinary tract functions. J Urol 1979;121:551-4.
19. Sand PK, Bowen LW, Pangamiban R, Ostergard DR. The low pressure urethra as a factor in failed retropubic urethropexy. Obstet Gynecol 1987;69:399-402.
20. Bergman A, Koonings PP, Ballard CA. Negative Q-tip test as a risk factor for failed incontinence surgery in women. J Reprod Med 1989;34:193-7.
21. Sand PK, Bowen LW, Ostergard DR, Nakanishi AM. Hysterectomy and prior incontinence surgery as risk factors for failed retropubic cystourethropexy. J Reprod Med 1988;33:171-4.
22. Beck RP, McCormick S, Nordstrom L. Intraurethral intravesical cough pressure spike difference in 267 patients surgically cured of genuine stress incontinence of urine. Obstet Gynecol 1988;72:302-6.
23. Bump RC, Fantl AJ, Hart GW. The mechanism of urinary continence in women with severe uterovaginal prolapse: results of barrier studies. Obstet Gynecol 1988;72:291-5.
24. Hertogs K, Stanton SL. Mechanism of urinary continence after colposuspension: barrier studies. Brit J Urol 1985; 92:1184-7.

Saline solution amnioinfusion for oligohydramnios after premature rupture of the membranes

A preliminary report

Motoharu Imanaka, MD,[a] Sachio Ogita, MD,[a] and Tadashi Sugawa, MD[b]
Osaka, Japan

In the conservative management of premature rupture of the membranes, most patients have oligohydramnios caused by continuous leakage of amniotic fluid. To avoid unfavorable effects of oligohydramnios on fetal development and well-being, we infused physiologic saline solution continuously into the amniotic cavity at a flow rate of 10 to 20 ml/hr with a new cervical indwelling catheter (PROM-fence). As a result the average pocket size of amniotic cavity was 2.7 cm before amnioinfusion, 5.9 cm 1 day after, 5.8 cm 5 days after, and 5.0 cm 10 days after amnioinfusion. The saline solution amnioinfusion made it possible to keep the amniotic cavity fluid level adequate. Variable deceleration disappeared in one case. No side effects such as uterine contractions were observed. We recommend the method of saline solution amnioinfusion for its favorable effects on fetal environment during management of premature rupture of membranes. (Am J Obstet Gynecol 1989;161:102-6.)

Key words: Saline solution amnioinfusion, oligohydramnios, premature rupture of the membranes, cervical indwelling catheter

Premature rupture of the membranes (PROM) is a major cause of preterm delivery. Management of PROM remains one of the most important unsolved obstetric problems. When PROM is managed conservatively, most patients have oligohydramnios to a greater or lesser degree as a result of the continuous leakage of amniotic fluid. Neonates delivered from pregnancies complicated by oligohydramnios sometimes have pulmonary hypoplasia, retarded growth, or both, because physiologic fetal growth, especially fetal lung development, requires a critical volume of amniotic fluid.[1, 2] Theoretically, the absence of amniotic fluid allows the uterus to exert continuous pressure against the fetus, resulting in abnormal fetal growth and development.

Mosberg et al.[3] reported a high rate of cesarean section for fetal distress because of intrapartum fetal heart rate patterns consistent with umbilical cord compression. They speculated that the high incidence (76%) of severe variable deceleration in patients with fetal distress was a result of oligohydramnios.

There are no effective ways to treat oligohydramnios in the conservative management of PROM. Most patients are simply treated with bed rest in Trendelenburg's position.

We devised a new cervical indwelling catheter (PROM-fence) to solve the clinical difficulties associated with PROM. The catheter plugs the rupture and prevents the outflow of amniotic fluid and ascending infection.[4, 5] Even with this technique it is very difficult to restore the amniotic fluid volume, much of which has been lost before the insertion of the catheter. To prevent unfavorable effects on fetal development and health, saline solution containing dissolved antibiotics is infused continuously into the amniotic cavity.

Material and methods

The study group consisted of 10 women between 22 and 32 weeks of gestation with amniotic fluid loss (the pocket size as defined by Manning et al.[6] was <5 cm). After cervical cerclage, a cervical catheter was inserted into the cervix and fixed by inflating its balloons with antiseptic solution. Antiseptic solution sterilized the inside of the cervical canal and the vagina through an opening between the balloons at a flow rate of 20 ml/day.

We also infused saline-containing antibiotics directly into the amniotic cavity through the catheter. The amnioinfusion equipment included intravenous extension tubing, a three-way stopcock, and 500 ml saline solution containing an antibiotic (100 mg of latamoxef sodium). Saline was infused continuously through the extension

From the Department of Obstetrics and Gynecology, Osaka City Perinatal Center,[a] and the Department of Obstetrics and Gynecology, Medical School, Osaka City University.[b]
Received for publication July 21, 1988; revised December 21, 1988; accepted January 30, 1989.
Reprint requests: Motoharu Imanaka, MD, Department of Obstetrics and Gynecology, Osaka City Perinatal Center, 1-Bainan, Nishinari, Osaka, Japan, 557.

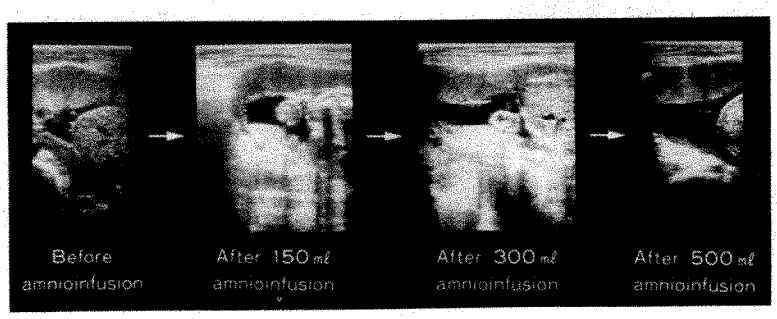


Fig. 1. Effect of amnioinfusion on amniotic cavity.

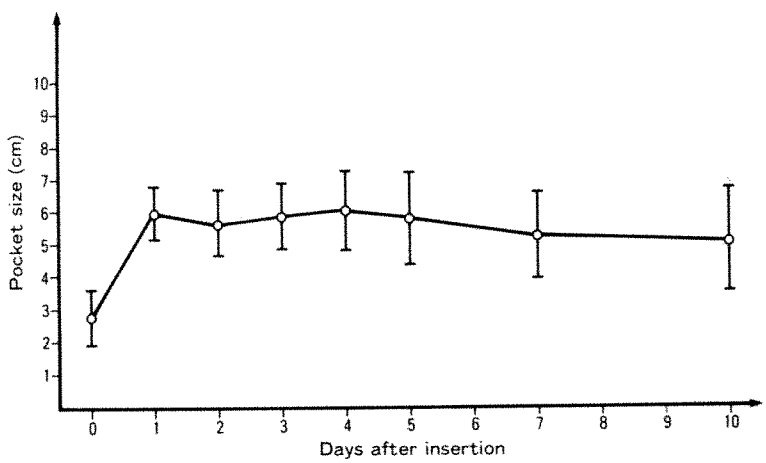


Fig. 2. Enlargement of pocket size following saline solution amnioinfusion.

Table I. Patients with PROM and condition of the neonates

No. of cases	10
Gestation at PROM (wk)	26.4 ± 3.2 (range 22.1-31.5)
Interval from rupture to catheter insertion (days)	2.6 ± 4.3 (range 0.2-14.7)
Duration of management with catheter (days)	8.3 ± 3.4 (range 2.2-13.0)
Mean birth weight (gm)	1084.2 ± 317.1 (range 660-1647)
Perinatal death	3/10 (30.0%)

Values are mean ± SEM.

tubing and the main duct of the catheter at the rate of 10 to 20 ml/hr until delivery.

During the amnioinfusion the amniotic fluid volume was measured periodically by the method of Manning et al.[6] Uterine contractions were recorded by an external tocodynamometer or by fetal heart rate tracings. The speed of infusion was regulated primarily according to the size of the amniotic cavity.

In 71 pregnant women between 12 and 41 weeks' gestation, amniotic fluid samples were obtained for measurement of the osmotic pressure. The results were compared with patients receiving saline solution amnioinfusion for 1 day.

Results

The mean time when PROM occurred was at 26.4 ± 3.2 weeks of gestation. The mean interval between rupture and insertion of the catheter was 2.6 days. Labor occurred or was induced 8.3 days after insertion; the mean birth weight was 1084 gm. The reasons for delivery were pulmonary maturation in six cases, and uterine contraction in four. Three of 10 deliveries (30%) resulted in perinatal death. Causes of death included multiple anomalies (one case), spontaneous abortion at 23 weeks of gestation in one case, and pulmonary hypoplasia in one (Table I). Two infants had mild respiratory distress syndrome. Seven infants, in-

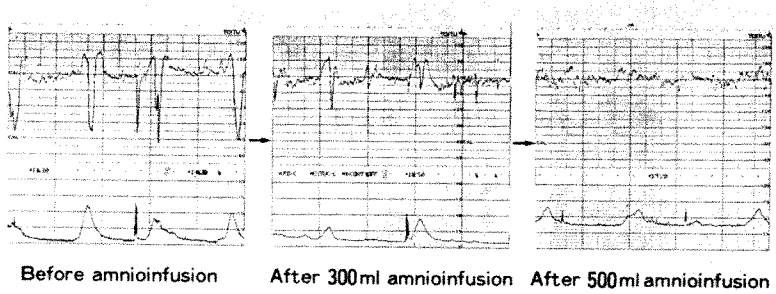


Fig. 3. Effect of amnioinfusion on fetal heart rate.

Table II. Effect of saline solution amnioinfusion on uterine contraction

Before amnioinfusion	*After 3 hr*	*After 12 hr*
Uterine contraction		
(−)	→ (−)	→ (−) (n = 3)
	↳ (+)	→ (−) (n = 4)
(+)	→ (+)	→ (−) (n = 3)
Total		(n = 10)

cluding the two with respiratory distress syndrome, developed normally with no clinical difficulties such as infection, electrolyte disorders, or jaundice.

Fig. 1 is an echogram of a patient with PROM taken at 25 weeks' gestation. Before insertion of the catheter, the pocket size was 2.4 cm and the cervix was dilated to 2.5 cm. After insertion, saline solution was infused at a rate of 10 ml/hr. The pocket size increased to 5.1 cm after infusion of 150 ml and to 6.0 cm after infusion of 300 ml and was kept at an adequate level thereafter. In this case, 6 days after catheter insertion, uterine contractions increased and the cervix dilated gradually. The catheter slipped out and amniotic fluid leaked freely. The pocket size decreased to 3.4 cm 12 hours later and to 2.1 cm 4 days later.

The average pocket size in those patients with PROM was 2.7 ± 0.9 cm before amnioinfusion, 5.9 ± 0.8 cm 1 day after, 5.8 ± 1.4 cm 5 days after, and 5.0 ± 1.6 cm 10 days after amnioinfusion (Fig. 2). Saline solution amnioinfusion had no effect on the fetal heart rate. Variable decelerations disappeared with enlargement of the amniotic cavity in one case (Fig. 3).

Amnioinfusion had little effect on uterine contractions (Table II). A patient with uterine contractions every 10 minutes or less was considered a positive contraction case. Of seven patients without uterine contractions before insertion, four were having positive contractions 3 hours after amnioinfusion, but by 12 hours after the insertion none had contractions.

Osmotic pressure gradually decreased as pregnancy progressed (269.2 ± 7.1 mOsm/kg before the twenty-third week, 261.2 ± 9.0 mOsm/kg between the twenty-fourth week and the thirty-fifth week, and 253.6 ± 9.9 mOsm/kg after the thirty-sixth week). Conversely, osmotic pressure after saline solution amnioinfusion was 273.7 ± 5.2 mOsm/kg, slightly higher than that of normal amniotic fluid (Fig. 4).

Comment

Oligohydramnios with PROM may cause fetal pulmonary hypoplasia, fetal distress, and is thought to be an uncomfortable environment for the fetus.

If a conservative approach to the management of PROM is taken and a prolonged latency period occurs, the suspected association between oligohydramnios and pulmonary hypoplasia becomes increasingly important. The greatest impact of prolonged membrane rupture on fetal development occurred in patients in whom membranes ruptured before 26 weeks' gestation and the duration of leakage was more than 5 weeks.[1]

Normal fetal lung development during pregnancy requires a critical volume of amniotic fluid. It is important to keep the amniotic cavity large enough for a fetus to breathe and move freely. Nakayama et al.[7] showed that the compressive effects of oligohydramnios inhibit normal fetal body and lung growth and that maintaining the amniotic cavity by saline solution amnioinfusion can prevent fetal pulmonary hypoplasia in rabbits.

Moreover, oligohydramnios resulting in cord or placental compression or both is probably the most common cause of variable decelerations. When variable decelerations occur after PROM, it is reasonable to enlarge the amniotic cavity to release cord or placental

compression. Miyazaki and Nevarez[8] performed intrauterine saline solution amnioinfusion in 49 patients when fetal repetitive variable decelerations did not respond to conventional therapy, such as maternal position changes and oxygen administration. Saline solution amnioinfusion effectively stopped repetitive variable decelerations in 25 of the 49 patients (51.0%); in the noninfusion group decelerations stopped in only 4.2% of patients.

Nageotte et al.[9] reported that prophylactic intrapartum amnioinfusion has significant benefit in reduction of the incidence of variable decelerations and improving the metabolic state in newborn infants born to women with PROM. This was supported by the fact that variable decelerations disappeared after saline amnioinfusion in our study.

However, their method cannot always be applied in the conservative management of PROM because it is difficult to keep the amniotic fluid volume adequate only by infusion of saline solution. Prevention of leakage of amniotic fluid with the catheter must accompany amnioinfusion of saline solution.

The volume of saline solution infused must be appropriate for the length of gestation and the degree of oligohydramnios. Saline solution was usually infused at a rate of 10 or 20 ml/hr. Because the rupture is not completely sealed, excess fluid flows out through the gap. The amniotic cavity can be maintained in its physiologic state of 5 to 8 cm of pocket size without excess enlargement. No effect on uterine contractions was observed at the flow rate we used.

The antibiotic in the saline solution used for amnioinfusion spreads so completely through the amniotic fluid that the level is high enough to inhibit bacterial growth in the amnion and the fetal skin, eyes, ears, genitalia, respiratory tract, and gastrointestinal tract without any significant systemic absorption of antibiotics.[10] In this study, 200 μg/ml of latamoxef sodium in the saline solution was considered adequate to destroy sensitive microorganisms in the uterine cavity.

Insertion of the catheter and saline solution amnioinfusion are thought to induce uterine contractions, but uterine contractions in all our cases were not strong and could be controlled. Amnioinfusion has no effect on uterine contractions; no patients had intrauterine pressure >10 mm Hg. The duration of time patients were managed with the saline solution amnioinfusion catheter was 8.3 days, which was not significantly different from patients treated with the catheter alone.

One infant died of pulmonary hypoplasia despite enlargement of the amniotic cavity. The time of the rupture was not known, and oligohydramnios had continued for over 2 weeks before saline solution amnioinfusion was started. There were two other perinatal deaths. If two cases (one malformation case and one abortion case) were excluded, however, the death rate was 12.5%. No deaths caused by infection or respiratory distress syndrome were observed. Seven infants developed normally.

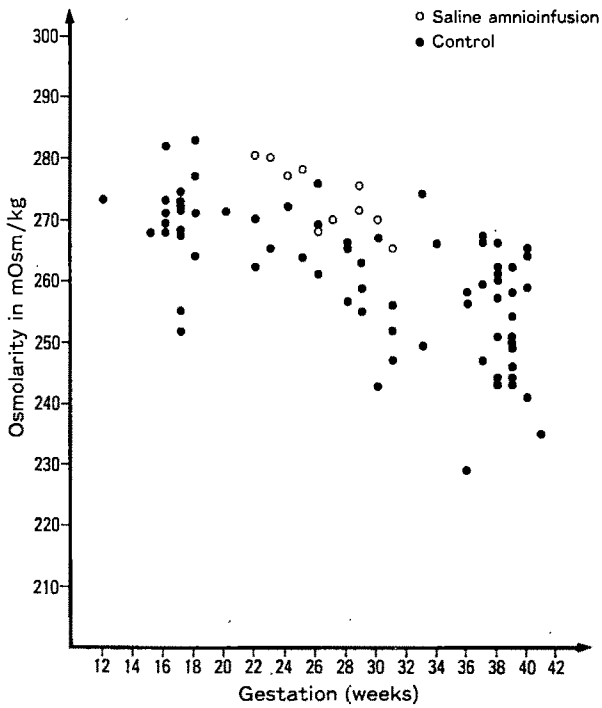


Fig. 4. Effect of saline solution amnioinfusion on osmotic pressure.

We used saline solution, which is as isotonic as serum, for the infusion fluid. Saline solution is considered suitable to prevent compression of the umbilical cord and pulmonary hypoplasia. However, physiologic saline solution is higher in osmolarity than natural amniotic fluid, and osmolarity after saline solution amnioinfusion increased slightly but was kept at most normal levels. Amniotic fluid samples could not be obtained before amnioinfusion for oligohydramnios; thus comparison of osmolarity of amniotic fluid before and after amnioinfusion could not be made. However, amniotic fluid contains various substances and plays an important role in fetal nutrition. Therefore fluid that contained various amino acids and electrolytes was deemed a better infusion solution.

The purpose of saline solution amnioinfusion is to maintain the physiologic intraamniotic environment, which is possible with the use of our cervical catheter. The method of saline solution amnioinfusion is recommended for its favorable effects on fetal environment during the management of PROM, although the cases in this preliminary study were limited.

REFERENCES

1. Nimrod C, Varela-Gittings F, Machin G, et al. The effect of very prolonged membrane rupture on fetal development. AM J OBSTET GYNECOL 1984;148:540-3.
2. Thibeault DW, Beatty EC, Hall RT, et al. Neonatal pulmonary hypoplasia with premature rupture of fetal membranes and oligohydramnios. J Pediatr 1985;107:273-7.
3. Mosberg LJ, Garite TJ, Freeman RK. Fetal heart rate patterns and fetal distress in patients with preterm premature rupture of membranes. Obstet Gynecol 1984; 64:60-4.
4. Ogita S, Imanaka M, Matsumoto M, Hatanaka K. Premature rupture of the membranes managed with a new cervical catheter. Lancet 1984;1:1330-1.
5. Ogita S, Mizuno M, Takeda Y, et al. Clinical effectiveness of newly devised cervical indwelling catheter in managing premature rupture of the membranes: a Japanese collaborative work. AM J OBSTET GYNECOL 1988;159:336-41.
6. Manning FA, Hill LM, Platt LD. Qualitative amniotic fluid volume determination by ultrasound: antepartum detection of intrauterine growth retardation. AM J OBSTET GYNECOL 1981;139:254-8.
7. Nakayama DK, Glick PL, Harrison MR, et al. Experimental pulmonary hypoplasia due to oligohydramnios and its reversal by relieving thoracic compression. J Pediatr Surg 1983;18:347-53.
8. Miyazaki FS, Nevarez FN. Saline amnioinfusion for relief of repetitive variable decelerations: a prospective randomized study. AM J OBSTET GYNECOL 1985;153:301-6.
9. Nageotte MP, Freeman RK, Garite TJ, Dorchester W. Prophylactic intrapartum amnioinfusion in patients with preterm premature rupture of membranes. AM J OBSTET GYNECOL 1985;153:557-62.
10. Ogita S, Imanaka M, Matsumoto M, et al. Transcervical amnioinfusion of antibiotics: a basic study for managing premature rupture of membranes. AM J OBSTET GYNECOL 1988;158:23-7.

Prematurity among insulin-requiring diabetic gravid women

Michael F. Greene, MD,[a,b] John W. Hare, MD,[b] Martha Krache, RN, MPH,[a] Mark Phillippe, MD, Vanessa A. Barss, MD,[a] Daniel H. Saltzman, MD,[a] Allan Nadel, MD, [a] M. Donna Younger, MD,[b] Linda Heffner, MD, PhD,[a] and J. Elizabeth Scherl, MD[a]

Boston, Massachusetts

From Jan. 1, 1983, through Dec. 31, 1987, 420 gravidas with insulin-requiring diabetes antedating pregnancy delivered on the Joslin Clinic service. Among them, 110 pregnancies (26.2% of the total) delivered before 37 completed weeks of gestation compared with a 9.7% incidence (906/9368) for the general population at the Brigham and Women's Hospital during calendar year 1985. Thirty-three percent of all premature deliveries were the result of the development of preeclampsia. The relative risk of prematurity for diabetic patients with any hypertensive complication was 2.0 (95% confidence interval, 1.40 to 2.87) compared with normotensive diabetic subjects. Compared with the general population, most of the excess risk of prematurity was confined to hypertensive diabetics and normotensive patients of more advanced White class. A history of having had a previous premature delivery, increasing duration of diabetes antedating pregnancy, and carrying a male fetus in the index pregnancy were significantly associated with premature delivery. Future efforts to reduce the incidence of prematurity among diabetic gravidas should be directed toward reducing the incidence of preeclampsia. (AM J OBSTET GYNECOL 1989;161:106-11.)

Key words: Diabetes, pregnancy, prematurity, hypertension

Diabetes mellitus has always been recognized as a serious complication in pregnancy. Twenty years after the introduction of insulin, Lawrence and Oakley[1] published their results from King's College Hospital and reviewed the existing literature. In summarizing the previously reported series, it was striking that although the maternal mortality rate had fallen to 2% to 5% after the introduction of insulin, the perinatal mortality rate of approximately 40% had not changed. Over the next 20 years, maternal mortality was eliminated as a significant problem, and the perinatal mortality rate was halved to approximately 18%. The sources of that mortality remained ketoacidosis, congenital anomalies, late intrauterine death, and prematurity. Ketoacidosis during pregnancy has become a distinctly unusual problem with regular medical care. Current methods of ante-

From the Department of Obstetrics and Gynecology, Brigham and Women's Hospital and Harvard Medical School,[a] and Joslin Clinic and Harvard Medical School.[b]

Received for publication August 31, 1987; revised August 1, 1988; accepted January 3, 1989.

Reprint requests: Michael F. Greene, MD, Department of Obstetrics and Gynecology, Brigham and Women's Hospital, 75 Francis St., Boston, MA 02115.

natal surveillance have reduced the incidence of late intrauterine death to probably less than 1%.[2] Congenital anomalies remain a problem, but efforts at rigorous metabolic control offer the hope that these can be reduced.[3] Although recent advances in neonatal care have substantially reduced the morbidity and mortality associated with prematurity, it still remains the most important determinant of neonatal morbidity in diabetic pregnancy.[4] Prematurity and its sources in a diabetic population have not been systematically addressed in recent years.

Before the advent of contemporary methods for antepartum monitoring, prematurity among infants of diabetic mothers was commonly iatrogenic in an effort to avoid late death. Although this source of prematurity has been largely eliminated, the incidence of prematurity among infants of diabetic mothers remains high. Some recent reports have dealt with various aspects of prematurity among infants of diabetic mothers. Miodovnik et al.[5] reported a very high incidence of premature labor but did not mention the incidence of premature delivery resulting from that preterm labor. In a series reported by Zalut et al.[6] the incidence of both premature labor and resultant premature delivery was lower than that for a control nondiabetic population. Diamond et al.[7] examined the role of hypertension in prematurity among infants of diabetic mothers and concluded that it was not a significant problem. In a literature review of reported series, Cousins[8] examined the incidence of various complications among diabetic gravidas. Some of the complications that he tabulated could result in premature delivery, but prematurity was not the focus of his study. This report details the incidence, sources of, and some risk factors for prematurity in a large series of patients with insulin-requiring diabetes antedating their pregnancies.

Patients and methods

Data on all patients presenting for prenatal care at the Joslin Clinic prenatal clinic are recorded in a computer-based perinatal data base at the initial visit. Subsequently, data pertaining to the progress of their pregnancies are collected prospectively. Delivery data are obtained by review of the patients' charts within 24 hours of delivery. Data are accumulated on the postpartum course and neonate prospectively, and all mother and infant charts are finally reviewed after discharge.

This series includes all patients with insulin-requiring diabetes antedating pregnancy (White Classes B and greater) delivering between Jan. 1, 1983, and Dec. 31, 1987, at greater than 20 weeks' gestation. No patients with gestational insulin-requiring diabetes are included. Patients were classified according to the system of White as modified by Hare and White.[9] For purposes of these analyses, 16 patients with nephropathy (Class F), 39 patients with nephropathy and retinopathy (Class FR) and four patients with renal transplants (Class T) are grouped together as Class F.

As a matter of policy, insulin-requiring diabetic patients were generally delivered between 38 and 39 weeks. Nonstress tests were performed weekly from 32 weeks and twice weekly from 36 weeks until delivery. Patients delivering before 37 completed weeks were considered to have delivered prematurely. Gestational ages were determined by the best available criteria, last menstrual period, basal body temperature chart, or first-trimester ultrasound examination. For each premature delivery, the immediate cause for premature delivery was determined. Cases of premature labor with spontaneous rupture of the membranes were assigned to spontaneous rupture of the membranes as the cause of prematurity. All cases of preeclampsia met the standard criteria of blood pressure in excess of 140/90 on at least two occasions separated by 6 hours and proteinuria in excess of 300 mg per 24-hour period. Some patients had apparently simple preeclampsia, whereas others clearly had preeclampsia superimposed on chronic hypertension. Any patient with blood pressure measurements in excess of 140/90 on at least two occasions separated by at least 6 hours was considered to have hypertension.

Data regarding the gestational ages at the time of delivery for all the hospital's deliveries were accumulated in a separate data base. Gestational ages were assigned after reviewing the data provided for each patient by each attending obstetrician.

Statistical methods used included χ^2 analysis, the Mantel-Haenszel χ, and the stratified, weighted Mantel-Haenszel χ according to the technique of Rothman.[10] A probability of <0.05 was taken to be statistically significant.

Results

There were 413 singleton, six twin, and one triplet gestations delivered during the study period. Among them 110 pregnancies were delivered before 37 completed weeks (26.2% of the total). These included six of the seven multiple gestations. Two of the multiple gestations were delivered due to preeclampsia, two due to evidence of fetal distress, and one each due to spontaneous rupture of the membranes and abruptio placentae. The premature deliveries are listed in Table I according to the cause of the premature delivery. The four singleton intrauterine deaths occurred as follows: (1) at 22 weeks in a patient with severe chronic hypertension; (2) at 30½ weeks with chorioamnionitis and sepsis after amniocentesis in a patient with severe preeclampsia; (3) at 33½ weeks in a chronic alcoholic with acute pancreatitis, adult respiratory distress syndrome,

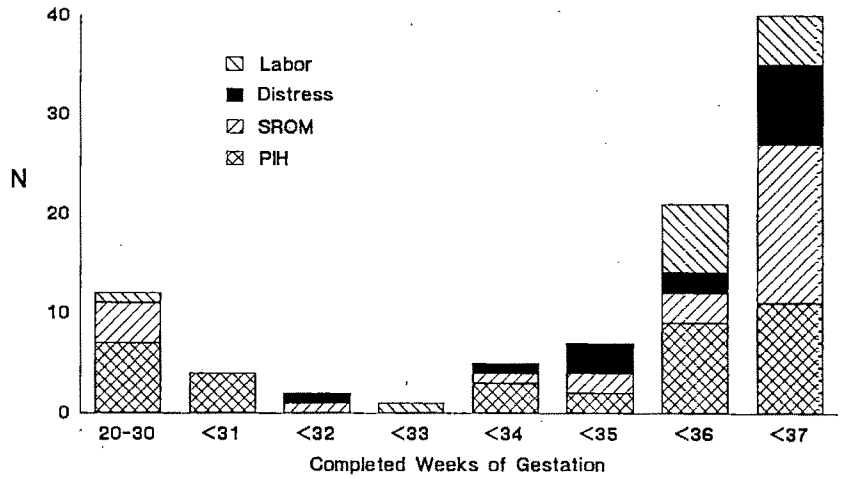


Fig. 1. Premature deliveries displayed according to gestational age and cause for four leading causes of prematurity. *Labor,* Premature labor; *Distress,* fetal distress; *SROM,* spontaneous rupture of membranes; *PIH,* preeclampsia.

Table I. Deliveries between 20 and 37 weeks of gestation by indication for delivery

	N	%
Preeclampsia	36	8.6
Spontaneous rupture of membranes	27	6.4
Fetal distress	15	3.6
Spontaneous onset of labor	14	3.3
Hemorrhage, abruptio, or previa	5	1.2
Intrauterine death	4	0.9
Polyhydramnios' maternal discomfort	4	0.9
Other	5	1.2
Total	110	26.2

acute renal failure, disseminated intravascular coagulopathy, and diabetic ketoacidosis; and (4) at 35 weeks in a patient with poor glycemic control but an otherwise unremarkable pregnancy. A fifth death occurred in a discordant small twin at 33½ weeks. The survivor had mature pulmonary indexes and was delivered for presumed fetal distress. The five patients listed as "other" included the following: (1) an induced abortion at 22 weeks for bilateral renal agenesis; (2) a spontaneous rupture of the uterus at 34½ weeks with fetal salvage in a patient with four prior cesarean sections; (3) a patient with "nephrogenic" diabetes insipidus at 35 weeks; (4) a patient with preeclampsia who had a grand mal seizure in association with a blood glucose level of 22 mg/dl at 35½ weeks; and (5) a patient with an intractable headache at 36 weeks.

The deliveries occurring between 20 and 37 weeks as a result of the four leading causes of prematurity are displayed graphically in Fig. 1. Clearly, preeclampsia is the single most important cause of prematurity in this population. Only 23 (5.5%) of the patients had hypertension antedating pregnancy, but these represent 18.4% of all patients with hypertensive disorders in pregnancy. Among these, 13 were delivered before 37 weeks, with nine the direct result of preeclampsia superimposed on their chronic hypertension. Thus of the 36 premature deliveries directly attributable to preeclampsia, one fourth were superimposed on chronic hypertension.

Although 23 patients were diagnosed as having premature labor with intact membranes and treated with tocolytic agents in this series, only 17 were actually delivered prematurely. Another 10 patients had premature labor with rupture of the membranes (all of whom delivered prematurely), for an overall incidence of premature labor of 7.8% (33/420). Polyhydramnios is difficult to quantitate and is most commonly diagnosed as a clinical impression from physical or ultrasonic examination. The extent to which it contributed to cases of preterm rupture of the membranes or labor is speculative. There were four cases in the 5 years of macrosomia (3500, 4200, 4700, and 5100 gm) associated with severe polyhydramnios, which led to significant maternal discomfort. In these cases amniocenteses at 35, 35½, 36, and 36½ weeks showed mature pulmonary indexes, and these patients were electively delivered before 37 weeks.

The distribution of the premature deliveries among the White Classes is shown in Table II. That this distribution is not random among the classes is demonstrated by a χ^2 analysis that is significant at less than the 0.005 level. The distributions of hypertensive complications and premature deliveries caused by preeclampsia are also shown in Table II. These risks parallel the risk of prematurity in aggregate and are also nonrandom by χ^2 analysis at the 0.005 level. Comparison of the risk of prematurity between hypertensive and normotensive diabetic patients via the Mantel-Haenszel χ

Table II. Prematurity, hypertensive complications, and prematurity caused by preeclampsia by White Class

Class	*No. of premature deliveries (%)*	*No. of hypertensive complications (%)*	*No. of premature deliveries due to preeclampsia*	*Total No.*
B	21 (20.4)	18 (17.5)	5	103
C	21 (17.4)	28 (23.1)	6	121
D	26 (25.7)	31 (30.7)	7	101
F	31 (52.2)	39 (66.1)	14	59
R	11 (30.5)	9 (25.0)	4	36
Total	110 (26.2)	125 (29.8)	36	420

test shows that the risk ratio is statistically significant at 2.00, with a 95% confidence interval of 1.40 to 2.87.

During calendar year 1985, there were 9368 deliveries at the hospital, of which 906 (9.7%) were before 37 completed weeks of gestation. The risk ratios for premature delivery for various groups of diabetics compared with the entire hospital population are shown in Table III. The entire diabetic population as a whole is considerably more likely to deliver prematurely than the general population. Virtually all subgroups of the diabetic population with the possible exception of normotensive White Class B and C patients are significantly more likely to deliver prematurely. Not surprisingly, the greatest risks for prematurity are found among patients with microvascular disease and a hypertensive complication.

No relationship was found between the risk of prematurity and the number of previous pregnancies, the number of previous term births, the number of living children, a history of previous perinatal mortality, or a history of one or more spontaneous or induced abortions. A history of having had a previous premature delivery was significantly associated with premature delivery in the current pregnancy (relative risk 1.60; 95% confidence interval, 1.02 to 2.50). Carrying a male fetus in the current pregnancy was also associated with an increased risk of prematurity (relative risk 1.43; 95% confidence interval, 1.02 to 2.02). Analyses of each of the individual causes of prematurity failed to reveal any one that in itself increased in pregnancies with male fetuses. There was no relationship between maternal age and prematurity. The number of years of diabetes antedating pregnancy was related to the risk of prematurity. Women with 20 or more years of diabetes antedating pregnancy ($N = 70$) were twice as likely to deliver prematurely as women with 19 or fewer years of diabetes (relative risk = 2.0; 95% confidence internal, 1.40 to 2.87).

Table III. Risk ratio for prematurity compared with all Brigham and Women's Hospital deliveries

	Relative risk	*95% Confidence interval*
All diabetics	3.22	2.76-3.75
Normotensive diabetics		
All classes	2.08	1.64-2.64
B	1.70	1.04-2.79
C	1.22	0.7 -2.15
D	2.51	1.62-3.90
F	3.10	1.50-6.39
R	2.68	1.36-5.28
Hypertensive diabetics		
All classes	5.19	4.31-6.25
B	4.02	2.09-7.72
C	3.69	2.13-6.41
D	3.00	1.66-5.43
F	6.63	4.78-9.18
R	4.59	1.97-10.73

Comment

This report is unique in recording the incidence and sources of prematurity in more than 400 insulin-requiring diabetic pregnancies from a single institution over only 5 years. Other reports of large numbers of pregnancies have either been accumulated over longer periods of time or from a large number of institutions. Furthermore, since all of the pregnancies in this cohort were managed by us, this series represents an unusual degree of homogeneity of care.

Despite the improvements that have been made in the care of diabetic pregnant patients, they remain at increased risk of premature delivery. If this risk is to be reduced by addressing its sources, then those sources must be identified. It is clear from the present series that hypertension or preeclampsia is a common problem among these patients. More than one fourth of the patients had a clinically significant hypertensive disorder. One third of all of the premature deliveries were the result of preeclampsia. Of all patients with a hypertensive complication, 29% had a premature delivery. The incidence of hypertensive complications in this series is quite high. It is higher than that reported by Kitzmiller et al.[11] but very similar to that reported for a comparable population by Gabbe et al.[12] In his 20-year compilation of data from the literature, Cousins[8] found a 14.5% incidence of total hypertension among Classes B and C and a 30.9% incidence for Classes D, F, and R. These figures compare with 20.5% among

Class B and C patients in this series and 40.3% among Class D, F, and R patients.

A recent study[7] called attention to the fact that the issues of perinatal morbidity and mortality related to hypertensive complications in diabetic pregnancy had not been addressed in the context of contemporary perinatal medicine. That study examined 199 insulin-dependent diabetic pregnancies occurring over 7 years at one institution. The authors concluded that hypertensive complications were not a source for major concern with modern management. However, examination of their data shows that there were only 59 hypertensive patients and 33 patients in White Classes D, F, and R. The data from the present study indicate that it is largely hypertensive patients and those normotensive patients in White Classes D, F, and R who are at significant risk for prematurity and hence the morbidity associated with prematurity. Furthermore, the small number of patients in the more advanced classes indicates that there were relatively few patients with diabetes of long duration, which is another important risk factor for prematurity. Since the number of patients at risk in their series was small, their conclusion should not be accepted without reservation.

Urinary albumin excretion increases during both normal pregnancies[13] and those complicated by diabetic nephropathy.[14] It was common among patients in this series to find clinically significant proteinuria develop early in pregnancy in patients with long-standing diabetes but not previously known to have renal involvement. Frequently these patients also developed modest but significant elevations in blood pressure near term. It is unclear whether this truly represents preeclampsia. The designation of preeclampsia in Class F patients who start pregnancy with hypertension and proteinuria is completely arbitrary.

Two recently published, randomized, controlled trials of antiplatelet therapy in high-risk nondiabetic pregnant subjects have shown a decreased incidence of preeclampsia among treated subjects.[15, 16] Neither trial was large enough to convincingly demonstrate a significant reduction in the incidence of prematurity. A diabetic population such as the present one would seem to be an excellent group to study the efficacy of antiplatelet therapy. If it proved efficacious in reducing the incidence of prematurity, it could be a valuable addition to the care of these patients.

The incidence of premature labor with or without rupture of the membranes was 7.8% in this series. This is dramatically different from the 29.9% rate recently found in another series.[5] It would be interesting to know what was the incidence of actual preterm delivery caused by that preterm labor. In this series only 6.4% (27/420) of the patients delivered prematurely in association with premature labor with or without premature rupture of the membranes. The low incidence of premature labor and the high percentage of those diagnosed who then deliver prematurely (82%; 27/33) are probably the result of the strict criteria used to diagnose premature labor. For any group of patients, the more stringent the diagnostic criteria for premature labor, the lower the frequency will appear to be and the lower the number of false-positive diagnoses will be. The hyperglycemia that results from β-agonist tocolytic therapy in diabetic patients makes us reluctant to diagnose premature labor with intact membranes without a clear change in the cervical examination. For these reasons, no conclusions should be drawn from these data with respect to the relative frequency of premature labor or efficacy of its therapy compared with a nondiabetic population.

These findings with respect to risk of premature delivery and past obstetric history are in accordance with those reported elsewhere in a general obstetric population. The relationship between carrying a male fetus and prematurity has also been previously noted.[17] More male than female infants were delivered prematurely in each of the four leading categories of causes of prematurity. However, this effect was modest enough that when broken down into such small data cells, no statistical significance could be found for any one. Interestingly, all four women requiring premature delivery for severe polyhydramnios carried male fetuses. The physiologic mechanisms underlying this sex difference remains a matter of speculation.

The continued prognostic use of the White classification has occasionally been questioned. However, it is clear from this analysis that patients with known nephropathy or long-standing disease and probable subclinical nephropathy have a significantly greater risk of developing a hypertensive complication in pregnancy and a higher likelihood of delivering prematurely as a result. Thus White's classification is useful clinically for an individual patient to alert the physician to the possibility of hypertensive complications. It is also useful in characterizing a patient population because the incidence of prematurity to be expected in that population will depend on the distribution of patients among White's classes.

REFERENCES

1. Lawrence RD, Oakley W. Pregnancy and diabetes. Q J Med 1942;11:45-75.
2. Jorge CS, Artal R, Paul RH, et al. Antepartum fetal surveillance in diabetic pregnant patients. Am J Obstet Gynecol 1981;141:641-5.
3. Fuhrmann K, Reiher H, Semmler K, Fischer F, Fischer M, Glockner E. Prevention of congenital malformations in infants of insulin-dependent diabetic mothers. Diabetes Care 1983;6:219-23.
4. Hanson U, Persson B, Stangenberg M. Factors influencing neonatal morbidity in diabetic pregnancy. Diabetes Res 1986;3:71-6.
5. Miodovnik M, Mimouni F, Siddiqi TA, Holroyde J, Tsang RC. High spontaneous premature labor rate in insulin-

dependent diabetic (IDD) pregnant women: an association with poor glycemic control. Presented at the Seventh Annual Meeting of the Society of Perinatal Obstetricians, Lake Buena Vista, Florida, February 5-7, 1987.
6. Zalut T, Reed KL, Shenker L. Incidence of premature labor in diabetic patients. Am J Perinatol 1985;2:276-8.
7. Diamond MP, Shah DM, Hester RA, Vaughn WK, Cotton RB, Boehm FH. Complication of insulin-dependent diabetic pregnancies by preeclampsia and/or chronic hypertension: analysis of outcome. Am J Perinatol 1985;2: 263-7.
8. Cousins L. Pregnancy complications among diabetic women: review 1965-1985. Obstet Gynecol Surv 1987; 42:140-9.
9. Hare JW, White P. Gestational diabetes and the White classification. Diabetes Care 1980;3:294.
10. Rothman KJ. Modern epidemiology. Boston: Little, Brown & Co, 1986:186-215.
11. Kitzmiller JL, Cloherty JP, Younger MD, et al. Diabetic pregnancy and perinatal morbidity. AM J OBSTET GYNECOL 1978;131:560-80.
12. Gabbe SG, Mestman JH, Freeman RK, et al. Management and outcome of pregnancy in diabetes mellitus, Classes B to R. AM J OBSTET GYNECOL 1977;129:723-31.
13. Wright A, Steele P, Bennett JR, Watts G, Polak A. The urinary excretion of albumin in normal pregnancy. Br J Obstet Gynecol 1987;94:408-12.
14. Kitzmiller JL, Brown ER, Phillippe M, et al. Diabetic nephropathy and perinatal outcome. AM J OBSTET GYNECOL 1981;141:741-51.
15. Beaufils M, Donsimoni R, Uzan S, Colau JC. Prevention of pre-eclampsia by early antiplatelet therapy. Lancet 1985;i:840-2.
16. Wallenburg HCS, Markovitz JW, Dekker GA, Rotmans P. Low-dose aspirin prevents pregnancy-induced hypertension and pre-eclampsia in angiotensin-sensitive primigravidae. Lancet 1986;i:1-4.
17. van den Berg BJ, Oechsli FW. Prematurity. In: Bracken MB, ed. Perinatal epidemiology. New York; Oxford University Press, 1984:69-85.

Management of the nonvertex second twin: Primary cesarean section, external version, or primary breech extraction

Stephen E. Gocke, MD,[a] Michael P. Nageotte, MD,[b] Thomas Garite, MD,[b] Craig V. Towers, MD,[b] and Wendy Dorcester, PhD[b]

Fresno and Long Beach, California

Six hundred eighty-two consecutive twin deliveries were reviewed. Included in the study were 136 sets of vertex-nonvertex twins with birth weights >1500 gm. A primary attempt at delivery of the second twin by external version was performed on 41 twins, 55 twins underwent attempted breech extraction, and 40 patients had a primary cesarean section solely because of physician preference. There were no differences in the incidence of neonatal morbidity or mortality among the modes of delivery. External version was associated with a higher failure rate than primary breech extraction ($p < 0.01$). External version was associated with complications (fetal distress, cord prolapse, and compound presentation) that were not seen in the other two groups. Primary breech extraction of the second nonvertex twin weighing >1500 gm appears to be a reasonable alternative to either cesarean section or external version. (AM J OBSTET GYNECOL 1989;161:111-4.)

Key words: Second twin, cesarean section, external version, breech extraction

The optimal mode of delivery of the nonvertex second twin remains controversial. Certain reports have advocated cesarean delivery if the second twin is nonvertex, suggesting increased perinatal morbidity and mortality in nonvertex twins delivered vaginally.[1-4] More recent reports have suggested that vaginal delivery by breech extraction or assisted breech delivery of the second nonvertex twin is not associated with an increase in perinatal morbidity if limited to infants with birth weights >1500 gm.[5-8] Chervenak et al.[9] advocated the alternative approach of external version of the second nonvertex twin to achieve delivery of the second twin as a vertex presentation. In an effort to examine this controversy we report a comparison of maternal and neonatal outcomes in the second nonvertex twin delivered by cesarean section, external version, or breech extraction for our institutions.

From the Valley Medical Center of Fresno[a] and Women's Hospital, Memorial Medical Center,[b] University of California, Irvine.
Received for publication March 27, 1988; revised October 22, 1988; accepted January 30, 1989.
Reprint requests: Stephen E. Gocke, MD, Women's Hospital, Memorial Medical Center, 2801 Atlantic Ave., Long Beach, CA 90801.

Material and methods

A retrospective review of all 682 twin deliveries occurring in the authors' two institutions from January 1982 through December 1987 was performed. All 202

Table I. Maternal variables

	Group A (n = 41)	*Group B (n = 55)*	*Group C (n = 40)*	*p*
Age (yr)	28.0	26.7	27.8	NS
Gestational age (wk)	35.5	36.7	36.2	NS
Para 0	15 (37%)	14 (26%)	15 (38%)	NS
Maternal postpartum length of stay (days)	3.7	2.4	4.4	<0.05
Postpartum hemorrhage	1 (2%)	3 (5%)	2 (5%)	NS
Endometritis	2 (5%)	3 (5%)	1 (2%)	NS

sets of vertex-nonvertex twins with birth weights >1500 gm were eligible for the study. Sixty-six sets of vertex-nonvertex twins who were delivered by cesarean section for failure to progress ($n = 9$), fetal distress ($n = 17$), repeat cesarean section ($n = 28$), cord prolapse ($n = 3$), or other intrapartum complications ($n = 9$) were excluded. The remaining 136 sets of twins (96 sets from Long Beach Memorial Hospital and 40 sets from Valley Medical Center of Fresno) were divided into three groups. Group A ($n = 41$) underwent a primary attempt at delivery of the second twin by external version. Group B ($n = 55$) had a primary attempt at breech extraction. Group C ($n = 40$) consisted of patients in whom a primary cesarean section was performed solely because of physician's preference because of the presence of a nonvertex second twin.

External version was performed according to the guidelines suggested by Chervenak et al.[9] Epidural anesthesia was used in most cases to facilitate abdominal wall relaxation. All versions were attempted under double set-up conditions with immediate access to cesarean section. Real-time ultrasonography was used in every case in the delivery room to determine fetal presentation of the second twin. Fetal heart rate was monitored throughout labor and delivery. Version was accomplished either by using the transducer to guide the infant into vertex presentation in the birth canal or by manually attempting either a forward or backward roll of the fetal vertex into the pelvic inlet. After successful version, membranes were often ruptured and pitocin augmentation was employed to facilitate descent of the vertex.

Breech extraction was performed under double set-up conditions in which immediate access to cesarean section was available. Epidural or halothane anesthesia was used to provide abdominal and uterine wall relaxation. In most cases, real-time ultrasonography was available for determination of fetal lie. The breech extractions were either "assisted," with spontaneous delivery of the breech, or "total," with the entire body of the infant delivered by the obstetrician.

The following maternal variables were considered: maternal age, parity, estimated gestational age, postpartum length of hospital stay, postpartum hemorrhage, and endometritis. Postpartum hemorrhage was defined as any blood loss that required a transfusion. Endometritis was defined as two temperature readings >38° C exclusive of the first 24 hours after delivery and findings on examination of a tender uterus, purulent cervical discharge, or both. The following neonatal variables were compared: birth weights of the first and second twins, Apgar scores at 1 and 5 minutes, incidence of neonatal intensive care unit admissions, respiratory distress syndrome, intraventricular hemorrhage, neonatal deaths, birth trauma, length of infant hospital stay, and length of infant hospital stay minus maternal length of hospital stay. Complications that occurred with each type of delivery were also recorded.

Statistical evaluation of the data was accomplished by the χ^2 test (with Yates correction) and Kruskal-Wallis test in appropriate cases. Differences were considered to be statistically significant at $p < 0.05$.

Results

Comparison of maternal variables among the three groups (Table I) showed no significant difference in maternal age, gestational age, incidence of primiparous women, incidence of postpartum hemorrhage, or incidence of endometritis. There was a significantly shorter maternal postpartum length of hospital stay in those patients who underwent an attempt at breech extraction (2.4 days) than in patients who underwent an attempt at external version (3.7 days) or primary cesarean section (4.4 days) ($p < 0.05$).

Neonatal variables were compared in Table II. There was no difference in birth weights of twin 1 or 2, 1-minute Apgar scores <4, 5-minute Apgar scores <7, incidence of neonatal intensive care unit admissions, respiratory distress syndrome, birth trauma, neonatal deaths, or postpartum infant length of hospital stay after the maternal postpartum length of stay was subtracted from the infant's length of stay. Before the maternal length of stay was considered, there was a significantly longer length of hospital stay for infants delivered by external version (10.1 days) or cesarean section (10.2 days) than in infants delivered by breech extraction (5.0 days). This increase in the length of infant stay in the hospital was a direct result of the increased maternal length of postpartum stay because of the increased incidence of cesarean section in groups A (39.0%) and C (100%) (Table III). The one infant in the primary breech extraction group with a 5-minute Apgar score of 6 had a birth weight of 2160 gm, was not admitted to the neonatal intensive care unit, and went home on postpartum day 3.

Table III shows that patients who underwent a primary attempt at external version had a significantly

Table II. Neonatal variables

	Group A (n = 41)	*Group B (n = 55)*	*Group C (n = 40)*	*p*
Birth weight of first twin (gm)	2399	2544	2356	NS
Birth weight of second twin (gm)	2365	2569	2347	NS
1 min Apgar score <4	5	5	1	NS
5 min Apgar score <7	0	1	0	NS
Neonatal intensive care unit admission (%)	18	13	18	NS
Respiratory distress syndrome	0	2	1	NS
Birth trauma	0	0	0	NS
Intraventricular hemorrhage	0	0	0	NS
Neonatal death	0	0	0	NS
Postpartum infant length of hospital stay (days)	10.1	5.0	10.2	<0.05
Postpartum infant length of hospital stay minus maternal stay	6.4	2.6	5.8	NS

Table III. Success of primary delivery attempt

	Group A (n = 41)	*Group B (n = 55)*	*p*
Successful vaginal delivery (vertex vaginal delivery after version or breech vaginal delivery after primary breech extraction)	19/41 (46%)	53/55 (95%)	<0.001
Cesarean section	16/41* (39%)	2/55 (4%)	<0.001
Successful secondary breech extraction procedure	6/41 (15%)	—	—
TOTAL	41/41 (100%)	55/55 (100%)	—

*Seven successful and nine unsuccessful versions required cesarean section.

higher incidence of cesarean section and were less likely to deliver vaginally as a result of the version attempt than patients who underwent primary breech extraction. In the external version group, the use of epidural anesthesia was not associated with an increase in successful external version (Table IV). Complications during the delivery attempts are listed in Table V. Of the seven successful external versions that required cesarean section, six of the seven operations were performed under emergency circumstances (2 cases for fetal distress, 3 cases of cord prolapse, and 1 compound presentation). The two failed attempts at breech extraction occurred in women who did not receive epidural anesthesia. In both of these cases the uterus contracted, which made breech extraction difficult. One of these patients received halothane anesthesia during the failed attempt at breech extraction. Neither patient received a β-sympathomimetic agent to facilitate uterine relaxation. Neither infant suffered any trauma from the failed attempt at extraction.

Table IV. Epidural anesthesia in external version patients

	Successful version	*Unsuccessful version*
Epidural	20	7
No epidural	6	8

$\chi^2 = 2.64, p > 0.10$

Comment

External version of the second nonvertex twin is a relatively new approach to management of the nonvertex twin gestation. Our results do not reflect this as a completely innocuous procedure. Patients in whom an external version was attempted had a vertex vaginal delivery rate of 46% but overall had a cesarean section rate of 39%. Indeed, in 41 attempts at external version, there were 26 successes but only 19 second twins delivered vaginally as a vertex. Because of the higher cesarean section rate, the external version group had a longer postpartum length of stay than patients who underwent primary breech extraction. Although there was no difference in neonatal morbidity or mortality among the three groups, cesarean section was performed for the second twin 39% of the time in patients having an attempt at external version. Approximately one third of these cesarean sections were emergencies for fetal distress, cord prolapse, or compound presentation.

There is a growing body of evidence to suggest that breech vaginal delivery of the second nonvertex twin is a reasonable alternative in infants with birth weights >1500 gm.[5-8] Earlier papers that suggested a worse outcome for the second twin included infants <1500 gm and were reported during a time period when the sec-

Table V. Delivery complications

	Complication	*Outcome*
External version		
Successful	Fetal distress	C/S
Successful	Fetal distress	C/S
Successful	Cord prolapse	C/S
Successful	Cord prolapse	C/S
Successful	Cord prolapse	C/S
Successful	Failure to descend	C/S
Successful	Compound presentation	C/S
Breech extraction		
Unsuccessful	Lower uterine segment contracted	C/S
Unsuccessful	Lower uterine segment contracted	C/S

C/S, Cesarean section.

ond twin was often undiagnosed before delivery.[1, 3, 4] Furthermore, most of these reports were before the use of continuous fetal monitoring and the modern anesthetic techniques of conduction or general anesthesia. In our study, 12 of the 41 infants in the version group and 8 of the 55 breech extraction infants had birth weights <2000 gm. The neonatal and maternal outcomes of these 20 infants were no different from the remainder of the infants in the study.

It should be noted that deliveries at Memorial Medical Center of Long Beach were attended by obstetricians and perinatologists. At Valley Medical Center deliveries were performed by residents, usually without the presence of staff. Thirty-five of the 55 breech extractions and three of the external version patients were from Valley Medical Center. If a bias existed for a worse outcome because of the level of operator experience, it would have been against the breech extraction group because more breech extractions than external versions were performed by residents in training.

The trend toward more successful versions with epidural anesthesia, although not statistically significant, is still striking considering the relatively low numbers (Table IV). Relaxation of the abdominal musculature improves the ability to move the fetal pole and would appear to improve the success rate of external version, even without the use of specific uterine tocolytic therapy. It is possible that such refinements and greater experience may improve the overall success rate with external version of the second twin. However, until better comparative outcome is found and demonstrated, it would be difficult to recommend external version as the preferred method for delivery of the nonvertex second twin. Our data suggest that breech extraction is both a safe and successful primary approach to the delivery of nonvertex second twins weighing more than 1500 gm. Further, these results at least suggest that attempted breech extraction represents a reasonable secondary approach to vaginal delivery of the second nonvertex twin if the external version attempt is unsuccessful.

In conclusion, attempts at external version were associated with a higher failure rate than primary breech extraction. External version was associated with complications that were not seen in either the elective cesarean section or breech extraction groups. There were no differences in terms of neonatal morbidity among the routes of delivery. Primary breech extraction of the second nonvertex twin >1500 gm appears to be a reasonable alternative to either cesarean section or external version.

REFERENCES

1. Kelsick F, Minkoff H. Management of the breech second twin. Am J Obstet Gynecol 1982;144:873.
2. Cetrulo CI, Ingardia CJ, Sbarra AJ. Management of multiple gestation. Clin Obstet Gynecol 1980;23:533.
3. Ho SK, Wu PYK. Perinatal factors and neonatal morbidity in twin pregnancy. Am J Obstet Gynecol 1975;122:979.
4. Ware HH. The second twin. Am J Obstet Gynecol 1971;100:865.
5. Acker D, Lieberman M, Holbrook RH, et al. Delivery of the second twin. Obstet Gynecol 1982;59:710.
6. Rabinovici J, Barkai G, Reichman B, et al. Randomized management of the second non-vertex twin: vaginal delivery or cesarean section. Am J Obstet Gynecol 1987; 156:52.
7. Chervenak FA, Johnson RE, Berkowitz RL, et al. Is routine cesarean section necessary for vertex-breech and vertex-transverse twin gestations? Am J Obstet Gynecol 1984; 148:1.
8. Chervenak FA, Johnson RE, Youcha S, et al. Intrapartum management of twin gestation. Obstet Gynecol 1985;65: 119.
9. Chervenak FA, Johnson RE, Berkowitz FL, et al. Intrapartum external version of the second twin. Obstet Gynecol 1983;62:160.

Magnesium supplementation during pregnancy: A double-blind randomized controlled clinical trial

Baha M. Sibai, MD, Marco A. Villar L., MD, and Eileen Bray, RNC

Memphis, Tennessee

Four hundred young normotensive primigravid women between 13 and 24 weeks' gestation were randomly allocated to one of two study groups. One group received placebo tablets and the other group received 365 mg of elemental magnesium daily (as magnesium aspartate hydrochloride). Three hundred seventy-four patients completed the study, 189 in the placebo group and 185 in the treatment group. There were no significant differences between the two groups regarding serum calcium, uric acid, or electrolyte levels. However, the magnesium-supplemented group had significantly higher magnesium levels at delivery (1.68 ± 0.03 mg/dl vs. 1.56 ± 0.03 mg/dl, $p < 0.01$). There were no significant differences in either systolic or diastolic blood pressures between both groups either at time of enrollment or at subsequent gestational ages later during pregnancy. Analysis of variance for repeated measurements and Fisher's least significant difference testing indicated a significant increase ($p < 0.01$) in blood pressure from the level at the time of enrollment to the level achieved at or beyond 37 weeks' gestation in each group. There were no significant differences between the two groups regarding any of the following parameters: incidences of preeclampsia, fetal growth retardation, preterm labor, birth weight, gestational age at delivery, or number of infants admitted to the special care unit. Magnesium supplementation during pregnancy did not improve pregnancy outcome in our population. (Am J Obstet Gynecol 1989;161:115-9.)

Key words: Oral magnesium aspartate hydrochloride, preeclampsia

The relationship between dietary magnesium deficiency and hypertension has been the subject of several recent experimental and observational studies.[1-5] In addition, in vitro studies on isolated umbilical arteries and veins obtained from term pregnancies showed that the basal tension of these vessels increased when magnesium was withdrawn from the solution and decreased when the magnesium concentration was raised.[6] These experimental findings led Altura et al.[6] to speculate that "progressive hypomagnesemia during pregnancy could produce progressive vasoconstriction resulting in spasm of umbilical and placental arteries and veins, and of other peripheral vessels in the pregnant mother and fetus."

Dietary magnesium deficiency during pregnancy has been implicated in the pathogenesis of preeclampsia, fetal growth retardation, and preterm delivery.[6-9] Data from recent studies reported a decreased incidence of preeclampsia,[6] fetal growth retardation,[7,8] and preterm delivery[8-10] in subjects who received oral magnesium supplementation during pregnancy.

From the Maternal-Fetal Division, Department of Obstetrics and Gynecology, University of Tennessee, Memphis.

Supported in part by a grant from Ciba-Geigy Corporation, Summit, N.J.

Received for publication July 22, 1988; revised December 8, 1988; accepted January 23, 1989.

Reprint requests: Baha M. Sibai, MD, University of Tennessee, 853 Jefferson Ave., Memphis, TN 38103.

To address these issues, we conducted a double-blind randomized, controlled clinical trial designed to investigate the effects of oral magnesium supplementation during pregnancy on maternal systolic and diastolic blood pressures and the incidence of preeclampsia as primary outcomes. Other outcome variables that were analyzed included changes in serum electrolytes, frequency of preterm delivery, postterm delivery, and fetal growth retardation.

Material and methods

The study population included 400 young (13 to 25 years) normotensive primigravid women who were recruited in our prenatal clinics. All patients were enrolled at 13 to 24 weeks of gestation as determined by obstetric dates and confirmed by ultrasonographic examinations. Patients with associated medical and obstetric complications and those beyond 24 weeks of gestation at the time of the first prenatal visit were excluded from the study. The study was a double-blind randomized, controlled clinical trial that was previously approved by both the Institutional Review Board and the Food and Drug Administration under an investigational new drug number.

After the consent form was signed, patients were allocated randomly to one of two groups, based on a computer-generated list of numbers, using a randomization schedule prepared in advance for the complete population. The same randomization code, standard-

Table I. Clinical characteristics

	Placebo (N = 189)	*Magnesium (N = 185)*
Age (yr)	18.5 ± 0.2	17.8 ± 0.2
Gestational age (wk)	17.8 ± 0.3	17.9 ± 0.4
Systolic blood pressure (mm Hg)		
At entry	110 ± 0.8	110 ± 0.8
At delivery	125 ± 1.3	126 ± 1.4
Diastolic blood pressure (mm Hg)		
At entry	61 ± 0.7	59 ± 0.8
At delivery	77 ± 1.3	77 ± 1.2
Total weight gain (lbs)	23.8 ± 0.9	25.9 ± 0.9

All values represent mean ± SEM.

ization procedures, and tablets were used in all patients. The code was kept in the outpatient pharmacy department. The women in the magnesium group received six tablets of magnesium-aspartate hydrochloride per day. Each of these tablets contained 60.8 mg of elemental magnesium (a total of 365 mg/day). The placebo group received six matching tablets containing aspartic acid only. The six tablets were divided into three daily doses and were continued until delivery. In addition, all patients received prenatal vitamins containing 200 mg of elemental calcium and 100 mg of elemental magnesium per day.

Total nutrient intake, including detailed description of magnesium intake, was evaluated three times during pregnancy with the 24-hour recall method. At each prenatal visit, patients were questioned regarding tablet intake, compliance, and side effects of medication. Patients were contacted by the research nurse as needed during the study to check compliance and to ensure that they come for their scheduled prenatal visits. On return visits tablet intake and compliance was assessed by a research nurse who performed a tablet count and recorded on the data form the total number of tablets used since the last prenatal visit. Serum magnesium levels at time of delivery also were used to confirm compliance with tablet intake. All patients were followed in our prenatal clinics. Blood pressure and weight were carefully measured at each prenatal visit. Systolic blood pressure was recorded with the appearance of the first Korotkoff sound, and diastolic blood pressure was recorded with the disappearance of the fourth Korotkoff sound. The result of all blood pressure measurements taken between the thirteenth and twenty-fourth weeks of gestation were pooled; the averages were used as baseline values in the adjusted analysis. Laboratory analyses of serum magnesium, calcium, uric acid, and electrolytes were performed at the time of delivery. All clinical decisions regarding management and timing of delivery were made by the attending faculty and house staff.

Analysis of data included comparisons of maternal blood pressures throughout pregnancy and the development of preeclampsia. Preeclampsia was defined as a systolic blood pressure reading of ≥140 mm Hg, diastolic blood pressure of ≥90 mm Hg on two occasions at least 6 hours apart with or without proteinuria, or both. Preeclampsia was classified as mild or severe according to the criteria of the American College of Obstetricians and Gynecologists. All such patients received intravenous magnesium sulfate as prophylaxis against convulsions. Pregnancy outcome included frequency of preterm labor with or without preterm premature rupture of membranes and frequency of postterm pregnancy. Perinatal outcome included gestational age at delivery, birth weight, placental weight, Apgar scores, number of infants admitted to the special care nursery, and the incidence of small-for-gestational-age infants.

Our primary hypothesis was that daily supplementation of 365 mg of elemental magnesium started in the second trimester of pregnancy would reduce the incidence of preeclampsia in a special population with an expected incidence of 20%. A sample size of 400 individuals has a power of 80% to detect a 50% reduction in the incidence of preeclampsia with an $\alpha = 0.05$.

For various reasons (refusal to start tablets, family wishes, to be seen by private physicians or in another clinic) 26 patients never took the medication after enrollment into the study. Eleven were in the placebo group and 15 were in the magnesium group. These subjects were excluded from the study from the onset. All remaining subjects were included in the treatment group to which they were originally randomized, regardless of any follow-up experience.

Statistical analysis used Student's t test, unpaired t test, χ^2, and Fisher's exact test when applicable. For serial blood pressure changes we used analysis of variance for repeated measurements followed by Fisher's least significant difference testing. A p value <0.05 was considered significant.

Results

Twenty-six subjects (11 in the placebo group and 15 in the magnesium group) were excluded for never starting the medication for various reasons. Information was subsequently obtained on 24 of these pregnancies. Two were complicated by preeclampsia and one other pregnancy resulted in fetal demise caused by congenital syphilis. The remainder of the analysis focuses on the 374 patients (189 in the placebo group and 185 in the magnesium group) who started the medication and completed their prenatal care in our clinics.

Table I compares relevant clinical characteristics in the two groups. There were no significant differences between the two groups regarding any of the categories

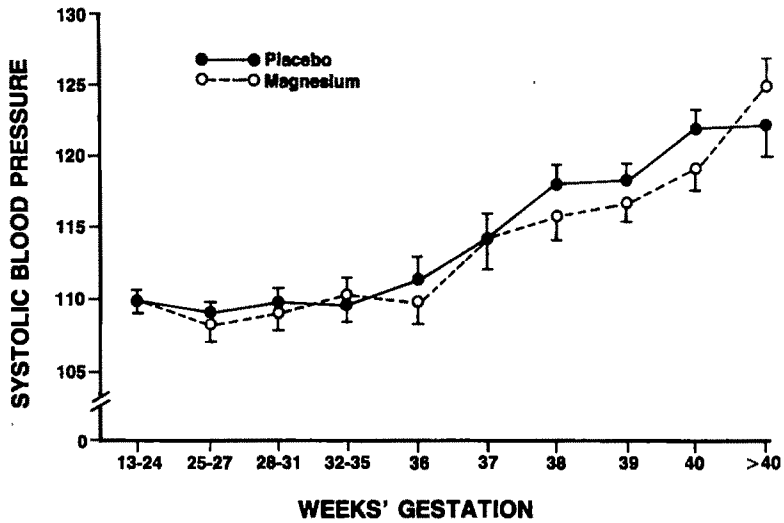


Fig. 1. A comparison of systolic blood pressures between placebo (●——●) and magnesium (o - - - - o). There were no significant differences in systolic blood pressures at any time during pregnancy.

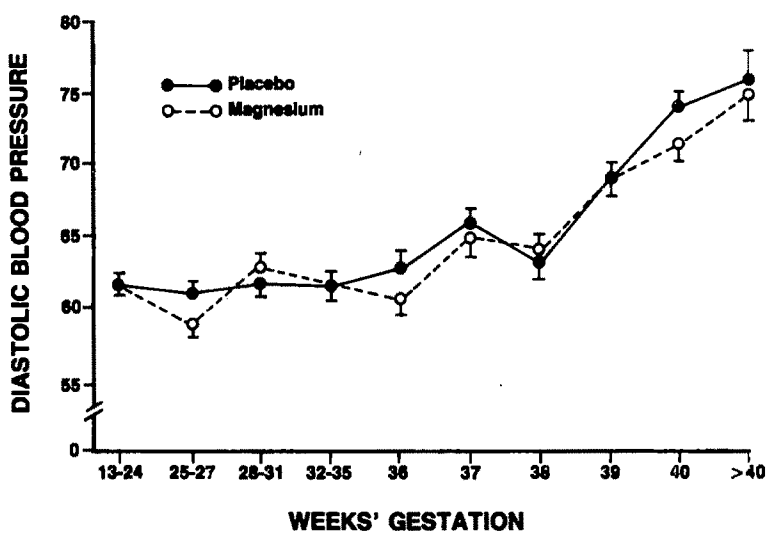


Fig. 2. A comparison of diastolic blood pressures between placebo (●——●) and magnesium (o - - - - o). There were no significant differences in diastolic blood pressures at any time during pregnancy.

studied. In addition there was no difference in racial distribution (90% in the placebo group versus 91% in the magnesium group).

Analysis of the 24-hour recall data revealed no significant difference between the two groups regarding intake of calories, protein, calcium, or magnesium. The mean calcium and magnesium intakes by these patients were 1000 and 260 mg/dl, respectively.

Fourteen subjects in the placebo group and 11 in the magnesium group discontinued intake of tablets before the beginning of the third trimester. The remaining subjects took approximately 90% of the possible number of tablets, which were continued until onset of labor. The major reasons for stopping the medications were gastrointestinal symptoms (nausea, vomiting, diarrhea) and difficulty in swallowing the tablets. The incidence of gastrointestinal symptoms were similar in both groups (7% in the placebo vs. 6% in the magnesium group).

There were no significant differences in either systolic or diastolic blood pressure between the two treatment groups either at time of enrollment or at time of delivery. Figures 1 and 2 describe the mean systolic and diastolic blood pressure values at various gestational

Table II. Laboratory findings at time of delivery

Serum	*Placebo (N = 112)*	*Magnesium (N = 105)*
Magnesium (mg/dl)*	1.56 ± 0.03	1.68 ± 0.05
Calcium (mg/dl)	9.34 ± 0.61	9.31 ± 0.68
Potassium (mEq/L)	4.0 ± 0.47	4.0 ± 0.44
Sodium (mEq/L)	139 ± 4.5	139 ± 4.4
Uric acid (mg/dl)	5.3 ± 1.4	5.2 ± 1.2

All values represent mean ± SEM.
*$p < 0.01$.

Table III. Pregnancy outcome

	*Placebo**		*Magnesium**	
	No.	*%*	*No.*	*%*
Preeclampsia	35	18.5	32	17.3
Mild	28	14.8	28	15.1
Severe	7	3.7	4	2.2
Abruptio placentae	2	1.1	3	1.6
Preterm labor	14	7.4	13	7.0
Delivery (gestational age)				
≤36 wk	18	9.5	19	10.2
≥42 wk	6	3.2	6	3.8

*No significant difference for any of the categories studied.

ages in both groups. There were no significant differences between the two groups regarding blood pressure changes throughout gestation (by analysis of variance for repeated measurements). However, within each group analysis of data with analysis of variance for repeated measurements followed by Fisher's least significant difference testing revealed a significant increase ($p < 0.01$) of both systolic and diastolic blood pressure values recorded at the time of enrollment compared to those recorded at or beyond 37 weeks of gestation.

Calculations were repeated after excluding patients who did not take medications during the third trimester from the analysis. These exclusions did not affect the above changes in blood pressure. Similar calculations were repeated after patients in both groups who developed preeclampsia were excluded. There were no differences in either systolic or diastolic blood pressures in subjects who remained normotensive in the two treatment groups.

Serum levels of magnesium, calcium, sodium, potassium, and uric acid were obtained in the majority of patients who continued medication throughout pregnancy. Table II compares the respective serum levels of these measurements in the two groups. The magnesium-supplemented group had statistically significantly higher serum magnesium levels than the placebo group. However, the serum magnesium levels achieved in the magnesium group were in the ranges that have no clinical significance.

Table IV. Perinatal outcome

	Placebo (N = 190†)*	*Magnesium* (N = 187‡)*
Birth weight (gm) (mean ± SEM)	3066 ± 43	3126 ± 49
Placental weight (gm) (mean ± SEM)	659 ± 13	677 ± 13
Gestational age (wk) (mean ± SEM)	39.0 ± 0.2	39.0 ± 0.2
SGA (No. and %)	12 (6.3%)	9 (4.9%)
Admitted to NSCU (No. and %)	13 (6.8%)	14 (7.5%)
Apgar scores		
<5 at 1 min	16 (8.4%)	13 (7.0%)
<7 at 5 min	5 (2.6%)	3 (1.6%)
Miscarriage	1	1
Neonatal deaths	1	1

SGA, Small for gestational age; *NSCU*, neonatal special care unit.
*No significant difference for any of the categories studied.
†One set of twins.
‡Two sets of twins.

Table III summarizes the pregnancy outcome in the two groups. The incidence of preeclampsia was 18.5% in the placebo group and 17.3% in the magnesium group. Calculations were repeated after excluding three patients from the placebo group and two from the magnesium group who developed preeclampsia, and patients who stopped medications before the third trimester. The incidence of preeclampsia remained similar in both groups. We observed no statistically significant differences between treatment groups with respect to preterm labor, delivery at or before 36 weeks' gestation, and postterm pregnancy.

Table IV compares the perinatal outcome of the two treatment groups. There were no statistically significant differences between the treatment groups with respect to birth weight, incidence of fetal growth retardation, Apgar score, or number of infants admitted to the special care nursery. There was one miscarriage at 17 weeks' gestation and one neonatal death at 26 weeks' gestation in the placebo group. In the magnesium group, there was one miscarriage at 20 weeks' gestation and a neonatal death from multiple congenital anomalies at 35 weeks' gestation.

Comment

Serum magnesium levels are usually lower during pregnancy compared with the nonpregnant state, and are further reduced in the presence of preterm labor.[10] In patients with preeclampsia magnesium levels were reported to be lower[11] or similar to[12] values observed in normotensive pregnancies. Because parenteral magnesium sulfate is the drug of choice in the treatment of preeclampsia, some authors suggested an etiologic relationship between magnesium deficiency and preeclampsia.[6, 7]

In a retrospective study, Conradt et al.[7] compared the pregnancy outcome in 4023 low-risk pregnancies to that in 882 pregnancies considered to be at risk for preterm delivery and fetal growth retardation. The high-risk group was treated with β-sympathomimetic agents in combination with magnesium aspartate hydrochloride. The incidence of preeclampsia was 2% in the no-treatment group and 0% in the treated group. The incidence of fetal growth retardation in the treated group was similar to that in the low-risk group. The authors concluded that magnesium supplementation prevents preeclampsia and fetal growth retardation.

In their double-blind study of magnesium supplementation in pregnancy, Spätling and Spätling[9] found that magnesium supplementation was associated with significantly fewer maternal hospitalizations and with a reduction in both preterm labor and number of newborns admitted to the intensive care unit. The differences in outcome between the magnesium and placebo groups were primarily related to the significantly higher number of patients in the placebo group with a diagnosis of antepartum hemorrhage (17 vs 4) and incompetent cervix (17 vs 8). It is very unlikely that magnesium supplementation will prevent such complications. Hence, differences in outcome were related to the fact that the two groups were not comparable.

The recommended daily allowance for dietary magnesium during pregnancy is 450 mg/day.[5] Ashe et al.[13] observed that in middle-class pregnant women (students and personnel at the University of Tennessee, Knoxville) the magnesium intake was only 60% of the recommended allowance of pregnant women. It is conceivable that in indigent pregnant women, nutritional magnesium deficiency is much worse. In a preliminary study of 500 patients at our prenatal clinics, approximately 75% of the patients took less than two thirds of the recommended dietary allowances of magnesium.

Our study was designed to evaluate the effects of oral magnesium supplementation during pregnancy on the incidence of preeclampsia in a group of patients judged to be at a very high risk for the development of the disease (expected incidence of 20%). The population included a homogeneous group of young primigravid women who were indigent and mostly black (90%).

Our findings support no evidence of any benefit of magnesium supplementation on the frequency of either preeclampsia or fetal growth retardation. In addition, magnesium supplementation did not influence either the systolic or diastolic blood pressure in these patients. These findings are similar to those reported recently by Spätling and Spätling.[9] In contrast, we did not find any differences in frequency of preterm delivery and number of admissions to a special care nursery between the placebo and the magnesium supplemented group. Our study did not have adequate size to detect small differences in the incidence of preterm labor. However, when the incidence of preterm labor in both groups is considered, a sample size 10 times larger than the number studied here would not have shown any difference.

The results of the present investigation do not support the conclusion of the investigation by Conradt et al.[7] Perhaps the differences between both studies may be related to study design (retrospective in nature), the population studied (heterogeneous group of patients with various diagnoses) and the use of β-sympathomimetic agents in the study by Conradt et al.

In summary, magnesium supplementation during pregnancy did not reduce the incidence of either preeclampsia or fetal growth retardation in our population.

We thank Dr. Frank Douglas and Ms. Rita Geiser, Ciba-Geigy Corporation, for their contribution to this investigation.

REFERENCES

1. Altura BM, Altura BT, Gebrewold A, et al. Magnesium deficiency and hypertension: correlation between magnesium-deficient-diets and microcirculatory changes in situ. Science 1984;223:1315-7.
2. Dyckner T, Wester PO. Effect of magnesium on blood pressure. Br Med J 1983;286:1847-8.
3. McCarron DA. Calcium and magnesium nutrition in human hypertension. Ann Intern Med 1983;98(part 2): 800-5.
4. Levine BS, Coburn JW. Magnesium, the mimic/antagonist of calcium. N Engl J Med 1984;310:1253-5.
5. Graber TW. Role of magnesium in health and disease. Nutrition 1987;13:29-35.
6. Altura BM, Altura BT, Carella A. Magnesium deficiency-induced spasm of umbilical vessels: relation to preeclampsia, hypertension, growth retardation. Science 1983; 221:376-8.
7. Conradt A, Weidinger H, Algayer H. On the role of magnesium in fetal hypotrophy, pregnancy-induced hypertension and preeclampsia. Mag Bull 1984;6:68-76.
8. Kiss V, Balasz M, Morvay F, et al. Effect of maternal magnesium supply on spontaneous abortion and premature birth and on intrauterine foetal development: experimental epidemiological study. Mag Bull 1981;3:73-9.
9. Spätling L, Spätling G. Magnesium supplementation in pregnancy. A double-blind study. Br J Obstet Gynaecol 1988;95:120-5.
10. Martin RW, Morrison JC. Oral magnesium for tocolysis. Contemp Ob/Gyn 1987;30(4):111-8.
11. Weaver K. In: Cantin M, Seelig MS, eds. Magnesium in health and disease. New York: Spectrum, 1980:833.
12. Cruikshank DP, Pitkin RM, Reynolds WA, Williams GA, Hargis GK. Effects of magnesium sulfate treatment on perinatal calcium metabolism. I. Maternal and fetal responses. Am J Obstet Gynecol 1979;134:243.
13. Ashe JR, Schofield FA, Gram MR. The retention of calcium, iron, phosphorus, and magnesium during pregnancy: the adequacy of prenatal diets with and without supplementation. Am J Clin Nutr 1979;32:286.

Methicillin-resistant staphylococcal mastitis

Charles Kalstone, MD
Miami, Florida

A postpartum patient had a unilateral breast infection that responded to cephalosporin treatment. During therapy, the contralateral breast developed a methicillin-resistant *Staphylococcus aureus* infection. The patient was hospitalized and treated successfully with intravenous vancomycin. Obstetricians should be alert to this possibility when treating patients with postpartum mastitis. (AM J OBSTET GYNECOL 1989;161:120.)

Key words: Postpartum mastitis, *Staphylococcus aureus*, methicillin-resistant organism

We report a case of postpartum mastitis caused by a methicillin-resistant *Staphylococcus aureus* infection. When such infection occurs, the usual antimicrobial therapy for mastitis may not be effective.

Case report

A 24-year-old primiparous woman who had a normal vaginal delivery 1 month previously was breast-feeding and developed mastitis in the left breast as evidenced by unilateral pain, tenderness, redness, and a fever of 102° F. Cephalexin, 500 mg every 6 hours, was prescribed and within 24 hours the patient was afebrile, felt well, and the breast tenderness was resolving. She continued to nurse. Two days later, while she was still taking cephalexin, she developed a fever of 103° F, pain, tenderness, and redness in the opposite breast. The initial (left) breast infection had completely resolved.

The development of mastitis in the contralateral breast while the patient was taking cephalosporin was thought to be caused by a methicillin-resistant *Staphylococcus aureus* infection. The patient was admitted to the hospital and treated with intravenous vancomycin, 500 mg every 6 hours. Cephalexin was discontinued. Two blood cultures were obtained and these subsequently revealed no growth. A Gram stain of the milk expressed from the inflamed right breast did not show any organisms, but the culture later grew a methicillin-resistant *Staphylococcus aureus*, sensitive to vancomycin and Bactrim. The patient was advised to discontinue nursing and was given bromocriptine mesylate and was treated with intravenous vancomycin for 5 days. Within 24 hours she was afebrile and remained so; the breast inflammation resolved and she was discharged with Bactrim for 5 additional days. She had no further problems. Her infant had no signs of staphylococcal infection. An epidemiologic search did not reveal the source of the maternal infection.

Comment

Since the incidence of methicillin-resistant staphylococcal infections is increasing, it seems logical that they will be encountered in postpartum mastitis. It had been standard practice to prescribe ampicillin in such cases. Even though the organism is usually a penicillinase-producing *Staphylococcus aureus*, the mastitis usually responds.[1, 2] Because of concerns about penicillin-resistant *Staphylococcus aureus* strains, standard treatment changed to a cephalosporin or cloxacillin.[3] This case demonstrates that postpartum mastitis may not respond to these drugs or that it may develop in the opposite breast despite them. These patients should have cultures and be treated for a methicillin-resistant *Staphylococcus aureus* infection, either with intravenous vancomycin or oral Bactrim, if the organisms are sensitive.

I thank William J. Ledger, MD, Professor and Chairman, Department of Obstetrics and Gynecology, Cornell Medical Center, New York, for his assistance.

From the Department of Obstetrics and Gynecology, South Miami Hospital.
Received for publication July 12, 1988; revised November 15, 1988; accepted February 21, 1989.
Reprint requests: Charles Kalstone, MD, 6280 Sunset Dr., Suite 500, South Miami, FL 33143.

REFERENCES

1. Marshall BR, Hepper JK, Zirbel CC. Sporadic puerperal mastitis. JAMA 1975;233:1377.
2. Niebyl J, Spence M, Parmely T. Sporadic (nonepidemic) puerperal mastitis. J Reprod Med 1978;20:97.
3. Ledger WJ. Infection in the female. Philadelphia: Lea & Febiger, 1986:269.

Gestational trophoblastic disease: A case-control study from the People's Republic of China

Louise A. Brinton, PhD,[a] Bao-Zhen Wu, MD,[b] Wen Wang, MD,[c] Abby G. Ershow, ScD,[d] Hong-Zhao Song, MD,[b] Jun-Yao Li, MD,[c] Michael B. Bracken, PhD,[e] and William J. Blot, PhD[d]

Bethesda, Maryland, Beijing, People's Republic of China, and New Haven, Connecticut

A case-control study involving 331 patients with complete hydatidiform mole and 662 community controls matched to the cases on age and timing of pregnancy was conducted in Beijing, China. A history of a term birth was associated with reduced risk (odds ratio = 0.6, 95% confidence interval 0.4 to 0.9), with some evidence of further decrease with multiple births. Previous spontaneous abortions were not related to risk, although those with a prior induced abortion were at elevated risk, particularly if two or more abortions were involved (odds ratio = 2.8, 95% confidence interval 1.4 to 5.7). A history of having sought medical advice for infertility was associated with reduced risk (odds ratio = 0.5, 95% confidence interval 0.2 to 0.8), but those who reported use of herbal medicines during a first trimester of a previous pregnancy were at excess risk (odds ratio = 2.2, 95% confidence interval 1.3 to 3.6). In addition, a statistically significant trend in risk was observed with years of oral contraceptive use (odds ratio = 2.6, 95% confidence interval 0.9 to 6.9 for ≥4 years of use). Dietary habits and family histories of cancer or trophoblastic disease were not related to risk in this study. (Am J Obstet Gynecol 1989;161:121-7.)

Key words: Hydatidiform mole, reproduction, infertility, contraception, epidemiology

Hydatidiform mole, an abnormal proliferation of the trophoblast, has long been of interest to clinicians as a complication of pregnancy that frequently progresses to invasive disease or choriocarcinoma. Hydatidiform mole can be classified into two distinct entities on the basis of pathologic and cytogenetic criteria.[1] The most clinically important entity, complete mole, usually has a 46,XX chromosomal constitution. This is a consequence of an anucleate egg being fertilized by a haploid sperm; subsequently cell division restores the normal chromosome number. In contrast, partial moles contain maternal chromosomes in the karyotype and have a triploid genotype.

Although the natural history of hydatidiform mole is relatively well understood, etiologic factors have received much less attention. It is recognized that risk increases significantly with late maternal age and to a lesser extent with pregnancies at very young ages.[2, 3] The influence of other reproductive factors, however, remains to be established. Other possible risk factors include Oriental race, low socioeconomic status, viral or parasitic infections, oral contraceptive use, consanguinity, and immunogenetic factors, although most of these variables have been suggested on the basis of case series rather than analytic studies.

An incidence survey of hydatidiform mole in more than three million women in the People's Republic of China[4] stimulated interest in further defining etiologic factors. Thus in 1982 a large case-control study was initiated in Beijing to evaluate the influence of a variety of environmental and genetic factors on risk of gestational trophoblastic disease.

Material and methods

This case-control study, which was begun in April 1984 after a 1-year pilot study, included 5 years of retrospectively ascertained and 2 years of prospectively ascertained cases from eight Beijing hospitals. Although the study identified all Beijing residents diagnosed with gestational trophoblastic disease during the study period, patients diagnosed with partial hydatidiform mole (i.e., those with embryonal tissue in the evacuated conceptus) were not enrolled because of small numbers ($n = 7$) and presumed differences in etiology and pathogenesis. Case definitions of study subjects were based on criteria established by the World Health Organization,[5] as reported in another investigation of Chinese women.[5] All cases of complete hydatidiform mole without invasive complications were pathologically confirmed. A lower proportion of malignant consequences were pathologically confirmed (36% of retrospective and 81% of prospective cases), primarily because of the nonsurgical treatment used to

From the Environmental Epidemiology Branch, National Cancer Institute[a]; the Peking Union Medical College Hospital[b]; the Cancer Institute; Chinese Academy of Medical Sciences[c]; the Biostatistics Branch, National Cancer Institute[d]; and the Department of Epidemiology and Public Health, Yale University School of Medicine.[e]

Received for publication August 31, 1988; revised January 11, 1989; accepted January 30, 1989.

Reprint requests: Louise A. Brinton, PhD, Environmental Epidemiology Branch, National Cancer Institute, Executive Plaza North, Room 443, Bethesda, MD 20892.

Table I. Distribution of cases and controls by selected sociodemographic factors

	Cases		*Controls*	
	No.	*%*	*No.*	*%*
Age at diagnosis* (yr)				
<24	30	9.1	39	5.9
24-26	119	36.0	217	32.8
27-29	97	29.3	225	34.0
30-32	25	7.5	73	11.0
≥33	60	18.1	108	16.3
Education				
No formal schooling	20	6.0	40	6.0
Primary school	65	19.6	103	15.6
Junior middle school	122	36.9	302	45.6
Senior middle school	93	28.1	163	24.6
College	20	6.0	35	5.3
Other	11	3.3	18	2.7
Unknown	0		1	0.2
Family income (yuan†) (per No. of people supported)				
<150	52	15.7	109	16.5
150-249	79	23.9	135	20.4
250-349	71	21.4	152	23.0
350-449	59	17.8	102	15.4
≥450	70	21.2	164	24.8

*Matching variable.
†In 1978, one U.S. dollar = 1.68 yuan.

preserve reproductive function. In the absence of pathologic confirmation, a diagnosis of invasive mole was assigned on the basis of human chorionic gonadotropin determinations, x-ray examinations, and the disease being preceded by a molar pregnancy within the previous year. A total of 260 patients with noninvasive complete hydatidiform mole, 157 with invasive mole, and 34 with choriocarcinoma were ascertained during the study period.

Each case was personally interviewed by trained interviewers. Interviews with prospectively ascertained cases were conducted in hospitals, while retrospectively ascertained patients were interviewed in their homes. Interviews took approximately 1 hour to complete and obtained detailed information on demographics, occupation, pregnancy history, infertility, menstrual history, contraceptive use, medical history, marriages, family history of hydatidiform mole and choriocarcinoma, and cancer, smoking, and dietary practices. Interviews were obtained from 358 of the 451 eligible cases (79.4%). Reasons for nonresponse related primarily to difficulties in locating subjects (15.5%), with few subjects being excluded because of death (1.8%) or refusal to participate (3.3%).

For each interviewed case in the study, two population controls were selected. Depending on whether the case resided in an urban or a rural area, the first unit of selection was the same street office or commune as that of the case. Within these areas, a random selection was made of one neighborhood committee or production brigade (residential units of approximately 900 families); these were visited to abstract information from census lists on all women who were within 2 years of age of the identified case. These potential controls were located and information on the timing of all of their pregnancies was obtained. Control subjects were then selected so that they had had a pregnancy within 1 year of the date of termination of the molar pregnancy of the matched case. Control subjects were interviewed in their homes by the same questionnaire approach as that used with retrospective cases.

Because the number of women with choriocarcinoma was small (n = 27), these subjects were not included in this analysis. Included for analysis were 331 patients with complete hydatidiform mole (189 with noninvasive mole and 142 with invasive mole) and 662 matched controls.

Since study subjects were interviewed at variable times after diagnosis, it was necessary to truncate exposure information in the analysis. Reproductive information considered only pregnancies occurring before the diagnostic molar pregnancy (and a comparable period for matched controls). Contraceptive information was truncated at a common date 6 months before the earliest termination date of the last non-molar pregnancy of the case or her matched controls.

The dietary history inquired about use of cooking oils and usual frequency of consumption of 32 food and beverage items 5 years before interview. The history focused on alcoholic beverages and foods high in vitamin A (retinol and carotene sources) and folic acid. The yearly frequencies of intake of various food items

Table II. Odds ratios of complete hydatidiform mole by selected reproductive variables

	Cases	*Controls*	*Odds ratio**	*95% Confidence interval*
No. of term births				
0	214	410	1.0	—
1	65	149	0.6	0.4-0.9
≥2	52	103	0.5	0.3-0.9
			p for trend = 0.01	
Spontaneous abortion				
No	289	598	1.0	—
Yes	42	64	1.3	0.8-2.0
No. of induced abortions				
0	250	540	1.0	—
1	57	102	1.2	0.8-1.8
≥2	24	20	2.8	1.4-5.7
			p for trend = 0.01	
Age at first pregnancy† (yr)				
<22	28	45	1.0	—
22-23	24	49	0.8	0.4-1.6
24-25	39	84	0.8	0.4-1.6
26-27	41	80	0.8	0.4-1.6
≥28	22	56	0.6	0.3-1.3
			p for trend = 0.3	
Ever breast-fed infant				
No	231	451	1.0	—
Yes	100	211	1.0	0.6-2.0

*Basic adjustment model included age, number of term births, number of abortions, infertility, herbal medicines, and years of use of oral contraceptives.

†Limited to previously pregnant women.

were adjusted for length of season and summed to form food groups such as staple foods, animal foods, high vitamin A foods, and fruits. The control distributions of the two case series were each divided into quartiles for use as exposure levels in risk estimates.

For evaluating effects of exposure factors related to complete hydatidiform mole, the measure of association used was the odds ratio, an estimate of the relative risk. Unmatched logistic regression analyses were performed to obtain maximum likelihood estimates of the odds ratios and 95% confidence intervals, adjusted for confounding variables. Two-tailed tests for trend in the logistic analyses were obtained by categorizing the exposure variable, assigning the score j to the *jth* exposure level of the categoric variable, and treating the scored variable as a continuous variable. Because matching was done in the study design, matched logistic regression analyses were also done. Since these analyses produced results nearly identical to those derived from the unmatched analyses, the latter were chosen for presentation.

Results

Table I shows selected sociodemographic variables for the patients with complete hydatidiform mole and their respective controls. The mean age of both the patients and the controls was 29.2 years.

Cases and controls were compared on a number of sociodemographic variables, including education, family size, total family income in 1978 (before diagnosis of disease in any study subjects), and income per person supported. No appreciable differences were seen between cases and controls for these variables.

Analyses of reproductive factors showed a protective effect associated with ever having had a term live birth or stillbirth (odds ratio = 0.6, 95% confidence interval 0.4 to 0.9). There was some indication of decreasing risk with increasing numbers of term births (Table II), although further decreases in risk were not observed for more than two births. After controlling for number of term births, there was no effect on risk of a history of prior spontaneous abortion. However, an elevated risk was associated with a history of induced abortion, particularly if two or more abortions were reported (odds ratio = 2.8, 95% confidence interval 1.4 to 5.7).

Although subjects were matched on age of index pregnancy, preventing assessment of this as a risk factor, it was of interest to examine among previously pregnant women whether risk was influenced by age at first pregnancy. There was an increase in risk associated with a first pregnancy before age 22, but there were no clear trends in risk beyond this age. No striking associations were found for factors related to reproduction, including ever having had a low-birth-weight baby (<2.3 kg) or a baby with a birth defect, delivery by cesarean section, or history of breast-feeding.

A history of having sought medical advice for infertility, although reported by a limited number of study

Table III. Odds ratios of complete hydatidiform mole by infertility and use of various preparations during first trimester of previous pregnancies

	Cases	*Controls*	*Odds ratio**	*95% Confidence interval*
Sought medical advice for infertility				
No	316	610	1.0	—
Yes	15	52	0.5	0.2-0.8
Use of herbal medicines during pregnancy				
No	295	621	1.0	—
Yes	36	41	2.2	1.3-3.6
Use of vitamins during pregnancy				
No	316	639	1.0	—
Yes	15	23	1.0	0.5-2.2
Use of progestogens during pregnancy				
No	314	621	1.0	—
Yes	17	41	0.7	0.4-1.3

*Basic adjustment model included age, number of term births, number of abortions, infertility, herbal medicines, and years of use of oral contraceptives.

Table IV. Odds ratios of complete hydatidiform mole by years of oral contraceptive and IUD use

	Cases	*Controls*	*Odds ratio**	*95% Confidence interval*
Years of oral contraceptive use				
0	254	543	1.0	—
<2	56	92	1.3	0.9-2.0
2-3	10	17	1.4	0.6-3.2
≥4	11	10	2.6	0.9-6.9
			p for trend = 0.03	
Years of IUD use				
0	302	607	1.0	—
<2	16	34	0.9	0.4-1.7
≥2	13	21	1.4	0.6-2.9
			p for trend = 0.8	

Basic adjustment model included age, number of term births, number of abortions, infertility, herbal medicines, and years of use of oral contraceptives.

subjects, was associated with a significant reduction in risk (odds ratio = 0.5, 95% confidence interval 0.2 to 0.8) (Table III). Use of herbal medicines during the first trimester of prior pregnancies, however, was associated with an elevated risk (odds ratio = 2.2, 95% confidence interval 1.3 to 3.6). During prior pregnancies, comparable histories of exposure to vitamins, progestogens, and estrogens (the latter not shown because of limited numbers) revealed no significant differences between cases and controls.

Table IV presents results according to years of use of oral contraceptives and intrauterine contraceptive devices (IUDs). Risk increased significantly with duration of pill use, with the odds ratio being 2.6 (95% confidence interval 0.9 to 6.9) for users of 4 or more years. This association persisted after control for a variety of other factors, including infertility, which other studies have linked to oral contraceptive use. Similarly, the infertility relationships were unaltered when examined only among non–oral contraceptive users. Years of use of IUDs did not significantly relate to risk.

The occurrence of complete hydatidiform mole was evaluated further according to several menstrual and anthropometric factors (Table V). Age at menarche was not associated with risk, despite a fair amount of range in ages at onset. Menstrual irregularity, reported by only a small proportion of the study subjects, was also not associated with risk (odds ratio = 1.1). Other factors examined included whether sexual intercourse was avoided during menses and how often the perineum was washed, but neither of these behaviors was predictive of risk.

Adult height and weight before a first pregnancy did not appear to relate to risk of complete mole, nor did more refined measures of body mass, including Quetelet's index (weight/height^2).

Risk was evaluated in relation to a number of previous diseases, including several experienced during the first trimester of previous pregnancies (Table VI). Although no significant associations were observed with a variety of conditions or diseases during pregnancy, including toxemia, influenza, and other conditions, subjects reporting toxemia were at a nonsignificantly elevated risk (odds ratio = 1.7, 95% confidence interval

Table V. Odds ratios of complete hydatidiform mole by menstrual and anthropometric variables

	Cases	*Controls*	*Odds ratio**	*95% Confidence interval*
Age at menarche (yr)				
<14	90	169	1.0	—
14	57	113	1.0	0.6-1.5
15	67	143	1.0	0.6-1.4
16	60	130	0.9	0.6-1.3
≥17	57	107	1.0	0.6-1.5
			p for trend = 0.7	
Menstrual irregularity				
No	307	614	1.0	—
Yes	24	48	1.1	0.6-1.8
Weight (jin†)				
<100	92	200	1.0	—
100-109	96	164	1.2	0.8-1.8
110-119	81	144	1.3	0.9-1.9
≥120	61	154	0.9	0.6-1.4
Unknown	1			
			p for trend = 1.0	
Height (cm)				
<155	44	91	1.0	—
155-159	119	214	1.2	0.8-1.8
160-164	112	245	0.9	0.6-1.4
≥165	56	112	1.0	0.6-1.6
			p for trend = 0.5	

*Basic adjustment model included age, number of term births, number of abortions, infertility, herbal medicines, and years of use of oral contraceptives.

†One jin = 500 gm.

Table VI. Odds ratios of complete hydatidiform mole by prior medical history variables

	Cases	*Controls*	*Odds ratio**	*95% Confidence interval*
Diseases during prior pregnancies				
Toxemia				
No	298	613	1.0	—
Yes	33	49	1.7	0.9-2.8
Influenza during first trimester				
No	323	649	1.0	—
Yes	8	13	0.8	0.3-2.1
Other diseases during first trimester				
No	319	648	1.0	—
Yes	12	14	1.2	0.5-2.8
Other prior diseases				
Erosion of the cervix				
No	311	615	1.0	—
Yes	20	47	0.8	0.4-1.4
Abnormal genital bleeding				
No	326	646	1.0	—
Yes	4	14	0.6	0.2-1.9
Unknown	1	2	1.1	0.1-12.2
Anemia				
No	288	583	1.0	—
Yes	43	78	1.1	0.7-1.6
Unknown	0	1		

*Basic adjustment model included age, number of term births, number of abortions, infertility, herbal medicines, and years of use of oral contraceptives.

0.9 to 2.8). Diseases experienced at other times, including cervicitis, abnormal genital bleeding, and anemia, were not predictive of risk.

There was no apparent relationship between risk and yearly intake of staple foods, animal source foods, vegetables, legumes, or fruits. There also was no consistent dose-response relationship of risk with foods rich in retinol, carotene, ascorbic acid, folic acid, or riboflavin. Intake of crude cottonseed oil, a source of the reproductive toxin gossypol, was also generally not related to complete hydatidiform mole.

No clear trends of disease were observed with either

Table VII. Odds ratios of complete hydatidiform mole by age difference between subject and husband, family history of cancer, smoking, and alcohol consumption

	Cases	*Controls*	*Odds ratio**	*95% Confidence interval*
Age difference of father and mother (yr†)				
Same age	75	133	1.0	
1-2	103	238	0.8	0.5-1.1
3-4	52	138	0.6	0.4-1.0
≥5	38	56	1.3	0.8-2.2
Mother elder	53	87	1.1	0.7-1.7
Family history of cancer				
No	296	592	1.0	
Yes	32	66	0.9	0.6-1.4
Unknown	3	4	1.5	0.3-6.7
Ever smoked				
No	314	633	1.0	—
Yes	17	28	1.2	0.6-2.3
Unknown		1		
Alcohol consumption (drinks per year)				
None	217	438	1.0	—
<13	61	153	0.8	0.5-1.1
13-52	28	47	1.1	0.7-1.8
≥53	25	24	2.1	1.2-3.9
			p for trend = 0.1	

*Basic adjustment model included age, number of term births, number of abortions, infertility, herbal medicines, and years of use of oral contraceptives.

†Limited to subjects married only once. Unknowns eliminated from analysis.

age of the father at conception of the molar pregnancy (not shown) or with the age difference between the study subject and her husband (Table VII). Family histories of cancer in first-degree relatives or husbands were also not predictive of risk, nor were family histories of gestational trophoblastic diseases, which were not reported for any of the mothers or sisters of the study subjects.

Ever having smoked cigarettes was also not associated with disease risk, although few subjects reported such a history. There was some evidence that the subjects with the highest intake of alcohol (more than 52 drinks per year) were at an elevated risk (odds ratio = 2.1, 95% confidence interval 1.2 to 3.9), although alcohol consumption at lower levels was not related to risk.

Finally, relationships were examined according to whether the case was retrospectively or prospectively ascertained, as well as by the occurrence of invasive complications. No major differences in results were observed between the retrospective and prospective series. Number of prior abortions was a stronger risk factor for invasive mole, whereas oral contraceptive usage more adversely affected noninvasive mole. Other risk factors operated similarly between the two case series.

Comment

Similar to other studies,[6,7] we found that a history of a prior term birth was associated with reduced risk for complete hydatidiform mole (odds ratio = 0.6) and that there was some evidence of a decreasing trend in risk with number of births. Similar to one other study[6] but in contrast with others,[7,8] we found no relationship of risk with the occurrence of prior spontaneous abortions. It is of note that we observed a significant relationship of risk to prior induced abortions, particularly if two or more abortions were involved. Other studies have been unable to assess this association in detail because of limited reporting of the procedure. However, our finding was difficult to interpret because of the possibility of recall bias and the absence of reasons for the terminations. Further investigation appears warranted.

A history of infertility in this study was not associated with increased risk, as reported by La Vecchia et al.[8] but not by Messerli et al.[6] In fact, there was some evidence of a protective effect, although the nature of the infertility was somewhat unusual since all subjects were required to have been pregnant at least once. In addition, histories were limited to women who had sought medical advice for their problems, and numbers of such women were small. Of particular interest was an elevated risk associated with use of herbal medicines during the first trimester of previous pregnancies. Although recall bias or uncontrolled confounding cannot be ruled out, it is of interest that we did not find a similar association with other preparations used during pregnancy, including progestogens, estrogens, or vitamins. The absence of information on reasons for use of herbal medicines prevents an assessment of whether

the increased risk is associated with the indication for use or the actual preparation. A history of abnormal bleeding did not appear to explain the relationship with herbal medicines, but other underlying disorders such as nausea, vomiting, or threatened miscarriage cannot be dismissed. It is possible that the herbal medicines, which are composed of a complex of natural and synthetic constituents, may have mutagenic or carcinogenic properties. Although our findings are of interest given laboratory findings that choriocarcinoma can be induced in patas monkeys by exposure to ethylnitrosourea during pregnancy,[9] further analytic studies in humans are needed.

Of further interest is the significant relationship that we observed between years of oral contraceptive use and risk of complete mole, where users of ≥4 years were at a nonsignificant 2.6-fold risk. Although the finding was based on small numbers and a biologic mechanism is unclear, the association is consistent with an effect observed by Berkowitz et al.,[10] although in that study the relationship appeared to be significantly confounded by IUD use. Studies by Messerli et al.[6] and La Vecchia et al.,[8] however, failed to find an effect of oral contraceptives on the occurrence of hydatidiform mole. Other studies have suggested that oral contraceptives may increase the risk of malignant sequelae after molar evacuation by providing a tumor-stimulating effect.[11] In one study[12] this effect was restricted to users of high-dose estrogens, although in other studies[10,13] there was no effect of the pill on postmolar complications, regardless of the preparations used. However, in our study, pill associations were stronger for noninvasive mole than for invasive mole.

Our study also provided the opportunity to evaluate a number of risk factors suggested by other investigators. Although late paternal age has been suggested in one study to increase the risk of trophoblastic diseases,[14] other investigations,[6,15] as well as our own, failed to confirm this. Cigarette smoking has also been linked with the occurrence of trophoblastic diseases,[8] but this could not be fully examined in our study as so few Beijing women smoked cigarettes. Furthermore, our results failed to indicate a relationship of risk to any dietary variables, including carotene, which has been suggested as a protective factor for complete hydatidiform mole.[10] However, this may be due in part to the difficulty of studying dietary exposures in China, where the food supply comprises relatively few items in any one locale and there is little heterogeneity in intake among subjects. Finally, other studies have hinted that a family history of trophoblastic diseases[16] might be important etiologic factors, but our study was unable to detect such an effect.

Although genetic investigators indicate that complete hydatidiform mole originates at fertilization, our results suggest that influences operating earlier, possibly on the susceptibility of the genetic material in the ovum, may also be involved. It is well established that a major predictor of risk is maternal age, but our results indicate that even after controlling for this factor, other reproductive factors seem to be etiologically important. In particular, the protective effect of a term pregnancy on risk of a subsequent molar pregnancy was apparent, whereas unnaturally terminated pregnancies increased risk. Furthermore, this study raises new questions regarding chemical and hormonal influences, particularly oral contraceptives, supporting the need for further detailed investigations.

REFERENCES

1. Jacobs PA, Wilson CM, Sprenkle JA, Rosenshein NB, Migeon BK. Mechanism of origin of complete hydatidiform moles. Nature 1980;286:714.
2. Bracken MB, Brinton LA, Hayaski K. Epidemiology of hydatidiform mole and choriocarcinoma. Epidemiol Rev 1984;6:52.
3. World Health Organization. Gestational trophoblastic diseases. Geneva: World Health Organization, 1983:1; Technical Report Series 692.
4. Song H, Wu P. Hydatidiform mole in China: a preliminary survey of incidence in more than three million women. Bull WHO 1987;65:507.
5. Song H, Wu P, Wang Y, Yang X, Shu D. Pregnancy outcomes after successful chemotherapy for choriocarcinoma and invasive mole: long-term follow-up. Am J Obstet Gynecol 1988;158:538.
6. Messerli ML, Lilienfeld AM, Parmley T, Woodruff JD, Rosenshein NB. Risk factors for gestational trophoblastic neoplasia. Am J Obstet Gynecol 1985;153:294.
7. Parazzini F, La Vecchia C, Pampallona S, Francheschi S. Reproductive patterns and the risk of gestational trophoblastic disease. Am J Obstet Gynecol 1985;152:866.
8. La Vecchia C, Franceschi S, Parazzini F, et al. Risk factors for gestational trophoblastic disease in Italy. Am J Epidemiol 1985;121:457.
9. Rice JM, Williams GM, Palmer AE, London WT, Sly DL. Pathology of gestational choriocarcinoma induced in patas monkeys by ethylnitrosourea given during pregnancy. Placenta 1981;3(suppl):223.
10. Berkowitz RS, Cramer DW, Bernstein MR, Cassells S, Driscoll SG, Goldstein DP. Risk factors for complete molar pregnancy from a case-control study. Am J Obstet Gynecol 1985;152:1016.
11. Stone M, Dent J, Kardana A, Bagshawe KD. Relationship of oral contraception to development of trophoblastic tumour after evacuation of a hydatidiform mole. Br J Obstet Gynaecol 1976;83:913.
12. Yuen BH, Burch P. Relationship of oral contraceptives and the intrauterine contraceptive devices to the regression of concentrations of the beta subunit of human chorionic gonadotropin and invasive complications after molar pregnancy. Am J Obstet Gynecol 1983;145:214.
13. Morrow P, Nakamura R, Schlaerth J, Gaddis O, Eddy G. The influence of oral contraceptives on the postmolar human chorionic gonadotropin regression curve. Am J Obstet Gynecol 1985;151:906.
14. La Vecchia C, Parazzini F, Decarli A, et al. Age of parents and risk of gestational trophoblastic disease. JNCI 1984;73:639.
15. Matsuura J, Chiu D, Jacobs PA, Szulman AE. Complete hydatidiform mole in Hawaii: an epidemiological study. Genet Epidemiol 1984;1:271.
16. Parazzini F, La Vecchia C, Franceschi S, Mangili G. Familial trophoblastic disease: case report. Am J Obstet Gynecol 1984;149:382.

Randomized treatment of mucopurulent cervicitis with doxycycline or amoxicillin

Jorma Paavonen, MD, Pacita L. Roberts, MS, Claire E. Stevens, MA, PA, Pål Wølner-Hanssen, MD, Robert C. Brunham, MD, Sharon Hillier, PhD, Walter E. Stamm, MD, Cho-Chou Kuo, MD, Timothy DeRouen, PhD, King K. Holmes, MD, PhD, and David A. Eschenbach, MD

Seattle, Washington

A randomized trial of doxycycline versus amoxicillin was performed to treat mucopurulent cervicitis. *Chlamydia trachomatis*, the most common single agent associated with mucopurulent cervicitis, was isolated from 30 (47%) and *Neisseria gonorrhoeae* from five (8%) of 64 patients. Patients were followed up for 3 months, and the effect of treatment was assessed by clinical (presence of endocervical mucopus, cervicitis severity score, and number of polymorphonuclear leukocytes on Gram-stained smears of endocervical secretions) and microbiologic criteria. Doxycycline and amoxicillin were equally effective for treating chlamydial and nonchlamydial cervicitis. However, endocervical mucopus was still present in 18% of the patients in both treatment groups after 2 months and in 23% of the doxycycline group and 33% of the amoxicillin group after 3 months of therapy. The cause of persistent/recurrent mucopus after antimicrobial treatment was not explained by relapse or reinfection with *Chlamydia trachomatis, Neisseria gonorrhoeae*, genital mycoplasmas, or *Gardnerella vaginalis*, but persistence was associated with the degree of cervical ectopy. (AM J OBSTET GYNECOL 1989;161:128-35.)

Key words: *Chlamydia trachomatis, Neisseria gonorrhoeae,* mucopurulent cervicitis, treatment of mucopurulent cervicitis

Chlamydia trachomatis and *Neisseria gonorrhoeae* cause mucupurulent cervicitis.[103] Mucopurulent cervicitis represents an important reservoir for upper genital tract infection in women. Acute pelvic inflammatory disease develops in 10% to 20% of women with *C. trachomatis* or *N. gonorrhoeae* cervicitis.[4] However, only about half of women with mucopurulent cervicitis have chlamydial or gonococcal cervical infection,[2] and the cause of the cervicitis and the risk of pelvic inflammatory disease among women with nonchlamydial/nongonococcal cervicitis is unknown.

Women with mucopurulent cervicitis have cytologic,[5] colposcopic,[6] and histologic[7] abnormalities regardless of whether *C. trachomatis* or *N. gonorrhoeae* is present. Observation of the clinical characteristics of cervicitis provides the only practical manner to monitor the effects of antimicrobial therapy among patients not infected with these two organisms. The eradication of *C. trachomatis* and *N. gonorrhoeae* from the cervix by penicillin-tetracycline regimens has been well documented.[8] However, the effect of antimicrobial therapy on clinical features of cervicitis and the treatment of nonchlamydial, nongonococcal cervicitis has not been reported, and more information is required of the treatment of all varieties of cervicitis. In this study we compared the effects of two antimicrobials on the results of cervical cultures, the clinical features of cervicitis, and polymorphonuclear leukocytes observed on Gram-stained smears of cervical secretions.

Material and methods

Study population. Nonpregnant women with gross endocervical mucopus who attended the Seattle–King County Sexually Transmitted Diseases Clinic at Harborview Medical Center in Seattle were eligible for study. Consecutive women who agreed to the randomized treatment section of the study were selected from a larger group of women with cervicitis previously described.[2] Patients were included if they had a yellow mucopurulent endocervical discharge as observed on a white cotton swab as previously described.[1,2] Exclusion criteria were under 16 years old, allergy to either study drug, ingestion of antimicrobial drugs within the prior month, use of intrauterine contraceptive device, or history of pelvic inflammatory disease. Five additional women with menses were excluded because they could not be evaluated for the presence of endocervical mu-

From the Departments of Medicine, Biostatistics, Obstetrics and Gynecology, and Pathobiology, University of Washington, and the Harborview Medical Center.

Supported in part by National Institutes of Health grant AI-12192.
Received for publication June 21, 1988; revised November 18, 1988; accepted January 10, 1989.

Reprint requests: Jorma Paavonen, MD, Department of Obstetrics and Gynecology, University Central Hospital, Haartmaninketu 2, SF-00290, Helsinki, Finland.

Table I. Characteristics of women with mucopurulent cervicitis randomly treated with doxycycline or amoxicillin

Characteristics	*Doxycycline group (N = 37)*	*Amoxicillin group (N = 27)*
Yr of age (mean ± SD)	25.0 ± 6.1	23.1 ± 4.5
No. white (%)	27/36 (75)	24/27 (89)
No. single (%)	32/36 (89)	24/27 (89)
Yr of education (mean ± SD)	13.4 ± 2.7	13.1 ± 2.7
No. ever pregnant (%)	22/36 (61)	9/27 (33)
No. parous (%)	13/37 (35)	7/27 (26)
No. using oral contraceptives (%)	19/36 (53)	18/27 (67)
Age at first intercourse (mean ± SD)	17.5 ± 2.5	17.6 ± 2.8
Lifetime sex partners (median number)	6	7
No. with past history of STD (%)	26/35 (74)	20/25 (80)
Mean cervicitis severity score	4	5
No. (%) infected with:		
C. trachomatis alone	16/37 (43)	9/27 (33)
N. gonorrhoeae/*C. trachomatis*	2/37 (5)	3/37 (11)
U. urealyticum	29/35 (74)	20/26 (77)
G. vaginalis	25/34 (83)	17/25 (68)
Herpes simplex virus	2/37 (6)	1/27 (4)
T. vaginalis	1/23 (3)	4/27 (15)
No. with bacterial vaginosis (%)	8/23 (35)	7/16 (44)

STD, Sexually transmitted disease.

copus. Seventy-two women were initially enrolled into the study, and the 64 women who returned for one or more follow-up visits are included in these analyses.

Patient evaluation. A standardized interview was obtained concerning menstrual, contraceptive, sexual, and a prior sexually transmitted disease history. During the speculum examination, the appearance of the cervix was observed both before and after vaginal material was removed from the portio with a large swab. The color of cervical secretions viewed on a white cotton swab was classified as clear, white, cloudy, or yellow. The severity of inflammation of the cervical epithelium was assessed by scoring the degree of (1) mucosal bleeding induced by swabbing the cervix, (2) presence of erythema, and (3) presence of edema in the zone of ectopy and the transformation zone as observed with the unaided eye. Each finding was scored as 0 (none or normal, 1+ (mild), 2+ (moderate), or 3+ (severe), and the sum of these scores represented the cervicitis severity score.[1, 9] Mild bleeding was defined as a few two drops of blood after repetitive swab sampling of the cervix. Bleeding that soaked a Q-tip swab with the first or second swab sampling was defined as moderate bleeding, and bleeding down the face of the cervix with the first swab or vaginal pooling of blood was defined as severe bleeding. Erythema was defined as an abnormal red color in the area of ectopy (transformation zone) subjectively graded as mild, moderate, or severe redness. Edema was defined as an elevation of the area of ectopy above the level of surrounding ectocervical squamous epithelium and was subjectively graded as mild, moderate, or severe elevation.

Collection of specimens. Urethral and endocervical specimens were obtained for isolation of *C. trachomatis* as previously described.[2] Endocervical specimens were obtained for *C. trachomatis* with sterile Dacron swabs and for other organisms with sterile cotton swabs. The swabs for *C. trachomatis* and herpes simplex virus cultures were placed in transport media as described elsewhere.[2] The remaining swabs were directly placed on the following media: Thayer-Martin medium for *Neisseria gonorrhoeae,* human blood Tween (HBT) medium for *Gardnerella vaginalis,* Diamond's medium for *Trichomonas vaginalis,* and agar and broth media for *Ureaplasma urealyticum* and *Mycoplasma hominis,* as previously described.[2] Aliquots of vaginal fluid were mixed with saline solution and microscopically examined for the presence of clue cells and motile trichomonads. A diagnosis of bacterial vaginosis was made by previously described Gram stain characteristics[10] when the number of small gram-negative bacilli and gram-positive cocci was greater than the number of *Lactobacillus* morphotypes.

Definition of mucopurulent cervicitis. After the ectocervix was wiped clean with a large cotton swab, endocervical mucus was collected on a white-tipped swab, with care taken to avoid contamination by vaginal secretions. The presence of a yellow mucopurulent endocervical discharge (mucopus) as observed on the white cotton swab was used to diagnose mucopurulent cervicitis.[1] The swab was rolled onto a 2 cm^2 area of a glass microscope slide. The smear was air dried and Gram stained by the rapid method. Slides were scanned at a magnification of 100 to evaluate the presence and amount of cervical mucus, to look for squamous cells, and to identify areas of mucus that appeared to contain inflammatory cells. Most often polymorphonuclear leu-

Table II. Recovery of selected organisms among women with mucopurulent cervicitis treated with doxycycline or amoxicillin

Organism designated as presence (+) or absence (−) before treatment		*Women with organisms recovered/no. cultured at that visit*			*Total no. women with organisms recovered at any follow-up visit*
		*1 mo**	*2 mo*	*3 mo*	
Doxycycline group					
C. trachomatis	+ (*N* = 18)	0/15	1/12	0/10	1/17
C. trachomatis	− (*N* = 19)	1/17	0/15	0/16	1/19
U. urealyticum	+ (*N* = 29)	6/24	11/20	11/20	16/28
U. urealyticum	− (*N* = 6)	1/3	1/4	1/4	2/5
M. hominis	+ (*N* = 13)	3/9	5/9	4/9	8/13
M. hominis	− (*N* = 31)	2/18	4/15	4/14	8/20
G. vaginalis	+ (*N* = 25)	6/12	9/12	8/12	13/18
G. vaginalis	− (*N* = 9)	0/6	1/4	3/5	4/6
Amoxicillin group					
C. trachomatis	+ (*N* = 12)	1/10	0/10	1/7	2/11
C. trachomatis	− (*N* = 15)	1/15	0/12	1/10	2/15
U. urealyticum	+ (*N* = 20)	16/18	13/16	11/13	18/19
U. urealyticum	− (*N* = 6)	2/5	2/3	1/2	3/5
M. hominis	+ (*N* = 13)	8/13	5/12	6/12	11/13
M. hominis	− (*N* = 13)	2/11	3/9	1/4	3/12
G. vaginalis	+ (*N* = 17)	7/8	4/5	4/4	8/9
G. vaginalis	− (*N* = 8)	2/4	2/3	1/3	4/5

*Follow-up visits after initiation of treatment.

kocytes were distributed uniformly in cervical mucus, but in cases in which they were distributed in a patchy fashion, representative areas containing the densest concentration of such leukocytes were selected. The number of polymorphonuclear leukocytes per microscopic field at a magnification of 1000 in five nonadjacent fields was then established with use of an oil lens (polymorphonuclear leukocyte count). Details of the examination have been published.[1]

Microbiologic studies. *C. trachomatis* was isolated on idoxuridine treated McCoy cells, and *N. gonorrhoeae* was identified by sugar fermentation as described.[1] Specimens from inoculated Diamond's medium[11] were microscopically examined every other day for 1 week to identify *T. vaginalis*. The methods used to identify herpes simplex virus, *U. urealyticum*, *M. hominis*, and *G. vaginalis* have been described.[2]

Treatment and follow-up. Patients were randomly assigned by a consecutive closed envelope draw to receive 100 mg of oral doxycycline twice daily for 14 days or oral amoxicillin, 3 gm, with 1 gm probenecid as an initial dose, followed by 500 mg three times daily for 14 days. Patients were asked to avoid sexual activity during the treatment, encourage their sexual partners of the past month to come for evaluation and treatment to the same clinic, use condoms until after the final follow-up visit, and return for follow-up visits at 4, 8, and 12 weeks after treatment was initiated. At follow-up visits, the presence or absence of mucopurulent cervicitis and other genital infections was reassessed, and all microscopic and microbiologic studies performed at the first visit were repeated.

Statistical analyses. Statistical comparisons between treatment groups for demographic, behavioral, clinical, and microbiologic variables used Fisher's exact test for categoric variables with dichotomous outcomes, and the Student *t* test for continuous variables. Comparisons between treatment groups for polymorphonuclear leukocyte counts and cervicitis scores were made at 4-, 8-, and 12-week visits with the use of the Mann-Whitney rank-sum test. Within treatment groups, statistical significance of changes between visits was determined with Wilcoxon's signed rank test for paired data. Logistic regression analysis was used to test effects while adjusting for potentially confounding variables.[12]

Results

Microbiologic results of therapy. Sixty-four patients with mucopurulent cervicitis were enrolled and returned for one or more follow-up visits; 37 received doxycycline and 27 received amoxicillin. Of these patients, 57 were examined at 1 month, 51 at 2 months, and 44 at 3 months. The treatment groups were comparable with respect to age, race, marital status, education, gravidity, parity, contraceptive use, age at first intercourse, number of lifetime sexual partners, and past history of sexually transmitted diseases (Table I). *C. trachomatis* alone was isolated from the cervix from 25 (39%) of the 64 patients, and both *C. trachomatis* and *N. gonorrhoeae* were isolated from five (8%) of the

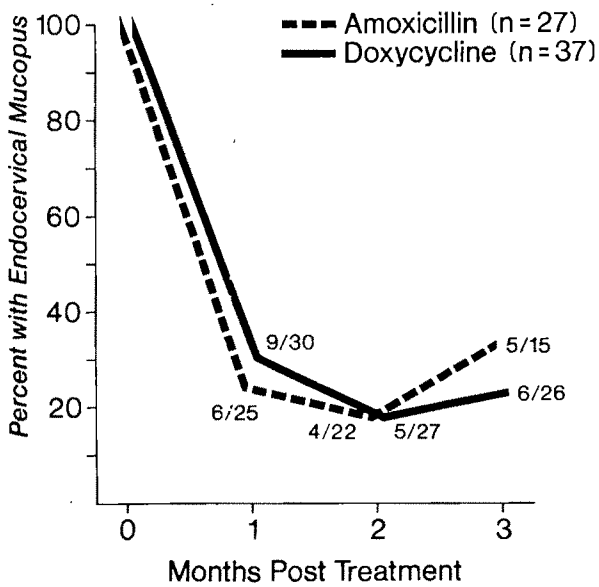


Fig. 1. Number of patients with endocervical mucopus over number of patients examined (%) before treatment and at 1, 2, and 3 month visits after starting treatment with doxycycline or amoxicillin.

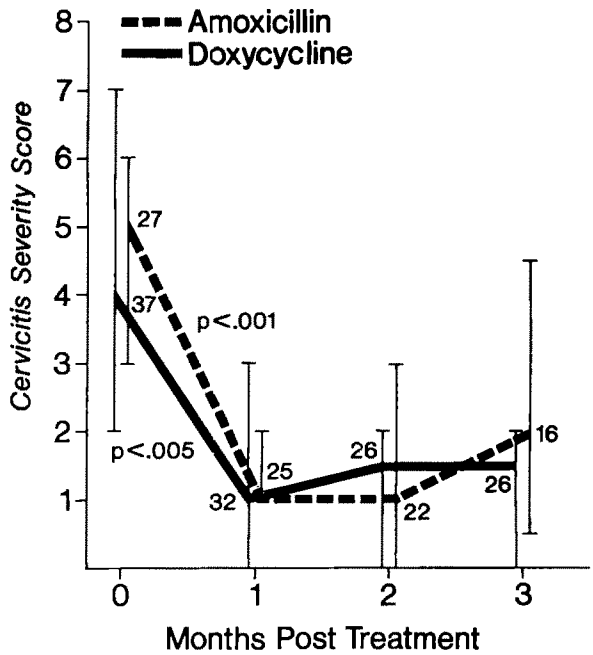


Fig. 2. Median cervicitis severity score with twenty-fifth and seventy-fifth percentiles before treatment and at 1, 2, and 3 months after starting treatment with doxycycline or amoxicillin (number indicates number of observations).

women. *N. gonorrhoeae* was not isolated without *C. trachomatis*. Neither organism was found in 34 (53%) of the women. At enrollment, the prevalence of *C. trachomatis, N. gonorrhoeae,* herpes simplex virus, *U. urealyticum, M. hominis,* and *G. vaginalis* was similar in the two treatment groups.

The presence of selected organisms at each of the follow-up visits stratified for the presence or absence of the organism at enrollment is tabulated in Table II for the doxycycline- and amoxicillin-treated groups. Both antibiotic regimens appeared effective in eradicating *C. trachomatis* and *N. gonorrhoeae* as determined at the 3-month follow-up visit. Among women positive for *C. trachomatis* at the initial visit, *C. trachomatis* was reisolated at any follow-up visit in one (6%) of 17 following doxycycline treatment and in two (18%) of 11 following amoxicillin treatment (NS). Among women negative for *C. trachomatis* at the enrollment visit, *C. trachomatis* was isolated at any follow-up visit from one (5%) of 19 patients given doxycycline and two (13%) of 15 given amoxicillin (NS). Thus *C. trachomatis* was isolated from two (6%) of 36 patients after doxycycline treatment and from four (15%) of 26 patients after amoxicillin treatment (NS). *N. gonorrhoeae* was not reisolated after therapy.

Neither regimen effectively eradicated *U. urealyticum, M. hominis,* or *G. vaginalis* (Table II). Among patients who were initially *U. urealyticum* positive, *U. urealyticum* was reisolated after therapy from 16 (57%) of 28 patients treated with doxycycline and from 18 (95%) of 19 patients treated with amoxicillin ($p = 0.005$). Although doxycycline was significantly more effective than amoxicillin in the eradication of *U. urealyticum,* the persistence of mucopurulent cervicitis was not significantly related to subsequent *U. urealyticum* isolation. Also, no association was found between mucopurulent cervicitis after therapy and the reisolation of *M. hominis* or *G. vaginalis.*

Effects of therapy on mucopus and cervical appearance. Fig. 1 shows the proportion of patients with endocervical mucopus in the two treatment groups at the initial and each of the three follow-up visits. Since some patients missed one or more follow-up visits, a "time to cure" could not be clearly defined for every patient. As shown in Fig. 1, the proportion of patients with mucopus decreased during the first month after therapy month, reached a minimum of 18% at 2 months in both groups, and increased at 3 months to 33% in the amoxicillin group and 23% in the doxycycline group. No significant difference between treatment groups was found in the proportion of women with mucopus at any of the visits.

The vast majority of persistent or recurrent mucopus after antimicrobial treatment could not be explained by the reisolation of *C. trachomatis, N. gonorrhoeae, U. urealyticum, M. hominis,* or *G. vaginalis* for follow-up. *C. trachomatis* was only isolated from four (27%) patients, and *N. gonorrhoeae* was isolated from none of the

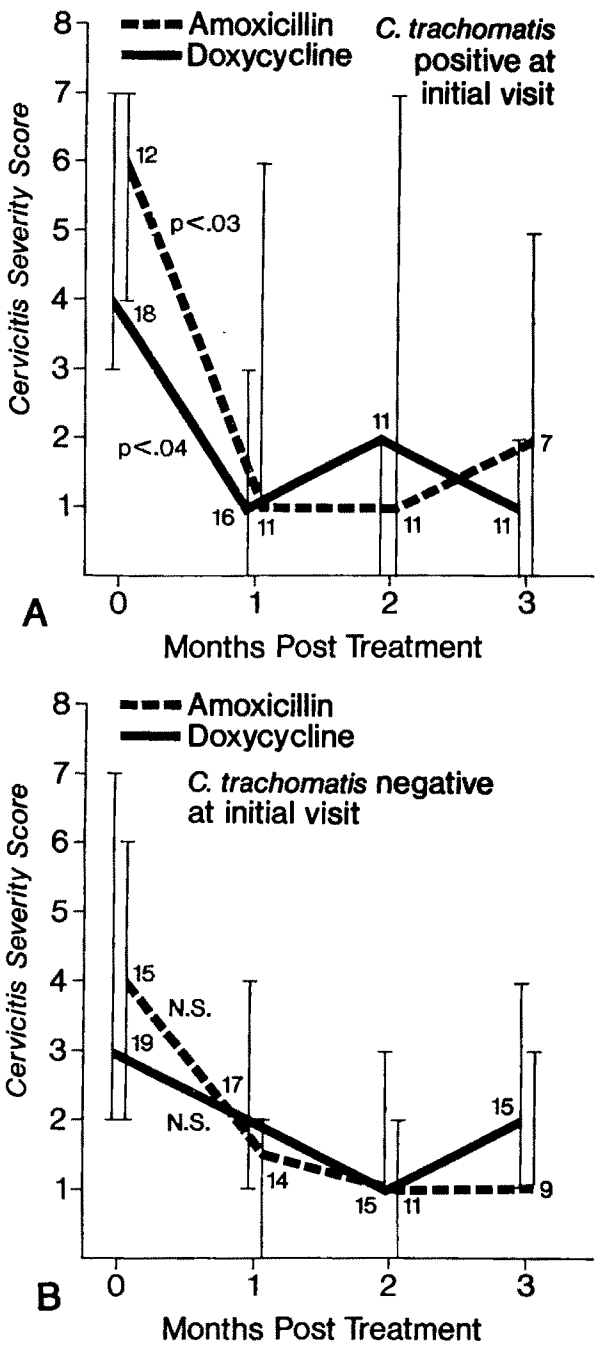


Fig. 3. Median cervicitis severity score with twenty-fifth and seventy-fifth percentiles before treatment and at 1, 2, and 3 months after starting treatment with doxycycline or amoxicillin among patients with positive (**A**) and negative (**B**) cervical culture for *C. trachomatis* (number indicates number of observations).

15 patients who had mucopus at the 2- or 3-month visit. The reisolation of none of the other organisms sought in this study correlated with mucopus on follow-up.

As shown in Fig. 2, the cervicitis severity score declined significantly after treatment in both treatment groups at 1 month ($p < 0.005$), and the improvement in severity score persisted throughout the follow-up period. The two antibiotic regimens had comparable effects on the cervicitis severity score of patients with *C. trachomatis*–positive (Fig. 3, *A*) and *C. trachomatis*–negative (Fig. 3, *B*) cervicitis. However, a statistically significant decrease in the cervicitis severity score only occurred among patients who were *C. trachomatis* positive. The follow-up severity score was not related to the isolation of *U. urealyticum*, *M. hominis*, and *G. vaginalis* on follow-up.

We also studied the effect of therapy on polymorphonuclear leukocyte counts observed in Gram-stained endocervical smears. As shown in Fig. 4, both doxycycline and amoxicillin therapy effectively lowered the polymorphonuclear leukocyte counts or the cervical mucus, and this effect persisted throughout the follow-up period. There was no relationship between the polymorphonuclear leukocyte count at follow-up and the initial diagnosis of chlamydial versus nonchlamydial mocupurulent cervicitis or the isolation of *U. urealyticum*, *M. hominis*, or *G. vaginalis* at follow-up.

Association of ectopy with persistent mucopus. Among women not initially infected with *C. trachomatis* or *N. gonorrhoeae*, oral contraceptives were used by nine (81%) of the 11 patients with persistent or recurrent mucopus and by three (25%) of 12 patients without persistent or recurrent mucopus ($p = 0.01$). This association was not seen among women who initially had *C. trachomatis* isolated. To further study factors that may be responsible for persistent mucopus, 24 patients with persistent mucopus and 20 patients without mucopus at each follow-up visit were further evaluated. The median areas of the ectocervix that showed ectopy ranged from 30% to 50% for those with persistent mucopus and from 10% to 20% for those without mucopus over the follow-up visits ($p = 0.01$), and more of them used oral contraceptives ($p = 0.04$) than women without mucopus on follow-up. Oral contraceptive use has been related to both ectopy and *C. trachomatis* isolation in other studies. Therefore a logistic regression analysis was performed with mucopus as the dependent variable and with ectopy, *U. urealyticum*, oral contraceptive use, and age as independent variables. Persistent mucopus remained associated with a greater area of ectopy ($p < 0.01$) after controlling for the other variables listed, whereas persistent mucopus was not significantly related to any of the other variables in this analysis.

Side effects. Side effects during the treatment were more common with doxycycline (46%) than with amoxicillin (26%) administration (Table III). However, side effects were generally mild and did not necessitate discontinuation of therapy in any patient.

Treatment of the male partner. Physical examinations were performed on 27 (42%) of the 64 potential male partners, including 17 (46%) partners of women

Table III. Reported side effects of the antimicrobial medications

Side effect	*Doxycycline (N = 37)*	*Amoxicillin (N = 27)*
Diarrhea	1	3
Nausea	6	0
Diarrhea and nausea	2	0
Heartburn	2	0
Yeast vaginitis	4	4
Skin rash	2	0
Any side effect	17 (46%)	7 (26%)

from the doxycycline group and 10 (37%) partners of women from the amoxicillin group. Of male partners, 15 had nongonococcal urethritis (one had *C. trachomatis*), one had both *C. trachomatis* and recurrent herpes, one had an asymptomatic *C. trachomatis* urethritis, and two other partners had gonococcal urethritis, one of whom also had *C. trachomatis*. Thus 19 (70%) of the 27 male partners of women with mucopurulent cervicitis had either clinical or culture evidence of urethritis. Persistence of endocervical mucopus was more common in women whose male partners had not received treatment (eight of 20) compared with women whose partners had received antibiotic treatment (five of 24), although the difference was not statistically significant.

Comment

Mucopurulent cervicitis is one of the most frequent genital infections among women. Approximately half of the women with mucopurulent cervicitis have had *C. trachomatis* or *N. gonorrhoeae* isolated from the cervix, and these organisms are well-established causes of the infection.[1, 2] *C. trachomatis* has been isolated four to five times more commonly than *N. gonorrhoeae* among women with mucopurulent cervicitis in our studies.[1, 2, 5, 6]

The recognition and proper treatment of endocervical infection by *C. trachomatis* or *N. gonorrhoeae* is considered important to prevent salpingitis and its sequelae. Tetracycline, erythromycin, sulfatrimethoprim, and to a lesser degree ampicillin have been successfully used to treat cervicitis caused by these two organisms.[13] However, previous treatment studies of cervicitis have left several issues unresolved. First, cure has been defined only by the eradication of *C. trachomatis* or *N. gonorrhoeae*. In men with nongonococcal urethritis, it has been conventional to demonstrate a reduction of clinical symptoms and urethral polymorphonuclear leukocytes as measures of therapeutic response. In contrast, the effects of antibiotic therapy on mucopus per se or on cervical mucus polymorphonuclear leukocytes have not been previously assessed in women either among those who initially had *C. trachomatis* or *N. gonorrhoeae* or among those without these two organisms. Second, long-term follow-up has not been used to study relapse or late recurrences. There is growing concern that conventional 1-week courses of therapy for chlamydial cervicitis may result in late recurrence that could persist for a prolonged time,[14, 15] causing ectopic pregnancy[16] or chronic salpingitis.[17] A comparison of short-term versus long-term tetracycline therapy, with an extended follow-up of perhaps 6 to 12 months, is required to further address the optimal duration of tetracycline therapy for cervical *C. trachomatis* infection.

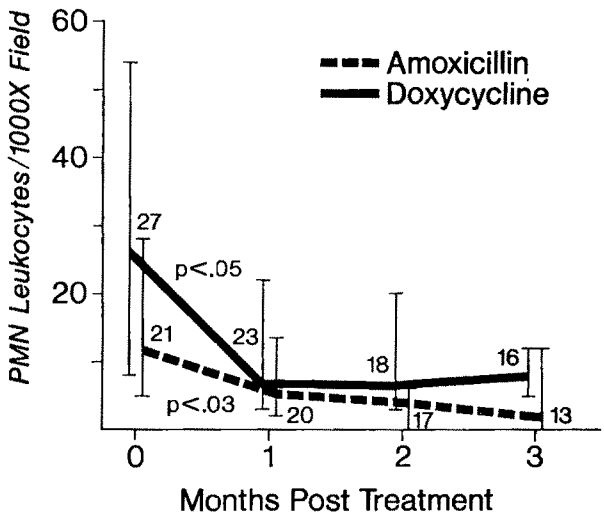


Fig. 4. Median number of polymorphonuclear leukocytes per ×1000 field in endocervical specimens with twenty-fifth and seventy-fifth percentiles obtained before treatment and 1, 2, and 3 months after treatment with doxycycline or amoxicillin (number indicates number of observations).

We chose amoxicillin to compare with doxycycline in this study because both are generally well tolerated and commonly used to treat cervicitis. Also, it was possible that differential therapy could provide clues to the cause of cervicitis among women without *C. trachomatis* or *N. gonorrhoeae*. Both antibiotics were expected to eradicate *N. gonorrhoeae* at the time the study was conducted in Seattle. Although both antibiotics have been reported to cure genital *C. trachomatis* infections, amoxicillin is less active than doxycycline against *C. trachomatis in vitro*,[13] and its use has been reported in relatively few women with *C. trachomatis*. Therefore we anticipated a lower efficacy for amoxicillin than doxycycline in the treatment of cervical mucopus, particularly among women with chlamydial cervicitis.

C. trachomatis was reisolated from two of 11 women treated with amoxicillin who were initially infected and from two of 15 women who were initially culture negative for the organism. Although these results are not significantly different from the results with doxycycline therapy, the power to detect a difference in the treat-

ment of *C. trachomatis* between the two antibiotics is low in this study. The failure rate in the amoxicillin group would have to have been 40% higher than that in the doxycycline group for the difference to be statistically significant (at a power of 80% and significance level of 5%), given the sample size and the *C. trachomatis* failure rate with doxycycline observed in this study. In a mouse model, amoxicillin has been more protective than ampicillin and oxytetracycline against intranasal *C. trachomatis*.[18] Amoxicillin, 750 mg, given three times daily for 7 or 10 days, eliminated *C. trachomatis* from the male urethra in previous small studies.[19, 20] However, there is continuing concern because low doses of amoxicillin have been less effective than other drugs for *C. trachomatis* infection in women.[21, 22] It has been further suggested that aminopenicillins may suppress *C. trachomatis* without killing the organism. Our results cannot definitively refute this notion, although *C. trachomatis* reisolation was infrequent for as long as 3 months after amoxicillin therapy. The effectiveness of amoxicillin and tetracycline against *C. trachomatis* needs to be compared in a larger study.

Since most women with mucopurulent cervicitis had neither *C. trachomatis* nor *N. gonorrhoeae,* it was of particular interest to find that the inflammatory response in this group diminished with antibiotic therapy as judged by mucopus, cervicitis severity score, or cervical polymorphonuclear leukocyte change after therapy. The cause of cervicitis among women without cervical infection by *N. gonorrhoeae* or *C. trachomatis* is not known, although the possibility remains that such cases actually have chlamydia or gonorrhea that was not detected by the methods used. In one study *U. urealyticum* was the only organism that significantly correlated with cervicitis after adjusting for *C. trachomatis*.[2] *U. urealyticum* has also been associated with non-chlamydial urethritis in men.[8] Persistent or recurrent mucopurulent cervicitis was not associated with the reisolation of *U. urealyticum* in this study, but too few patients were treated to exclude this possibility with certainty. Persistent or recurrent mucopurulent cervicitis was also not related to the reisolation of *M. hominis* or *G. vaginalis*. It is possible that persistent mucopus was caused by other antibiotic-sensitive organisms not identified in this study. However, the isolation of any organism from cervical cultures (except *N. gonorrhoeae* or *C. trachomatis*) must be interpreted with caution because of high contamination rates of these cultures with vaginal organisms. The natural history of nonchlamydial, nongonococcal mucopurulent cervicitis is uncertain, and it would be necessary to compare amoxicillin or doxycycline versus placebo to confirm conclusively the apparent effect of those antibiotics on the clinical course of this condition.

Larger studies are needed to determine whether the association between ectopy and mucopus among women with nonchlamydial, nongonococcal cervicitis represents an interaction between ectopy and an infectious agent or an independent effect of ectopy. It is possible that mucopus results from an inflammatory reaction caused by columnar cell contact with vaginal environment factors other than an infectious agent in patients without *C. trachomatis* or *N. gonorrhoeae*. Nevertheless, the weak association between the persistence of mucopus and no treatment of male partners lends support to the concept that even cervicitis not caused by *C. trachomatis* or *N. gonorrhoeae* may be attributed to an infectious agent. Histopathologic characteristics of cervicitis both before and after therapy may provide a better understanding of the causes of this important infection. More data are also required on the risk of women with nonchlamydial, nongonococcal cervicitis for upper genital tract infection.

Since many of the male sexual contacts of women with mucopurulent cervicitis had urethral infections, these data further support the Centers for Disease Control recommendations for male partner examination and treatment.[23] This article includes only nonpregnant women, and the findings are not necessarily applicable to pregnant women. Further study is required of the origin, treatment, and complications of mucopurulent cervicitis during pregnancy.

We thank Linda Cles and Judith Hale for performing microbiologic studies, the staff of Seattle–King County Sexually Transmitted Disease Clinic (H. Hunter Handsfield, MD, Director), and Laurie Johnson for manuscript preparation.

REFERENCES

1. Brunham RC, Paavonen J, Stevens CE, et al. Mucopurulent cervicitis: the ignored counterpart of urethritis in men. N Engl J Med 1984;311:1-6.
2. Paavonen J, Critchlow CW, DeRouen T, et al. Etiology of cervical inflammation. Am J Obstet Gynecol 1986; 154:556-64.
3. Rees E, Tait A, Hobson D, et al. Chlamydia in relation to cervical infection and pelvic inflammatory disease. In: Hobson D, Holmes KK, eds. Nongonococcal urethritis and related infections. Washington, DC: American Society for Microbiology, 1977:67-76.
4. Westrom L. Incidence, prevalence, and trends of acute pelvic inflammatory disease and its consequences in industrialized countries. Am J Obstet Gynecol 1980;138: 880-92.
5. Kiviat NB, Paavonen J, Brockway J, et al. Cytologic manifestations of cervical and vaginal infections. I. Epithelial and inflammatory cellular changes. JAMA 1985;253:989-96.
6. Paavonen J, Stevens CE, Wolner-Hanssen P, et al. Colposcopic manifestations of cervical and vaginal infections. Obstet Gynecol Surv 1988;43:573-9.
7. Kiviat NB, Paavonen J, Wolner-Hanssen P, et al. Histologic manifestation of chlamydial cervicitis. In: Oriel D, Ridgway G, Schachter J, Taylor-Robinson D, Ward M, eds. Chlamydial infections. London: Cambridge University Press, 1986:209-12.
8. Bowie WR, Wang S-P, Alexander ER, et al. Etiology of

nongonococcal urethritis: evidence for *Chlamydia trachomatis* and *Ureaplasma urealyticum*. J Clin Invest 1977; 59:735-42.
9. Brunham RC, Kuo C-C, Stevens CE, Holmes KK. Therapy of cervical chlamydial infection. Ann Intern Med 1982;97:97:216-9.
10. Spiegel CA, Amsel R, Holmes KK. Diagnosis of bacterial vaginosis by direct Gram stain of vaginal fluid. J Clin Microbiol 1983;18:170-7.
11. Fouts AC, Kraus JJ. *Trichomonas vaginalis:* reevaluation of its clinical presentation and laboratory diagnosis. J Infect Dis 1980;141:137-43.
12. Cox DR. The analysis of binary data. London: Methuen, 1970.
13. Sanders LL, Harrison HR, Washington AE. Treatment of sexually transmitted chlamydial infections. JAMA 1986; 255:1750-6.
14. Oriel JD. Chemotherapy. In: Oriel D, Ridgway G, Schachter J, Taylor-Robinson D, Ward M, eds. Chlamydial infections. Proceedings of the sixth international symposium on human chlamydial infections. London: Cambridge University Press, 1986:513-23.
15. Jones BR, Batteiger BE. Human immune response to *Chlamydia trachomatis* infections. In: Oriel D. Ridgway G, Schachter J, Taylor-Robinson D, Ward M, eds. Chlamydial infections. Proceedings of the sixth international symposium on human chlamydial infections. London: Cambridge University Press, 1986:423-32.
16. Brunham RC, Binns B, McDowell J, Paraskevas M. *Chlamydia trachomatis* infection in women with ectopic pregnancy. Obstet Gynecol 1096;67:722-6.
17. Henry-Suchet, Utzmann C, DeBrux J, Ardoin P, Catelan F. Microbiologic study of chronic inflammation associated with tubal factor infertility: role of *Chlamydia trachomatis*. Fertil Steril 1987;47:274-7.
18. Kramer MJ, Cleveland R, Grunberg E. Activity of oral amoxicillin, ampicillin, and oxytetracycline against infections with *Chlamydia trachomatis* in mice. J Infect Dis 1979;139:717-9.
19. Bowie WR, Alexander ER, Holmes KK. Eradication of *Chlamydia trachomatis* from the urethras of men with nongonococcal urethritis by treatment with amoxicillin. Sex Transm Dis 1981;8:79-81.
20. Csango PA, Gundersen T, Martinsen IM. Effect of amoxicillin on simultaneous *Chlamydia trachomatis* infections in men with gonococcal urethritis: comparison of three dosage regimens. Sex Transm Dis 1985;12:93-6.
21. Bowie WR, Manzon LM, Borrie-Hume CJ, Fawcett A, Jones HD. Efficacy of treatment regimens for lower urogenital *Chlamydia trachomatis* infection in women. Am J Obstet Gynecol 1982;142:125-9.
22. Brunham RC, Kuo C-C, Stevens CE, et al. Treatment of concomitant *Neisseria gonorrhoeae* and *Chlamydia trachomatis* infections in women: comparison of trimethoprim-sulfamethoxazole with ampicillin and probenecid. Rev Infect Dis 1982;4:491-9.
23. *Chlamydia trachomatis* infections: policy guidelines for prevention and control. MMWR 1985;34:53S-71S.

Right ventricular dysfunction in human fetal compromise

Juha Räsänen, MD, Pertti Kirkinen, MD, and Pentti Jouppila, MD
Oulu, Finland

Fetal M-mode echocardiography was performed in 13 pregnancies complicated by intrauterine distress. Absent end-diastolic blood velocity in the fetal descending aorta was documented by pulsed Doppler examinations. The right ventricular fractional shortening of these fetuses was decreased and the ratio of the right/left ventricular end-diastolic diameters was increased. The left ventricular size and myocardial contractility did not differ from normal pregnancy. These findings reveal that relative right ventricular heart dysfunction is associated with severe intrauterine distress. (Am J Obstet Gynecol 1989;161:136-40.)

Key words: Fetal heart, echocardiography, Doppler

Recent investigations with pulsed or continuous wave Doppler techniques have revealed that chronic fetal distress is commonly associated with hemodynamic changes in fetal and umbilical blood circulation. The diastolic flow in the fetal descending aorta and in the umbilical artery is decreased, whereas the fetal intracranial arterial diastolic flow components appear to be increased.[1-6] These changes are thought to reflect a redistribution of fetal cardiac output to the most vital fetal organs, but the pathophysiologic mechanisms are not known. The purpose of our study was to investigate fetal cardiac growth and myocardial function in conditions in which the fetus had developed a strongly abnormal blood flow pattern in connection with chronic intrauterine distress.

Material and methods

The study comprised 13 last-trimester pregnancies (pathologic pregnancy group) in which the Doppler velocity spectrum of the fetal descending thoracic aorta revealed that the end-diastolic blood velocity was decreased to 0 (no forward flow above the filter level during the last 2/10 of the heart cycle). In this group, severe preeclampsia was diagnosed in nine cases and cesarean section was later performed in seven cases because of a pathologic fetal heart rate pattern. In 11 cases, the birth weights of the newborns were < the 5th percentile, and in two cases, < the 10th percentile for normal pregnancies. The perinatal data of the material are presented in Table I.

Echocardiographic examination of these fetuses was made within 14 days (6.8 ± 5.6 days (mean ± SD), range of 0 to 14 days) before delivery, with a General Electric RT 3000 scanner with a 5 MHz sector probe. M-mode examination was done with a four-chamber view of the fetal heart by directing the M-mode level perpendicularly toward the interventricular septum at the level of the tips of the atrioventricular valves.[7,8] The end-systolic (ES) and end-diastolic (ED) diameters of the ventricles were recorded (from the endocardium to the interventricular septum) and the fractional shortenings of the ventricles were calculated [(ED − ES)/ED]. The mean circumferential shortenings of the ventricles, which allow the evaluation of the combined effect of fractional shortening with the duration of systole, were also calculated [(ED − ES)/ED × duration of systole]. The biventricular inner end-diastolic and end-systolic diameters (from endocardium to endocardium) were measured and the biventricular inner fractional change was calculated. The systolic period was restricted to the time when the atrioventricular valves were closed on the M-mode screen. Five correctly imaged consecutive heart cycles were analyzed and their mean values were used for further analysis. Heart growth was monitored by measuring the end-diastolic inner biventricular transversal diameter and inner end-diastolic transversal diameters of the ventricles. Biventricular, left and right ventricular fractional, and mean circumferential shortenings were calculated to assess the functional properties of the heart.

Immediately after the M-mode recording, the blood flow in the fetal descending thoracic aorta was examined with duplex pulsed Doppler equipment (Toshiba SAL-20, 3.5 MHz linear probe, and Pedof, 2 MHz probe, at a fixed 50-degree angle; 150 Hz filter).[3] Using the mean Doppler frequency shift, the time-averaged mean velocity, systolic peak velocity, end-diastolic velocity, pulsatility index [(peak velocity − end-diastolic velocity)/mean velocity during the cycle], and volumetric flow (ml/min) were calculated.[3] The flow parameters were based on registration of the mean blood velocity in the fetal descending thoracic aorta at

From the Department of Obstetrics and Gynecology, University of Oulu.
Received for publication March 8, 1988; revised January 4, 1989; accepted January 10, 1989.
Reprint requests: Juha Räsänen, MD, Department of Obstetrics and Gynecology, University of Oulu, 90220 Oulu, Finland.

Table I. Perinatal data

	No. of cases	*Ultrasonographic examination (gestational week)*	*Gestational age at delivery (wk)*	*Birth weight (gm)*	*Apgar scores (1 min)*
Normal pregnancy	51	35 ± 2.4	40 ± 2.3	3232 ± 668	8.8 ± 0.55
Pathologic pregnancy	13	34 ± 3.5	35 ± 4.0*	1742 ± 576*	7.4 ± 1.58*

All data represent mean ± SD.
*$p < 0.0005$.

Table II. Myocardial contractility and right/left relationship of cardiac ventricles

	Normal pregnancy	*Pathologic pregnancy*
Left ventricle		
Fractional shortening	33.54% ± 1.88%	33.44% ± 2.67%
Circumferential shortening	1.37 ± 0.15	1.36 ± 0.14
Right ventricle		
Fractional shortening	32.62% ± 2.35%	28.78% ± 1.97%*
Circumferential shortening	1.33 ± 0.13	1.16 ± 0.12†
Biventricular inner fractional change	26.90% ± 2.26%	25.22% ± 2.43%†
Right/left relation		
Inner diastolic dimension	1.01 ± 0.057	1.19 ± 0.132*
Fractional shortening	0.97 ± 0.062	0.87 ± 0.110*
Circumferential shortening	0.97 ± 0.066	0.86 ± 0.125*

All data represent mean ± SD.
*$p < 0.0005$.
†$p < 0.05$.

intervals of 5.2 msec over 10 consecutive cycles. All measurements were made with the mother lying in a left recumbent position. Cardiotocography was normal in all cases on the same day when examined by the above mentioned ultrasonographic methods. The baseline of the fetal heart rate varied between 120 and 160 beats/min.

A group of 51 women with normal pregnancies (normal pregnancy group) was examined during the last trimester. There was no maternal history of diseases that predisposed the fetus to abnormal fetal growth in this group. Birth weights of these newborns were between the 10th and 90th percentiles of normal weight curves. Doppler and echocardiographic examinations were made in these cases in connection with routine ultrasonographic controls, corresponding to the examinations made in the pathologic cases. All of the control cases had continuous forward aortic blood flow over the whole of diastole.

Comparison of the means and linear regression coefficients was done by Student t test; $p < 0.05$ was selected as the level of significance.

Results

Table II shows the comparison of the echocardiographic findings between the two groups. Pregnancies with absent end-diastolic blood velocities of the fetal aortic Doppler examinations showed decreased values of right ventricular fractional and mean circumferential shortenings compared with normal pregnancies ($p < 0.0005$ for the means) whereas the left ventricular values remained normal. Correspondingly, the relationships between the right and left ventricular fractional and mean circumferential shortenings were decreased from those in normal pregnancy ($p < 0.0005$ for the means). Pregnancies with abnormal blood flow showed increased right/left relationships of the inner end-diastolic dimension of the cardiac ventricles ($p < 0.0005$ for the means).

Table III. Results of the fetal aortic Doppler examinations

	Normal pregnancy	*Pathologic pregnancy*
Total blood flow (ml/min)	725.1 ± 224.6	408.5 ± 167.6*
Pulsatility index	2.07 ± 0.44	3.33 ± 0.77*
Time-averaged mean velocity (cm/sec)	32.0 ± 5.28	20.1 ± 4.93*
Time-averaged peak velocity (cm/sec)	79.0 ± 14.2	63.1 ± 15.3*

All data represent mean ± SD.
*$p < 0.0005$.

The results of the flow measurements in the fetal descending aorta are presented in Table III. The pulsatility index was increased in the group with abnormal

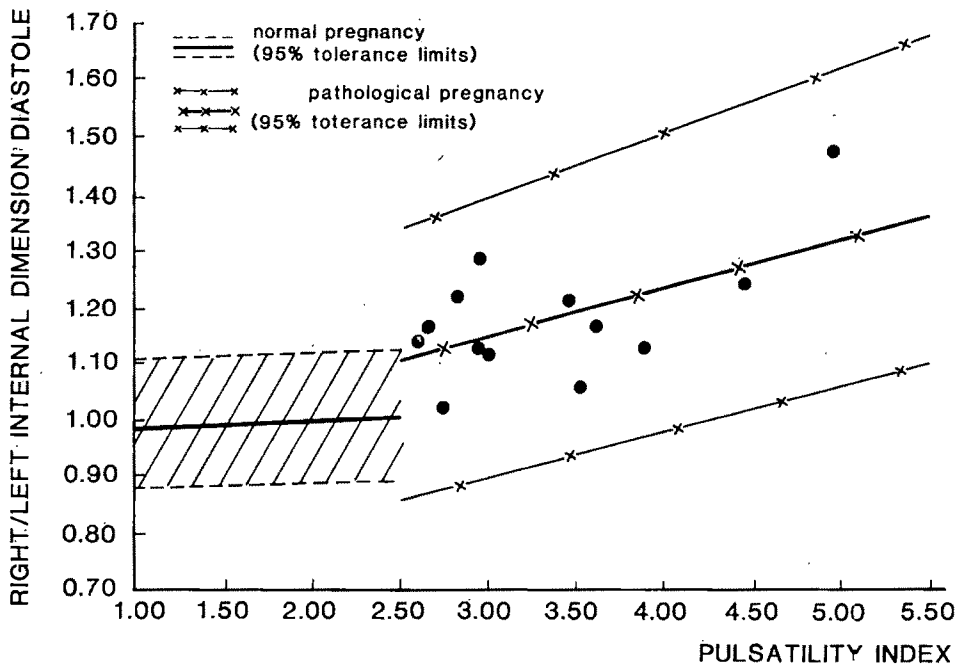


Fig. 1. Right/left ratio of ventricular inner end-diastolic dimensions plotted against corresponding pulsatility index in fetal descending thoracic aorta. •, Pathologic cases. Regression lines and 95% tolerance limits of both groups are presented. Significant difference between groups ($p < 0.02$).

flow ($p < 0.0005$ for the means) compared with normal pregnancies, whereas the blood velocities were decreased ($p < 0.0005$ for the means).

When the inner end-diastolic dimension of the right ventricle was plotted against the corresponding biparietal diameter, the cases with abnormal flow pattern showed increased right ventricular dimensions compared with normal cases ($p < 0.05$ for linear regression slopes). In 11 of the 13 abnormal cases, the right ventricular inner end-diastolic dimension was >50th percentile curve for normal fetuses. The left ventricular dimensions did not differ from those measured in the normal pregnancies.

In the normal pregnancies, the physiologic variation of the pulsatility index did not affect the right/left ratio of the ventricular inner end-diastolic dimensions. The cases with absent end-diastolic blood flow showed a significant increase in this ratio with increasing pulsatility index (Fig. 1; $p < 0.02$ for linear regression slopes). In fetuses with abnormal blood flow, the decrease in the blood velocities was associated with an increase in the right/left ratio of the ventricular inner end-diastolic dimensions compared with normal pregnancies (Fig. 2; $p < 0.03$ for linear regression slopes).

Comment

Our knowledge of human fetal myocardial function in intrauterine distress has largely been based on the evaluation of heart frequencies and their changes. In addition, some investigations by electrocardiography have been performed in cases of intrauterine distress.[9]

The characteristics of cardiac growth and myocardial function in uncomplicated pregnancies have been investigated by many authors.[7, 8] M-mode echocardiography has been accepted as a clinical tool for evaluation of suspected functional and structural disturbances of the fetal heart.[10-12] Recently much interest has been directed toward the use of continuous-wave and pulsed Doppler examination in obstetrics and the limitations and benefits of these techniques have been widely discussed.[13-15]

In all our cases with an abnormal aortic blood flow pattern, the cause was chronic placental insufficiency, primarily as a result of maternal vascular disease. This resulted in severe fetal growth retardation and intrauterine chronic distress. As commonly found with this type of intrauterine distress, these fetuses showed decreased velocities and increased waveform indexes in the Doppler measurements of descending aortic blood flow. It has been demonstrated that low mean blood velocity in the fetal descending aorta is associated with fetal hypoxia, hypercapnea, acidosis, and hyperlactemia. There have also been attempts to treat fetuses with intrauterine growth retardation by maternal oxygen therapy.[16, 17] In these conditions, the relationship between the systolic/diastolic flow components increases and a total block at end-diastole appears. On

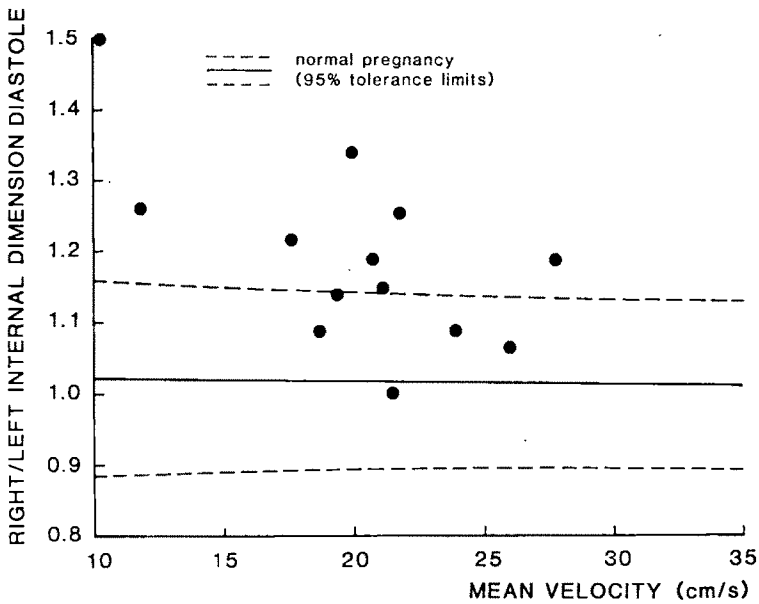


Fig. 2. Right/left ratio of ventricular inner end-diastolic dimensions plotted against time-averaged mean blood velocity in descending thoracic aorta. •, Pathologic cases. Significant difference between groups ($p < 0.03$).

the basis of this fact and the clinical outcome of our cases, we think that our cases represented severe intrauterine compromise, despite the fact that none of the fetuses died in utero.

Our cases demonstrated that the fetal heart reacts in such conditions by showing both an increased right/left relationship in ventricular dimensions and decreased contractility of the right ventricular myocardium. Left ventricular function appeared to remain normal. This may be a sign of fetal effort to maintain adequate cerebral and myocardial circulation as far as possible, since most of the left ventricular cardiac output is directed to these organs.[18] Thus relative right ventricular heart dysfunction of the fetus appears to develop. However, none of these cases had any signs of manifest decompensation such as ascites or edema. These fetal cardiac changes also seem to precede subsequent cardiotocographic abnormalities. DeVore[19] demonstrated right/left ventricular disproportion in the fetuses with abdominal circumferences ≤2 SD below the mean. Pulmonary hypertension was thought to be the cause of the right ventricular dilatation. It has been shown that most of the right ventricular cardiac output is ejected to the descending aorta via the pulmonary artery and the ductus arteriosus.[19] Thus it can be speculated that increased vascular resistance in the aorta could cause the dilatation of ductus arteriosus, pulmonary hypertension, and the changes in the right ventricular function. However, in our study we did not measure the transverse diameters of ductus arteriosus or pulmonary outflow tract.

In our previous study of hypertensive pregnancies, right-sided fetal cardiac ventricular dilatation was found to be associated with increased fetal vascular resistance in the descending aorta.[20] Our series showed that if vascular resistance is very severe, with loss of the end-diastolic aortic flow components, right ventricular myocardial contractility is also impaired.

We conclude that signs of increased peripheral vascular resistance in the fetal aorta in severe intrauterine distress seem to be connected with right ventricular dysfunction, without any evident disturbance of the left ventricle. However, these changes are very moderate with regard to clinical manifestations such as growth impairment and subsequent pathologic fetal heart rate patterns, which reflected the severity of fetal compromise in our material. This indicates that the human fetal heart has great functional reserves in chronic intrauterine distress.

REFERENCES

1. Griffin D, Bilardo K, Masini L, et al. Doppler blood flow waveforms in the descending thoracic aorta of the human fetus. Br J Obstet Gynaecol 1984;91:997-1006.
2. Trudinger BJ, Giles WB, Cook CM, Bombardieri J, Collins L. Fetal umbilical artery flow velocity waveforms and placental resistance: clinical significance. Br J Obstet Gynaecol 1985;92:23-30.
3. Jouppila P, Kirkinen P. Blood velocity waveforms of the fetal aorta in normal and hypertensive pregnancies. Obstet Gynecol 1986;67:856-60.
4. Tonge HM, Wladimiroff JW, Noordam MJ, van Kooten C. Blood flow velocity waveforms in the descending fetal aorta: comparison between normal and growth-retarded pregnancies. Obstet Gynecol 1986;67:851-5.
5. Wladimiroff JW, Tonge HM, Stewart PA. Doppler ultra-

sound assessment of cerebral blood flow in the human fetus. Br J Obstet Gynaecol 1986;93:471-5.
6. Laurin J, Lingman G, Marsal K, Persson P. Fetal blood flow in pregnancies complicated by intrauterine growth retardation. Obstet Gynecol 1987;69:895-902.
7. DeVore GR, Siassi B, Platt LD. Fetal echocardiography IV. M-mode assessment of ventricular size and contractility during the second and third trimesters of pregnancy in the normal fetus. Am J Obstet Gynecol 1984;150: 981-8.
8. Shime J, Gresser CD, Rakowski H. Quantitative two-dimensional echocardiographic assessment of fetal cardiac growth. Am J Obstet Gynecol 1986;154:294-300.
9. Symonds EM. Fetal electrocardiographic monitoring: past, present and future—a historical perspective. J Perinat Med 1986;14:385-90.
10. Allan LD, Anderson RH, Sullivan ID, Campbell S, Holt DW, Tynan M. Evaluation of fetal arrhythmias by echocardiography. Br Heart J 1983;50:240-5.
11. DeVore GR, Siassi B, Platt LD. Fetal echocardiography III. The diagnosis of cardiac arrhythmias using real-time–directed M-mode ultrasound. Am J Obstet Gynecol 1983;146:792-9.
12. Allan LD, Crawford DC, Anderson RH, Tynan M. Evaluation and treatment of fetal arrhythmias. Clin Cardiol 1984;7:467-73.
13. Eik-Nes S, Marsal K, Brubakk A. Ultrasonic measurement of human fetal blood flow. J Biomed Eng 1982;4:28-36.
14. Eik-Nes S, Marsal K, Kristoffersen K. Methodology and basic problems related to blood flow studies in the human fetus. Ultrasound Med Biol 1984;10:329-36.
15. Gill R. Measurement of blood flow by ultrasound: accuracy and sources of error. Ultrasound Med Biol 1985; 11:625-41.
16. Soothill PW, Nicolaides KH, Bilardo CM, Campbell S. Relation of fetal hypoxia in growth retardation to mean blood velocity in the fetal aorta. Lancet 1986;2:1118-20.
17. Nicolaides KH, Campbell S, Bradley RJ, Bilardo CM, Soothill PW, Gibb D. Maternal oxygen therapy for intrauterine growth retardation. Lancet 1987;1:942-5.
18. DeVore GR, Siassi B, Platt LD. Fetal echocardiography V. M-mode measurements of the aortic root and aortic valve in second- and third-trimester normal human fetuses. Am J Obstet Gynecol 1985;152:543-50.
19. DeVore GR. Examination of the fetal heart in the fetus with intrauterine growth retardation using M-mode echocardiography. Semin Perinatol 1988;12:66-79.
20. Räsänen J, Kirkinen P, Jouppila P. Fetal aortic blood flow and echocardiographic findings in human pregnancy. Eur J Obstet Gynecol Reprod Biol 1988;27:115-24.

Preterm and term fetal cardiac and movement responses to vibratory acoustic stimulation

Ronald L. Thomas, MD, Timothy R. B. Johnson, MD, Richard E. Besinger, MD, Deedra Rafkin, RN, Catharine Treanor, RN, and Donna Strobino, PhD

Baltimore, Maryland

To assess fetal response to vibratory acoustic stimulation, 24 preterm (group A; <36 weeks' gestation) and 30 term (group B; ≥36 weeks' gestation) pregnancies were studied. Study variables were perceived fetal movement, fetal heart rate, and fetal heart rate pattern. Observer- and patient-perceived fetal movement responses were noted in most cases but were slightly more common in term patients (group A: +fetal movement, patient 87.5%/observer 87.5%) group B: +fetal movement, patient 96.7%/observer 90%). Baseline changes in fetal heart rate (≥10 beats/min) were observed in 46% of preterm fetuses and 70% of term fetuses. Tachycardia (fetal heart rate >160 beats/min) was a common finding in both groups. In group A, tachycardia after vibratory acoustic stimulation persisted more than 1 minute in 29.2% and more than 5 minutes in 12.5% of patients. In group B tachycardia beyond 1 and 5 minutes was noted in 73.3% and 50% of patients, respectively. A significant shift to "awake" fetal heart rate patterns occurred in both groups; 29% to 79% was noted in group A ($p < 0.001$) and 46.7% to 90% in group B ($p < 0.001$). When vibratory acoustic stimulation was used, the high occurrence of increased baseline, tachycardia, and emergence of unusual fetal heart rate patterns must be recognized to adequately interpret fetal heart rate tracings. (Am J Obstet Gynecol 1989;161:141-5.)

Key words: Vibratory acoustic stimulation, fetal behavior

Observations on fetal response to external sound stimuli were first reported in the 1920s.[1,2] The concept of vibration/auditory interaction with the fetus was slowly rediscovered in the 1960s, 1970s, and 1980s. A number of recent studies[3-6] indicate that fetal acoustic stimulation has applicability in the evaluation of nonreactive nonstress tests. However, Gagnon et al.[7] showed evidence of alteration in fetal heart rate (FHR) and FHR patterns after vibratory acoustic stimulation in studying term gestations (36 to 40 weeks). Before the introduction of this technique into routine use at our facility, we conducted a pilot study to assess the effects of vibratory acoustic stimulation on FHR tracings and maternal and observer perceptions of fetal movements. A second concern was whether there was a differential effect of vibratory acoustic stimulation for term and preterm fetuses.

Material and methods

Fifty-four patients, including high-risk patients referred for nonstress testing (NST), contraction stress testing, or biophysical profiles, were studied. Each patient in the study was studied on only a single occasion. Because of previous work by Nijhuis et al.[8] suggesting development of organized fetal behavioral states at approximately 36 weeks, this gestation was chosen as the pivot point for our two groups. Study patients otherwise had routine and reassuring testing. A breakdown of referral indications is found in Table I.

Table I. Indications for referral

Indications for testing	*Preterm fetuses (%)*	*Term fetuses (%)*
IUGR	12.5	23.3
Hypertensive disease	25	13.3
Renal disease	8.3	0
Decreased movement	8.3	6.7
Diabetes	25	20
Bleeding hydramnios, lupus, Rh immunization, H/O loss, cardiac disease, miscellaneous	16.7	20
Postdates	0	20

IUGR, intrauterine growth retardation; *H/O*, history of.

Patients were selected from referrals to the Fetal Assessment Center over an 8-week period. Patient enrollment was based on scheduling load and the availability of additional monitoring time. Regardless of the indication for referral, all patients had an NST performed in standard fashion during the assessment. Five continuous minutes of monitoring were performed for

From the Division of Maternal-Fetal Medicine and Fetal Assessment Center, The Johns Hopkins Hospital and University School of Medicine.

The views expressed in this article are those of the authors and do not reflect the official policy or position of the Department of the Navy, Department of Defense, or the U.S. Government.

Received for publication May 26, 1988; accepted January 30, 1989.

Reprint requests: Ronald L. Thomas, MD, Department of Obstetrics and Gynecology, National Naval Medical Center, 8901 Wisconsin Ave., Bethesda, MD 20814-5011.

Table II. Definitions for FHR patterns

Neonatal/fetal behavioral state	*Gross description*	*Neonatal*	*Fetal*	*FHR pattern*
1/1F	Quiet sleep	Regular respirations, non-REM sleep	Regular respirations (if present), rare gross movements nondiscriminatory	(A) Variability <10 beat/min; isolated accelerations associated with movements
2/2F	Active sleep	REM sleep; irregular respirations, activity nondiscriminatory	Eye movements; irregular respirations (if present); small movements; gross movements nondiscriminatory	(B) Frequent 10 to 20 accelerations (not inherent to pattern); variability 10-20 beats/min
3/3F	Quiet awake	Regular respirations, no gross activity	Eye movements; regular respirations (if present); no gross movements	(C) No accelerations; rate oscillates more regularly than (B)
4/4F	Active awake	Physically active, irregular respirations	Eye movements; Irregular respirations (if present); gross movements and small movements	(D) "Unstable" FHR with variability up to 25 beats/min; accelerations of 25-30 beats/min; occasional sustained "tachycardia"
5/	Crying	Same as (4) with crying	—	—

See reference 9.
REM, Rapid eye movement.

each fetus before the application of vibratory acoustic stimulation. Stimulation of the fetuses was performed with an artificial larynx (Model 5-C; Western Electric). The instrument was applied to the fetal vertex, as identified by the Leopold maneuver, and two 2-second pulses of sound, which were separated by 30 seconds, were administered. Monitoring was continued on all patients until the FHR recorded had returned to the original baseline rate.

We measured FHR and FHR pattern before and after the vibratory acoustic stimulation using Hewlett-Packard fetal monitor Model HP 3040A. Fetal tachycardia was recorded if the FHR persisted above a rate of 160 beats/min for at least 1 minute; duration of tachycardia was also recorded. Definitions for FHR patterns were those described by Nijhuis et al.[8] and Martin[9] and appear in Table II. Patterns were categorized according to the criteria most clearly met by the tracing in the 5 minutes before and the 5 minutes immediately after acoustic stimulation.

In evaluating FHR patterns, we were interested in shifts that occurred from those patterns typically recorded in term fetuses (≥36 weeks) with sleep and awake states. Table II outlines the definitions and tracings of four FHR patterns used in our study and their association with "sleep" and "awake" states. FHR patterns do not form part of a defined behavioral state before 36 weeks; nonetheless, induced changes are observed.

Maternal and observer perceptions of fetal movements after vibratory acoustic stimulation were also recorded. Maternal perception of fetal movement was recorded as positive if the patient noted an immediate and obvious fetal kick or movement within 10 seconds of the stimulus. Observer perception of movement was recorded as positive if a definite sudden movement was palpated by a hand placed on the uterine fundus. Fetal movement was recorded as positive if movement was felt after either of the two sequential stimulations. The χ^2 test for independence was used to compare differences in the percentage of fetuses with tachycardia with those with a given heart rate pattern. The analysis was performed separately for term and preterm groups. Small numbers did not permit age and parity comparisons.

Results

The gestational ages in group A ranged from 26.9 weeks to 35.9 weeks, with a mean of 32.9 weeks. In group B fetuses ranged in gestational age from 36 weeks to 42.7 weeks, with a mean of 38.9 weeks. In response to vibratory acoustic stimulation, 19 (79.2%) of the preterm fetuses showed some increase in FHR, with an average rise of 16 beats/min. Eleven of 24

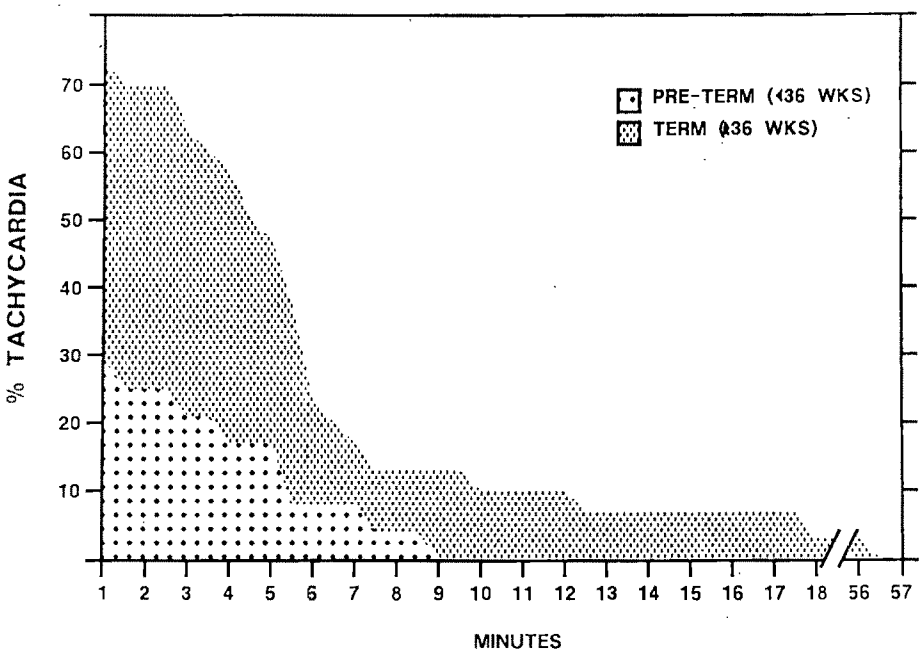


Fig. 1. Preterm and term tachycardiac responses to vibratory acoustic stimulation and their duration.

Table III. Data breakdown in study groups

	Group A	*Group B*	*Significance*
Gestational age range (wk)	26^{+6}-35^{+6}	36-42^{+5}	
Gestational age (mean ± 1 SD) (wk)	32.9 ± 2.2	38.9 ± 2.0	
Patient-perceived movement with VAS (%)	(87.5)	(96.7)	NS
Observer-perceived movement with VAS (%)	(87.5)	(90) $p < 0.05$	NS
Mean increase in FHR baseline with VAS ± 1 SD (beats/min)	12.3 ± 9.5	16.2 ± 10.6	NS
Baseline increases of 10 beats/min above baseline (%)	46	70	
Tachycardia ≥ min (%)	29.2	73.3	$p < 0.0001$
Tachycardia ≥ 5 min (%)	12.5	50	$p < 0.001$

VAS, Vibratory acoustic stimulation.

(46%) preterm fetuses had a rise in FHR of more than 10 beats/min. A decrease in FHR was noted in one case. Among term fetuses, 26 of 30 (86.7%) fetuses showed an increase in FHR in response to acoustic stimulation; the mean rise was 16.9 beats/min. The increase in FHR in 21 of 30 infants was more than 10 beats/min (70%). Again, one fetus showed a decrease in FHR in response to vibratory acoustic stimulation.

Among the preterm infants, seven of 24 (29.2%) fetuses had tachycardia lasting for at least 1 minute, and three (12.5%) had more than 5 minutes of tachycardia. In term fetuses, at least 1 minute of tachycardia was found in 22 of 30 fetuses (73.3%) and persisted for more than 5 minutes in 15 fetuses (50%) (Table III). The pattern of persisting tachycardia is seen in Fig. 1.

Figs. 2 and 3 demonstrate shifts in FHR patterns before and after vibratory acoustic stimulation for preterm and term fetuses, respectively. In preterm fetuses there was a significant shift from "sleep" patterns (FHR patterns A and B) to "awake" patterns (FHR patterns C and D). Before acoustic stimulation, 20% of fetuses in the preterm group had either FHR pattern C or D. The percentage increased markedly to 79% after vibratory acoustic stimulation. FHR patterns C and D were more commonly identified in term fetuses, but nonetheless, there was a significant shift in fetuses not already exhibiting one of the two "awake" patterns, from 46.7% to 90% after vibratory acoustic stimulation.

No difference was noted in fetal movements as perceived by an observer from those perceived by the pa-

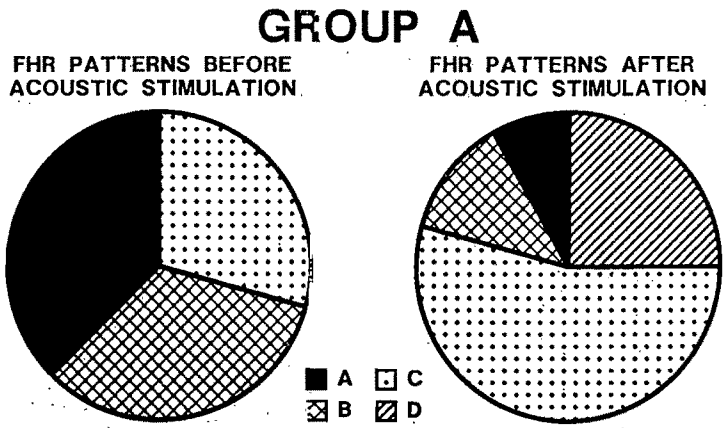


Fig. 2. FHR patterns in preterm fetuses before (FHR pattern A, 37.5%; FHR pattern B, 33.3%; FHR pattern C, 29.2%) and after vibratory acoustic stimulation (FHR pattern A, 8.3%; FHR pattern B, 12.5%; FHR pattern C, 54.2%; FHR pattern D, 25%).

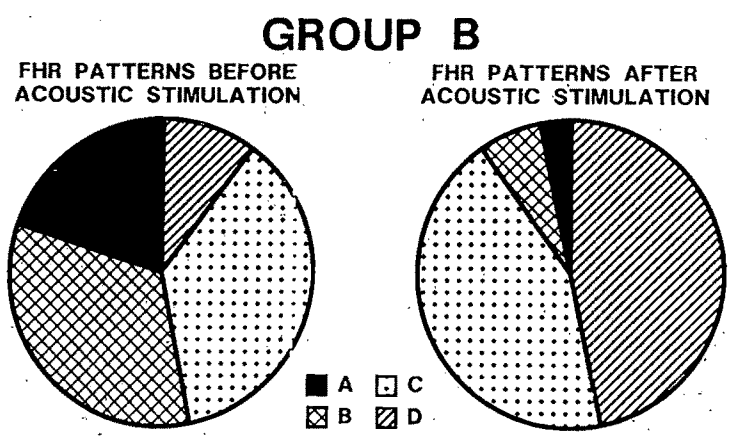


Fig. 3. FHR patterns in term fetuses before (FHR pattern A, 20%; FHR pattern B, 33.3%; FHR pattern C, 36.7%; FHR pattern D, 10%) and after vibratory acoustic stimulation (FHR pattern A, 3.3%; FHR pattern B, 6.7%; FHR pattern C, 43.3%; FHR pattern D, 46.7%).

tient. Only rarely was there a discordance noted in perception. In the term group the patient more commonly perceived fetal movement (96.7% versus 90%, $p < 0.05$) than the observer. Perception of movement was slightly more common in term fetuses.

Comment

Significant changes in baseline FHR have been noted after vibratory acoustic stimulation. The changes in baseline were as common in group A as in group B. This is contrary to the lack of baseline change reported by Gagnon et al.[10] (30 to 42 weeks). Observed tachycardia probably does not represent stress but a physiologic response to a nonphysiologic stimulus, vibratory acoustic stimulation. The tachycardia raises theoretic concerns that the severely compromised fetus might be further compromised by vibratory acoustic stimulation. Also, since "tachycardia" has been reported as an ominous sign by several investigators,[11, 12] its occurrence after vibratory acoustic stimulation without other complicating factors needs to be recognized as not being prognostically important. It also appears from our data that the FHR changes reported in term fetuses also occur in preterm fetuses. This implies that these changes may be independent of behavioral states that do not form before approximately 36 weeks. These findings confirm those previously reported by Gagnon et al.[7]

The shifts noted in FHR patterns, particularly from FHR pattern A to B, C, and D may explain the observations that we and others[3, 5] have noted of a decrease in nonreactive NSTs with vibratory acoustic stimulation applications. Baseline rate increase and tachycardia are also common responses. Vibratory acoustic stimulation during antepartum fetal testing has become popular during the past decade. Preliminary work shows it to

be efficacious and cost-effective. Thus far, antepartum vibratory acoustic stimulation has been limited in application to its influences on the NST. The physiologic mechanism of fetal response has not been delineated, but it is theorized that there may be an alteration in fetal behavioral state in response to vibratory acoustic stimulation.

Previous studies reported by Nijhuis et al.[8] have detailed behavioral studies in neonates and term and preterm fetuses. Their work reports organized behavioral states existing at 36 weeks; individual parameters of these states, such as FHR patterns, although not states, are definable before 36 weeks' gestation. Nevertheless, these patterns may be concordant with associated behavioral states in the term fetus and neonate (see Table II). Transitional patterns are found as shifts from one pattern to another occur. A computer-based analysis of FHR patterns has shown that a good correlation with individual visual classification exists.[13] In the normal fetus (and neonate), most of the time is spent in FHR patterns A and B. These patterns (at 36 weeks and beyond) are associated with sleep (nonrapid eye movement and rapid eye movement), and although FHR accelerations occur with movement in all states, they are less frequent during sleep. Gagnon et al.[7] hypothesize that the changes seen with vibratory acoustic stimulation, such as in antepartum fetal monitoring, may be a reflection of state change in the fetus.

There is an obvious fetal movement/startle response with vibratory acoustic stimulation. Ultrasonography has been used to document fetal "startle" responses to vibratory acoustic stimulation.[14] Given the high proportion of cases in which the patient and observer perceived nearly immediate response recognition, ultrasound documentation may be superfluous. This response in and of itself may provide a rapid assessment of gross fetal compromise. Westgren et al.[15] have recently reported on a series of patients to correlate fetal movement response with a reactive NST and show a sensitivity of 100%. There are also significant alterations in FHR pattern with vibratory acoustic stimulation including "tachycardia," which may be prolonged. Caution must be taken before universal application of this nonphysiologic stimulus. Whether the development of tachycardia or FHR pattern C or D is equivalent to a "reactive NST" is presently not known. Furthermore, whether perception of fetal movement in response to vibratory acoustic stimulation is equivalent to a reactive NST is also unknown. More detailed study is needed to better understand the highly unusual FHR responses that occur with vibratory acoustic stimulation. Application of non-FHR observations may provide additional tools to assess the fetus at risk for in utero compromise.

REFERENCES

1. Peiper A. Sinnesempfindungen des Kindes vor seiner Geburt, Monatsschr. Kinderheilk 1925;29:236.
2. Forbes HS, Forbes HB. Fetal sense reaction: hearing. J Comp Physiol Psychol 1927;7:353.
3. Smith CV, Phelan JP, Platt LD, et al. Fetal acoustic stimulation testing. II. A randomized clinical comparison with the nonstress test. AM J OBSTET GYNECOL 1986;155:131.
4. Smith CV, Phelan JP, Paul RH, et al. Fetal acoustic stimulation testing: a retrospective experience with the fetal acoustic stimulation test. AM J OBSTET GYNECOL 1985; 153:567.
5. Davey DA, Dommisse J, MacNab M, et al. The value of an auditory stimulatory test in antenatal fetal cardiotocography. Eur J Obstet Gynecol Reprod Biol 1984;18:273.
6. Serafini P, Lindsay MBJ, Nagey DA, et al. Antepartum fetal heart rate responses to sound stimulation: the acoustic stimulation test. AM J OBSTET GYNECOL 1984;148:41.
7. Gagnon R, Hunse C, Carmichael L, et al. External vibratory acoustic stimulation near term: fetal heart rate and heart rate variability response. AM J OBSTET GYNECOL 1987;156:323.
8. Nijhuis JG, Martin CB Jr, Prechtl HFR. Behavioral states of the human fetus. In: Prechtl HFR, ed. Clinics in developmental medicine, No. 94. Continuity of neural function from prenatal to postnatal life. Philadelphia: JB Lippincott Co, 1984, chapter 5.
9. Martin CB Jr. Behavioral states in the human fetus. J Reprod Med 1981;26:425.
10. Gagnon R, Patrick J, Foreman J, et al. Stimulation of human fetuses with sound and vibration. AM J OBSTET GYNECOL 1986;155:848.
11. Brady J, James LS. Fetal electrocardiographic studies; tachycardia as a sign of fetal distress. AM J OBSTET GYNECOL 1987;86:785.
12. Hager WD, Pauly TH. Fetal tachycardia as an indicator of maternal and neonatal morbidity. Obstet Gynecol 1985;66:191.
13. Jongsma HW, Nijhuis JG. Classification of fetal and neonatal heart rate patterns in relation to behavioural states. Eur J Obstet Gynecol Reprod Biol 1986;21:293.
14. Divon MY, Platt LD, Cantrell CJ, et al. Evoked fetal startle response: a possible intrauterine neurological examination. AM J OBSTET GYNECOL 1985;153:454.
15. Westgren M, Almstrom H, Nyman M, et al. Maternal perception of sound-provoked fetal movements as a measure of fetal well-being. Br J Obstet Gynaecol 1987;94:523.

Effect of physician characteristics on the cesarean birth rate

Gertrud S. Berkowitz, PhD,[a] Gale S. Fiarman, MD,[b] Michael A. Mojica, MD,[b] Jay Bauman, MD,[c] and Roberta Haynes de Regt, MD[d]
New York and Elmhurst, New York

The rising cesarean birth rate has become of increasing concern to the obstetric profession and the public. There is a general consensus that the major obstetric indications responsible for the rising rate are dystocia, fetal distress, breech presentation, and previous cesarean delivery. However, the role of sociologic or nonmedical factors has not been established. This retrospective study examines the effect of the physician's age, experience, gender, and practice setting (solo versus group practice) on the cesarean birth rate. The analysis is based on 6327 private deliveries performed by 48 attending obstetrician/gynecologists at a New York City Hospital from January 1983 through December 1985. Although no significant differences were found according to the gender or practice setting of the physicians, older, more experienced physicians performed significantly fewer cesarean sections for dystocia and a higher percentage of forceps deliveries and breech extractions. These results suggest that physician characteristics may affect the type of delivery that is performed. (Am J Obstet Gynecol 1989;161:146-9.)

Key words: Cesarean birth rate, forceps deliveries, physican characteristics

The cesarean birth rate in the United States has increased from 5.5% in 1970 to 22.7% in 1985.[1] This dramatic rise in the incidence of cesarean sections has caused concern both in the obstetric profession and the general public.[2, 3] There is general agreement that the most important obstetric indications responsible for this increase are dystocia, breech presentation, fetal distress, and previous cesarean delivery.[1-3] The underlying reasons behind the changing incidence and indications cannot be readily documented, but several factors have been suggested, including the relative safety of cesarean births for the mother and fetus, improved prognosis of low birth weight infants, increased proportions of nulliparous and older mothers, higher proportions of deliveries performed by obstetricians as opposed to general practitioners, improved third-party payment, and fear of malpractice suits.[2]

Variations in rates by geographic region and type and size of hospital have also been noted. The highest cesarean delivery rates have been recorded in the Northeast, teaching hospitals, and hospitals of 500 beds or more.[4, 5] In addition, private patients have been found to be more likely to undergo cesarean sections than their service[6] or health maintenance organization[7] counterparts. Although these data do much to emphasize the role of nonmedical factors, little is known about the influence of physician characteristics on the cesarean birth rate. The purpose of this article is to examine how physician age, experience, gender, and practice setting affect the clinical decision to perform a cesarean section at one New York City hospital.

From the Departments of Obstetrics, Gynecology and Reproductive Science,[a,c] and Community Medicine,[a] The Mount Sinai School of Medicine,[b] and the Department of Obstetrics and Gynecology, City Hospital Center at Elmhurst.[d]
Received for publication June 15, 1988; revised January 1, 1989; accepted January 10, 1989.
Reprints not available.

Material and methods

A total of 10,461 deliveries were performed at the Mount Sinai Hospital in New York City between January 1983 and December 1985. Private patients represented 63% of the deliveries, and clinic patients accounted for 37% of the deliveries. The overall cesarean birth rate for this period was 21.8%, which is comparable with that of the Northeast United States.[5] The cesarean rate was higher for private patients (24.0%) than for clinic patients (18.2%).

To reduce the number of factors that might influence the cesarean birth rate, this study was limited to deliveries of private patients. Excluded from the 54 physicians who delivered private patients were four full-time attending physicians who managed only high-risk patients and two physicians who shared the same surname and who were not consistently identified in the delivery log book. The remaining 48 physicians constituted the study population and accounted for 6327 deliveries during the study period. The great majority of private patients were white, married, over the age of 25 years, had some college education, and used third-party insurance payment.

The data were obtained from a delivery log book. The information abstracted comprised the primary physician responsible for the delivery, the mode of delivery, and the stated indication for each cesarean sec-

Table I. Description of physician characteristics

Characteristic	*No.*	*%*	*Mean age ± SD*	*Mean yr of experience ± SD*
Male	37	77.1	49.4 ± 11.2*	18.5 ± 11.5*
Female	11	22.9	34.6 ± 4.3	3.3 ± 2.8
Solo	23	47.9	49.6 ± 12.7*	18.0 ± 13.8
Group	25	52.1	42.3 ± 9.7	12.2 ± 9.7
Total	48	100.0	46.0 ± 11.9	15.0 ± 12.1

*$p < 0.05$.

tion. Indications for cesarean sections were classified as dystocia, fetal distress, breech, repeat or "other." The term dystocia included both failure to progress and cephalopelvic disproportion. The category of "other" included mostly herpes, placenta previa, placental abruption, prematurity, and previous uterine surgery. When multiple indications were present, the model derived by Anderson and Lomas[8] was used to determine the primary indication. Those cesarean sections without clear indications (approximately 10%) were cross-referenced with discharge summaries available in the Mount Sinai Hospital Department of Medical Records.

Data on the physicians' characteristics included gender, date of birth, residency program, date of completion of residency program, and whether the physician was in a solo or group practice. Age was calculated in years as of July 1984, the midpoint of the study period. Experience was defined as the number of years from the completion of an Obstetrics and Gynecology residency program to the midpoint of the study period. The influence of place of residency was not assessed, since 70.8% of the physicians studied were trained at Mount Sinai Hospital and an additional 12.5% trained at other teaching institutions in New York City.

The cesarean birth rate was defined as the percentage of total deliveries that were performed by cesarean section. The primary cesarean birth rate was expressed as the percentage of total deliveries, which represented cesearean sections for an indication other than repeat sections. Operative vaginal delivery rate was defined as the percentage of all deliveries that used forceps or were breech extractions. Overall operative delivery rate was defined as the percentage of total deliveries that used forceps, were breech extractions, or required cesarean sections. Finally, the four major indications for cesarean section were analyzed as a percentage of total cesarean sections.

All rates were based on the 48 physicians as the units of analysis. Student t test and the Pearson product-moment correlation coefficient were used for statistical evaluation. Where appropriate, nonparametric analysis with Wilcoxon rank sum test and Spearman's rank correlation coefficient was also performed. The level of significance was selected as $p = 0.05$. Computations were performed by the CLINFO statistical package at the Mount Sinai Hospital Department of Biomathematical Sciences.

Table II. Distribution of deliveries according to delivery mode and cesarean indication

Delivery mode	*No.*	*%*
Vaginal		
NSVD	3265	67.6*
Low forceps	1404	29.1
Mid forceps	100	2.1
Breech	57	1.2
Cesarean		
Dystocia	417	27.8†
Repeat	373	24.8
Fetal distress	207	13.8
Breech	197	13.1
Other	307	20.5
Total vaginal	4826	76.3
Total cesarean	1501	23.7
Total deliveries	6327	100.0

NSVD, Normal spontaneous vaginal delivery.
*Percentage of vaginal deliveries.
†Percentage of cesarean deliveries.

Results

Table I describes the characteristics of the 48 study physicians. Thirty-seven (77.1%) of the physicians were men and 11 (22.9%) were women. The male physicians were significantly older and more experienced than the female physicians. About half (52.1%) of all physicians participated in group practices. Those in solo practice were significantly older and slightly more experienced than those in group practices.

The 6327 deliveries performed by the 48 physicians during 1983 to 1985 are summarized in Table II. The cesarean birth rate was 23.7% with dystocia (27.8%) as the most common indication, followed by repeat sections (24.8%), fetal distress (13.8%), and breech presentations (13.1%). The indications for the remaining 20.5% of the cesarean sections were classified as "other." The primary cesarean section rate was 17.8% and the operative vaginal delivery rate was 24.7%.

Physician age did not correlate significantly with the cesarean section rate ($r = 0.06$), the primary cesarean section rate ($r = 0.07$), or the percentage of cesarean sections performed for repeat ($r = 0.18$), breech ($r =$

Table III. Operative delivery rates according to physician's gender

Variable	*Male (N = 37) mean rate (%)*	*SD*	*Female (N = 11) mean rate (%)*	*SD*	*p Value*
Cesarean sections	23.7	8.8	21.6	9.5	0.49
Primary cesarean sections	18.0	7.2	17.9	7.4	0.96
Operative vaginal deliveries	26.5	13.7	22.7	10.0	0.71
Overall operative deliveries	50.2	13.8	44.3	11.1	0.20
Cesarean section by indication					
Dystocia	27.0*	17.2	35.0	14.8	0.26
Repeat	22.6	13.5	15.9	11.3	0.46
Breech	12.1	7.0	10.0	13.6	0.48
Fetal distress	14.0	10.8	18.5	15.1	0.66

*Percentage of cesarean deliveries.

Table IV. Operative delivery rates according to practice setting

Variable	*Solo (N = 23) mean rate (%)*	*SD*	*Group (N = 25) mean rate (%)*	*SD*	*p Value*
Cesarean sections	24.6	9.8	22.0	7.9	0.56
Primary cesarean sections	19.4	7.5	16.7	6.7	0.20
Operative vaginal deliveries	25.7	16.5	25.6	9.0	0.70
Overall operative deliveries	50.2	16.4	47.6	10.0	0.50
Cesarean section by indication					
Dystocia	27.7*	21.3	29.8	11.8	0.63
Repeat	18.1	13.2	23.8	12.9	0.27
Breech	8.8	7.6	14.2	9.2	0.44
Fetal distress	16.1	16.1	14.0	6.1	0.78

*Percentage of cesarean deliveries.

0.04), or fetal distress ($r = 0.06$). A significant negative correlation was found between age and the percentage of cesarean sections performed for dystocia ($r = -0.38$; $p = 0.008$).The operative vaginal delivery rate increased significantly with age ($r = 0.43$; $p = 0.002$). The overall operative delivery rate was also positively correlated with age ($r = 0.46$; $p = 0.001$). Similar results were obtained when assessing the effect of years of experience, since age and years of experience were highly correlated ($r = 0.90$; $p = 0.0001$). Reanalysis of these variables with Spearman's rank correlation test did not alter the results.

As shown in Table III, male physicians tended to have slightly higher operative delivery rates and a lower proportion of cesarean sections for dystocia than did female physicians, but none of the comparisons were statistically significant. With respect to practice setting (Table IV), there was some suggestion that physicians in solo practice had higher primary cesarean section rates than those in group practice. However, no significant associations were observed between overall operative delivery rates and solo versus group practice.

Comment

Although no overall associations could be found between specific physician characteristics and cesarean section rates, physician's age and experience were positively correlated with higher operative vaginal delivery rates and negatively related tc the percentage of cesarean sections for dystocia. Age and experience were highly correlated, and individual effects could not be separated.

Increasing age and experience are two physician characteristics that may simply represent differences in management based on techniques acquired in training at a time when forceps and breech extraction deliveries were more commonly performed. Since more cesarean sections are being performed today for breech presentations or as an alternative to a difficult vaginal delivery, residents in training or younger physicians have not been exposed to as many operative vaginal techniques as their senior counterparts.[3]

Other physician characteristics that were examined included gender and practice setting. Gender was not found to be statistically associated with any of the operative delivery rates, although male physicians were both older and more experienced. There was some suggestion that male physicians had a higher operative delivery rate and a lower rate of cesarean sections performed for dystocia. The relatively small sample size, particularly of female physicians, may account for the lack of significant findings. Further study is warranted in the future when more women will have completed training programs.

Similarly, no significant effect of practice environment could be delineated. Since solo practitioners

tended to have a higher primary cesarean section rate than group practitioners, it is possible that significant differences would be obtained with a larger sample size. Nevertheless, the absolute differences in operative rates for physicians in solo as opposed to group practices were small. Although solo practitioners are probably more subject to the pressures of time constraints and fatigue than those in group practice, there is no convincing evidence that the "convenience factor" plays an important role in cesarean birth rates. Evans et al.[9] and Fraser et al.[10] concluded that physician convenience may have influenced the timing of "nonacute" or dystocia cesarean sections, but Phillips et al.[11] found no difference in the distribution of nonelective cesarean section indications between weekends and weekdays. Carpenter et al.[12] found lower overall cesarean rates and lower dystocia cesarean rates if physicians shared night call with at least two other colleagues. However, payment differential between vaginal and cesarean delivery, average annual deliveries per practice, and in contrast to our study, number of years since training were not correlated with cesarean rates.

The effect of the medicolegal climate on individual physicians and their operative rates is difficult to assess. If frustration or fear of litigation can affect the decision of *whether* to practice,[13] then it can be postulated to affect the decision of *how* to practice. Nevertheless, in the study by Carpenter et al.[12] physicians with a recent threat of a malpractice suit had lower cesarean rates than those who had not recently experienced medical liability.

Other physician characteristics that might potentially influence operative rates that deserve study include personality, fund of knowledge, and previous poor perinatal outcomes. The effect of the patient's risk status on operative delivery rates also needs to be considered in future studies.

What can be done to lower operative rates without sacrificing perinatal morbidity? Wennberg[14] found that feedback from professional committees could reduce variation in operative rates caused by physician characteristics. Porreco[15] and O'Driscoll and Foley[16] have shown that institutional standardization of clinical policy can decrease the cesarean birth rate without compromising favorable perinatal mortality rates. Other recommendations to reduce the effects of physician characteristics on perinatal decision-making include mandatory second-opinion consultation before surgery, the institution of peer-review panels, and physician continuing education programs. Since this study examined physician characteristics and operative rates in a fairly homogeneous study population, other centers could evaluate physicians with more diverse training backgrounds and varied physician characteristics.

This investigation was undertaken as partial fulfillment of the clerkship requirement in Community Medicine while Gale S. Fiarman and Michael H. Mojica were third-year students at Mount Sinai School of Medicine.

REFERENCES

1. Taffel SM, Placek PJ, Liss T. Trends in the United States cesarean section rate and reasons for the 1980-85 rise. Am J Public Health 1987;77:955-9.
2. Bottoms SF, Rosen MG, Sokol RJ. The increase in the cesarean birth rate. N Engl J Med 1980;302:559-63.
3. Minkoff HL, Schwartz RH. The rising cesarean section rate: can it safely be reversed? Obstet Gynecol 1980; 56:135-43.
4. Placek PJ, Taffel S, Moien M. Cesarean section delivery rates: United States, 1981. Am J Public Health 1983; 73:861-2.
5. Dennison CF. 1984 Summary: National Hospital Discharge Survey. Hyattsville, Maryland: National Center for Health Statistics, 1985; DHHS publication no. (PHS) 85-1250. (Vital and health statistics; no. 112).
6. DeRegt RH, Minkoff HL, Feldman J, Schwarz RH. Relation of private or clinic care to the cesarean birth rate. N Engl J Med 1986;315:619-24.
7. Wright CH, Gardin TH, Wright CL. Obstetric care in a health maintenance organization and private fee-for-service practice: a comparative analysis. Am J Obstet Gynecol 1984;149:848-56.
8. Anderson GM, Lomas J. Determinants of the increasing cesarean birth rate. N Engl J Med 1984;311:887-92.
9. Evans MI, Richardson DA, Shell JS, Johnson BA. Cesarean section: assessment of the convenience factor. J Reprod Med 1984;29:670-6.
10. Fraser W, Usher RH, McLean FH, et al. Temporal variation in rates of cesarean section for dystocia: does "convenience" play a role? Am J Obstet Gynecol 1987; 156:300-4.
11. Phillips RN, Thornton J, Gleicher N. Physician bias in cesarean sections. JAMA 1982;248:1082-4.
12. Carpenter MW, Soule D, Yates WT, Meeker CI. Practice environment is associated with obstetric decision making regarding abnormal labor. Obstet Gynecol 1987;70:657-62.
13. Riffer J. Malpractice crisis threatens obstetric care. Hospitals 1986;60(Feb 5):60.
14. Wennberg JE. Dealing with medical practice variations: a proposal for action. Health Aff (Milwood) 1984;3:6-32.
15. Porreco CP. High cesarean section rate: a new perspective. Obstet Gynecol 1985;65:307-11.
16. O'Driscoll K, Foley M. Correlation of decrease in perinatal mortality and increase in cesarean section rates. Obstet Gynecol 1983;61:1-5.

Court-ordered cesarean section: An analysis of ethical concerns in compelling cases

Thomas E. Elkins, MD,[a] H. Frank Andersen, MD,[a] Mel Barclay, MD,[a] Tina Mason, MD,[a] Noelle Bowdler, MD,[a] and Garland Anderson, MD[b]

Ann Arbor, Michigan, and Memphis, Tennessee

Two previously unreported cases are presented in which court-ordered cesarean sections were considered appropriate by physicians. An analysis of the factors that compel physicians to deem court-ordered intervention appropriate is presented. When the significance of a third-trimester fetal death or a lifetime physical or mental disability is balanced against the demand to uphold maternal autonomy at all costs, the recognized ethical principles of beneficence, nonmaleficence, justice, obstetric contract keeping, and acting in the patients' best interests combine, in rare situations, to override concerns for individual maternal autonomy and justify court-ordered intervention. (Am J Obstet Gynecol 1989;161:150-4.)

Key words: Ethics, cesarean section, medical-legal, fetal death

The following case presentations represent previously unreported situations in which court-ordered cesarean sections were considered appropriate by physicians. They are actual accounts of patient care dilemmas in which court orders were considered or obtained. In these situations, multiple coercive/pleading arguments were presented to the patients, and lengthy deliberations were made before court orders were sought. An analysis of the factors that compel physicians to deem court-ordered intervention appropriate in these instances follows the case presentations.

Case reports

Case No. 1. In 1975 a 27-year-old, unmarried, white woman, gravida 1, para 0, was first seen at Planned Parenthood where she requested pregnancy termination. Because of her advanced gestation, approximately 30 to 34 weeks, she was advised that pregnancy termination was not possible. However, because she had a blood pressure of 180/110, she was immediately transferred to a tertiary care hospital. At the time of admission, the patient was noted to have similar blood pressures, along with other findings suggestive of severe preeclampsia. In the hospital she again asked that an abortion be performed. She was initially managed with bed rest; however, the following morning a decision was made to induce labor.

From the Department of Obstetrics and Gynecology, University of Michigan Medical Center,[a] and the Department of Obstetrics and Gynecology, University of Tennessee.[b]

Received for publication October 17, 1988; accepted January 11, 1989.

Reprint rqeuests: Thomas E. Elkins, MD, Department of Obstetrics and Gynecology, University of Michigan Medical Center, 1500 E. Medical Center Dr., D2234 MPB, Box 0718, Ann Arbor, MI 48109-0718.

Cervical dilatation was 2 to 3 cm. Her mentation and affect were appropriate. An intravenous infusion of magnesium sulfate was started. External fetal monitoring demonstrated spontaneous rhythmic uterine activity with late decelerations. Membranes were ruptured artificially and internal fetal monitors were placed. Internal monitoring confirmed loss of baseline variability and moderate-to-severe late decelerations. A scalp pH was performed and was 7.09. The patient agreed to these medical procedures without objections.

The patient was advised that there was fetal distress and that she should be delivered immediately by cesarean section. She refused the operation, stating that she did not want the baby. Despite repeated requests from house officers, staff, and nursing personnel, the patient continued to refuse surgical delivery. Oxytocin augmentation was begun in the hope that a vaginal delivery could be accomplished. During this time the fetal heart rate tracing continued to deteriorate, showing severe late decelerations, absent variability, and prolonged episodes of bradycardia. A sister of the patient, who was located by telephone, came to the hospital, and attempted to convince the patient that a surgical delivery was needed.

Two and one-half hours after the first scalp pH, a second scalp pH was reported was 6.71. Labor continued. Finally, a stillborn female infant, weighing 2140 gm, was delivered spontaneously.

After the patient's initial refusal of the cesarean delivery, the hospital attorney was contacted. The attorney spoke with three local judges, each of whom refused to hear the case.

Post partum the patient commented that she felt the staff had disapproved of her refusal of cesarean section for fetal indications. Although the hypertension and proteinuria had not resolved, she signed out of the hospital on the third postpartum day against medical advice.

Case 2. A 24-year-old, nonindigent, white woman, gravida 1, para 0, was admitted at 34 weeks' gestation dilated 2 to 3 cm with ruptured membranes and frequent contractions. The patient had left another hospital against medical advice because a cesarean section was recommended. She had hypertension (blood pressure 160/110), 3+ edema, and 3+ proteinuria. Electronic fetal monitoring showed absent variability, persistent late decelerations, and intermittent episodes of severe bradycardia. Heavy meconium was noted. The patient again refused all initial recommendations for cesarean section. She appeared almost noncommunicative, with a flat affect, and was unwilling to discuss her decision. Her mother was contacted and said that the patient had appeared very depressed and withdrawn for a couple of weeks and that the mother was also unable to communicate with her. The patient allowed fetal scalp pH determinations to be done. The first showed a pH of 7.10. A second scalp pH, performed an hour later, was 6.96. Even with these indications of fetal distress, the patient would not agree to cesarean section. After a total of 4 hours of pleading, a juvenile court judge was contacted directly by the attending obstetrician and a court order was obtained to proceed with cesarean section. The patient remained uncooperative.

The patient was forcefully restrained while general anesthesia was induced. A cesarean section was then performed, which resulted in the delivery of a 4-pound male infant with Apgar scores of 2 at 1 minute, 5 at 5 minutes, and 7 at 10 minutes. The operation was otherwise uncomplicated, as was the mother's postoperative course. The infant initially suffered from seizures but stabilized within 48 hours and did well thereafter.

Postpartum the patient requested custody of her infant and indicated that she understood in retrospect that surgery had been necessary. She has been followed closely by social service agencies and appears to be functioning appropriately in relation to her infant. At 1 year of age, the infant appears developmentally within the normal range.

Comment

A number of articles have recently appeared in opposition to obstetric intervention of any kind without maternal consent and especially to court-ordered cesarean section for fetal benefit when maternal consent is not obtained.[1-3] Several arguments have been presented to justify such opposition to court-mandated obstetric management. Individual patient situations have revealed errors in physicians' clinical assessments, resulting in the pursuit of court orders for intervention that appeared unnecessary in retrospect.[6, 7] Other authors have emphasized respect for the principles of bodily integrity, privacy, and autonomy that are valued as near absolutes in our society.[8] The right of competent adults to refuse medical therapy of any kind is also noted.[9]

Others have proposed that the fetus is the property of the mother who should have the sole right of decision-making in any obstetric question.[10] This is consistent with literature that states that any consideration of fetal rights is philosophically nonjustifiable.[11]

General agreement exists that extensive seeking of court opinions to force women to comply with nonemergent medical care is unwarranted. Many fear that opening the door to forced cesarean sections will encourage mandated behavior on the part of pregnant women in general and that such forced interventions could have a negative effect on maternal participation in prenatal and intrapartum care.[12]

The Committee on Bioethics of the American College of Obstetricians and Gynecologists has recently stated: The use of courts to resolve these conflicts is almost never warranted . . . obstetricians should refrain from performing procedures that are unwanted by a pregnant woman.[13] Even the strength of this statement in its opposition to forced obstetric intervention has been criticized by some, who object to the word "almost" in this sentence.

Although the authors concur in general with these concerns against court-ordered intervention and the opinion of the American College of Obstetricians and Gynecologists Bioethics Committee, it is their belief that there are rare circumstances in which court-ordered intervention on behalf of the third-trimester fetus is warranted. An analysis of the patient management situations in this article gives some rationale for the reluctance of many obstetricians to condemn court-ordered intervention in all situations.

Certain standard arguments in maternal-fetal conflicts have not been helpful to physicians, including relying on legal arguments and reasoning with false analogies. Many physicians prefer to rely on prior court decisions or federal or state legislation as guides to medical decision-making in an era of concern for malpractice liability. However, in the particular problem of maternal-fetal conflict, a reliance on the law as a guide to medical decision-making is not helpful. Case law and statutes that have established a doctrine of informed consent in our society would appear to support any consenting adult's right to refuse medical treatment.[14] However, multiple court decisions since *Roe v Wade*[15] have emphasized societal concern for the well-being of the viable third-trimester fetus. Case precedents show that the fetus has some rights of well-being in pregnancy for which damages might later be sought,[16] a right of inheritance that may legally be pursued after birth, and the legal status of personhood in some narrowly worded decisions.[17] In practical terms, physicians who seek to practice defensive medicine realize very quickly that they may be held liable for their decision to treat a patient against her will in the third trimester

or for a decision to agree to nontreatment that then leads to either death of a third-trimester fetus or birth of a severely handicapped newborn.[18] Therefore those who suggest a decision-making model that is protective for the physician in a legal sense offer no guidance in the area of maternal-fetal conflict.

Second, arguments for and against intervention have been based on equating the maternal-fetal relationship with other familial relationships. Some have argued that the maternal-fetal relationship is analogous to a parent-child relationship and that any maternal action that brings harm to the third-trimester fetus constitutes child abuse or neglect.[3] In contradistinction, others have tried to equate maternal-fetal concerns with those of siblings, one of whom needs a bone marrow transplant. Since society has not forced one sibling to undergo minor surgery for bone marrow or organ transplantation to help the other sibling, they reason that gestational mothers should not be compelled to aid the third-trimester fetus.[10] Without undue discussion, it may simply be stated that neither of these situations may be equated to the extreme interdependency of the mother and fetus in the third trimester when the life of both parties is dependent on the medical condition of the other. Therefore in extreme situations obstetricians may be faced with difficult decisions in which they must weigh numerous concerns involving both mother and fetus.

Although case No. 1 occurred before emergency court-ordered interventions had been reported, the similarities in these two cases allow a single analysis of the ethical priorities of the physicians involved in both cases.

(a) In both cases, there was no question that fetal distress existed and that fetal death or severe brain damage to the newborn would occur without surgical intervention. Therefore in this instance, erroneous clinical judgment, cannot be considered a factor that would speak against pursuit of court-ordered intervention.

(b) Oftentimes in cases of maternal-fetal conflict, there is reason to pursue court-ordered intervention for the physical well-being of both mother and fetus. This is true in cases of central placenta previa or gross fetal pelvic disproportion in which delivery of a healthy fetus by cesarean section is only one reason to pursue a cesarean section, since attempted vaginal delivery might also result in severe physical harm and even death to the mother. In the cases presented here, expedient delivery was indicated in each situation for severe preeclampsia. However, in both of these cases, cesarean section was sought primarily for fetal benefit because of the presence of fetal distress.

(c) In neither of the cases presented here was there a prolonged physician-patient relationship at the referral hospital where delivery occurred. Neither patient had attended prenatal care clinics in the hospital system, and consequently, neither patient had been able to discuss her concerns about medical intervention in pregnancy before admission for labor and delivery.

An analysis of the ethical concerns of the physicians who sought court-ordered intervention in each of these cases reveals four distinct areas of reasoning.

(1) There is concern for the innate value of fetal life in the third trimester that motivates forced intervention on rare occasions based on the principle of beneficence. There has been a general consensus explained most distinctly in the language of *Roe v Wade*, that society shares a protective concern for the viable third-trimester fetus.[15] Arguments based on theologic tenets and moral philosophy amplify at least this degree of concern for the fetus.[19] Regardless of the source of one's opinion of postviability fetal value, physicians have traditionally been reluctant to perform in a fashion that ignores the value of fetal life at term. Such a respect for fetal life was described in a policy statement published by the American College of Obstetricians and Gynecologists in 1977. A portion of that statement sought to explain the concept of fetal value, which is especially pertinent to discussions of the third-trimester fetus.

> Since the status of the fetus involves widely divergent theological and philosophical opinions in a pluralistic society, unanimity should not be expected. However, there appears to be a greater agreement on this matter than some physician statements suggest. This agreement is to be found in a general consensus in at least two main areas: 1) without going at length into the basis of the difference, the fetus has a qualitatively different nature and value from that of other human tissues or organs and 2) this value derives, at least in part, from its potential for developing into an obvious member of the human family. The value of the fetus is thus related to that placed on all human beings. It is therefore not possible fully to dissociate fetal life from human life so as to disvalue the one without posing some measure of threat, however subtle, to the value of the other.[20]

(2) The unique relationship of mother and fetus, especially in the third trimester, makes the physician-patient obligation a motivating factor in obstetric decision-making. The obstetrician has long recognized the presence of two pateints to whom the physician is obligated in the third trimester. Advances in perinatology have emphasized electronic, ultrasonographic, and biochemical fetal surveillance techniques that identify the viable fetus as a patient for whom the obstetrician cares.[21] Contemporary ethical theories of contractualism recognize that uniquely dual obligations inherent to the obstetric contract between physician and

patient exist once a woman allows her pregnancy to progress to a point of fetal viability or near-term gestation.[22] Even authors who oppose court-ordered intervention admit the unique maternal-fetal contractual approach to obstetric decision-making.[23] No other dual patient relationship approximates the total interdependence of mother and fetus at term gestation. Therefore those who propose that therapy always be directed toward the patient's (or patients' in this situation) best interests are compelled to take both mother and fetus into consideration during the third trimester.

(3) The physician must assess the reasonableness and rationality of patient demands. Ready acquiescence to the demands of a patient possibly suffering from an affective disorder, such as in case No. 2, would be problematic for any physician who viewed her request as being irrational. Patient requests for termination of viable fetal life (not just an end to pregnancy), as that reported in case 1, may be viewed as being unreasonable by physicians and many others in society.[24] As noted by Veatch,[25] a demand for reasonableness is essential to adequate medical decision-making in our society. In the face of requests that appear so opposed to societal norms, physicians may feel compelled to seek to overrule a patient's demands with court-ordered intervention. Failure to intervene, as in case 1, results in an unacceptable loss of fetal life at term and a profound dissatisfaction among the health care team with the care provided. In case 2, after delivery the mother requested to keep her newborn baby and indicated after the fact an understanding of the intervention needed for her baby to survive.

(4) The potentially permanent damage to a fellow person born disabled as a result of aberrant in utero decision-making is a heavy burden for physicians, society as a whole, the family, and especially the newborn potentially subjected to a lifetime of suffering. Physicians who favor a "burdens v benefit" ratio approach to these types of medical decision-making dilemmas are extremely concerned about nonintervention when such a result is possible. In such a direct medical management approach, or a burdens versus benefits approach, cesarean section would be selected when the risk of nonintervention is seen as excessively high (resulting in either fetal death or newborn disability) and the risk of maternal morbidity/mortality sufficiently low to justify surgical intervention.[26] The Committee on Bioethics of the American Academy of Pediatrics has recently published a statement on maternal-fetal conflict that supports this very premise.[27] In keeping with this model, some physicians feel compelled to intervene because of the history and tradition of principles emphasized by long-standing codes of medical ethics, such as the Hippocratic Oath, that encourage beneficence and nonmaleficence in physician actions.[28] The very real chance that lifelong physical or mental impairment of a newborn could develop from nonintervention creates concerns about both individual and distributive justice, as well as economic utility.

We oppose seeking court-ordered intervention except in the most unusual and atypical of situations. Because of a deep respect for maternal autonomy, such times should be exceedingly rare. For example, court intervention has been sought in less than one case per 10,000 deliveries at the two institutions involved in these cases. However, even in an era when autonomy, individual rights and privacy have achieved a high priority in contemporary ethical theory, the maternal-fetal conflicts discussed demand a balancing emphasis on the obligations found in the concepts of community, commitment, family life, and childbearing that others have shown to limit the absoluteness of autonomy.[29, 30] Only total denial of fetal value and sole concern for maternal autonomy will ever justify complete prohibition of court-approved medical intervention to assist a third-trimester fetus against maternal wishes in all situations.

The cases in this article represent unusual instances in which physicians felt compelled to seek court-ordered intervention for the many reasons mentioned. Such compelling situations are rare. Physician awareness of these dilemmas can frequently prevent such situations. However, even if prenatal discussions of such issues become routine for all patients, there will continue to be an occasional situation that must be managed individually, with methods that may include court-ordered intervention, to provide medically reasonable and ethically acceptable obstetric care. As shown by these cases, the rare instance occurs in which reasonable certainty of in utero death or newborn disability is present for a term fetus without surgical intervention, reasonable expectation is present for the surgery to result in the birth of a live newborn with a good prognosis, and maternal refusal of surgery all coexist. When the significance of a third-trimester fetal death or a lifetime of permanent physical or mental disability is balanced against the demand to uphold maternal autonomy at all costs, the recognized ethical principles of beneficence, non-maleficence, justice, obstetric contract keeping, and acting in the patients' best interests combine in rare situations to challenge and override concerns for individual maternal autonomy.

REFERENCES

1. Nelson LJ, Milliken N. Compelled medical treatment of pregnant women: life, liberty, and law in conflict. JAMA 1988;259:1060-6.
2. Kolder VE, Gallagher J, Parsons MT. Court-ordered obstetrical interventions. N Engl J Med 1987;316:1192-6.
3. Annas GJ. Forced cesareans: the most unkindest cut of all. Hastings Cent Repo 1982;12:16-17, 45.

4. Rhoden N. The judge in the delivery room: the emergence of court-ordered cesareans. Calif Law Review 1986;74:1951-2030.
5. Fletcher JC. Ethical considerations in an beyond experimental fetal therapy. Sem Perinat 1985;9:130-5.
6. Jefferson v Griffin Spalding County Hospital Authority, 274 SE 2d 457 (Ga 1981).
7. In re Baby Jeffries, No. 14004 (Jackson County P Ct Mich, May 24, 1982).
8. Jonsen AR, Siegler M, Winslade WJ. Clinical ethics. New York: MacMillan Publishing, 1986:47-51.
9. Bouvia v Superior Court, 179 Cal App 3d 1127 (Cal App 1986).
10. Andrews L. My body, my property. Hastings Cent Rep 1986;16(5):28-38.
11. Gallagher J. Prenatal invasions and interventions: what's wrong with fetal rights. Harvard Women's Law 1987;10:9-58.
12. Holder AR. Maternal fetal conflicts and the law. Female Patient 1985;10:80-90.
13. American College of Obstetricians and Gynecologists. Patient choice: maternal-fetal conflict. American College of Obstetricians and Gynecologists policy statement. Washington, DC: American College of Obstetricians and Gynecologists, 1987.
14. Dal Pozzo EE, Marsh FH. Psychosis and pregnancy: some new ethical and legal dilemmas for the physician. AM J OBSTET GYNECOL 1987;156:425-7.
15. Roe v Wade, 410 US 113 (1973).
16. Engelhardt HT. Current controversies in obstetrics: wrongful life and forced fetal surgical procedures. AM J OBSTET GYNECOL 1985;151:313.
17. Lieberman JR, Mazo M, Chaim W, et al. The fetal right to live. Obstet Gynecol 1979;53:515.
18. Holt v Nelson, 523 P 2d 211 (Wash Ct App 1974).
19. Congregation for the Doctrine of the Faith. Instruction on respect for human life in its origin and on the dignity of procreation. Vatican City: Vatican Polyglot Press, 1987.
20. American College of Obstetricians and Gynecologists. Further consideration in induced abortion: American College of Obstetricians and Gynecologists policy statement. Washington, DC: American College of Obstetricians and Gynecologists, 1977.
21. Lenow JL. The fetus as a patient: emerging rights as a person? Am J Law Med 1983;9:1-29.
22. Ruddick W, Wilcox W. Operating on the fetus. Hastings Cent Rep 1982;12:10-3.
23. Hallisey PL. The fetal patient and the unwilling mother: a standard for judicial intervention. Pacific Law J 1983; 14:1065-78.
24. Chervenak FA, Farley MA, Walters L, Hobbins JC, Mahoney MJ. When is termination of pregnancy during the third trimester morally justifiable? N Engl J Med 1984; 310:501-4.
25. Veatch R. Limits of guardian treatment refusal: a reasonable standard. Am J Law Med 1984;9:427-68.
26. Cervenak FA, McCullough LB. Perinatal ethics: a practical analysis of obligation to mother and fetus. Obstet Gynecol 1985;66:442-6.
27. American Academy of Pediatrics Committee on Bioethics. Fetal therapy: ethical considerations. Pediatrics 1988; 81:898-9.
28. Beauchamp TL, Childress JF. Principles of biomedical ethics. New York: Oxford University Press, 1979:97.
29. Callahan D. Autonomy: a moral good, not a moral obsession. Hastings Cent Rep 1984;14:40-2.
30. Hauerwas S. Truthfulness and tragedy. Part two: survival, community, and the demand for truthfulness. London: University of Notre Dame Press, 1977;101-46.

Removing a retained placenta by oxytocin—A controlled study

Charlotte Wilken-Jensen, MD, Verner Strøm, MD, Meta Damkjær Nielsen, MSc, and Bo Rosenkilde-Gram, MD

Gentofte, Denmark

No increase in maternal plasma oxytocin concentration was detected after administration of 100 IU oxytocin into the umbilical veins of seven women immediately after delivery. The delivery of the placenta was accelerated after umbilical vein injection of 100 IU oxytocin in a placebo-controlled study of 40 women: 12 minutes (4 to 40) in the oxytocin group versus 40 minutes (29 to 40) in the placebo group (median and interquartile ranges), $p < 0.05$. (AM J OBSTET GYNECOL 1989;161:155-6.)

Key words: Oxytocin, retained placenta, umbilical vein administration

In 1983 Golan et al.[1] published a new method for removal of a retained placenta by injecting 100 IU of oxytocin into the vein of the umbilical cord. The work of Golan et al. was an open study and the originally published dose of 100 IU of oxytocin was later corrected to 10 IU. This dose was ineffective in a later published study.[2] We have studied oxytocin concentration in the maternal circulation after a bolus injection of 100 IU of oxytocin into the vein of the umbilical cord and performed a prospective, double-blind, randomized trial that was based on that dose.

Material and methods

Design and participants. To study the passage of oxytocin across the placenta, 10 ml of oxytocin (Syntocinon, 10 IU/ml, Sandoz, Basel, Switzerland) diluted in 20 ml of 0.9% sodium chloride was injected into the umbilical vein immediately after delivery in seven patients. Blood samples from the laboring women were collected before the injection and without intervals consecutively until delivery of the placenta.

Oxytocin and placebo ampules were supplied by Sandoz A/S, Denmark.

In the second part of the study 40 consecutive laboring women with a retained placenta were randomized to injection of either 10 ml oxytocin (10 IU/ml) mixed with 20 ml of 0.9% sodium chloride or 30 ml of sodium chloride into the vein of the umbilical cord. The placenta was regarded as retained if it was not expelled within 20 minutes after delivery of the infant, applying intermittent traction on the umbilical cord and light suprapubic pressure. All deliveries were vaginal, either spontaneously or by vacuum extraction. Methylergotamine, 0.2 mg, was given intramuscularly at delivery of the first shoulder.

The two groups were comparable with regard to age, gestational age, parity, former retained placenta, oxytocin augmentation in labor, duration of labor, number of vacuum extractions, sex, and weight of the infant ($p > 0.05$).

Blood pressure, pulse rate, and blood loss were noted, both before and after the injection. Heavy bleeding that demanded immediate removal of the placenta was the only criterion for exclusion. If the placenta was not delivered within 40 minutes after the injection, it was removed manually with the patient under general anesthesia.

Informed consent was obtained from all patients. The study was approved by the local ethics committee. In the first part of the study oxytocin in the maternal blood was measured by radioimmunoassay after acetone extraction and intensive purification.

Statistics. Wilcoxon matched-pairs rank-sum test was used to evaluate the open part of the study. The Mann-Whitney two-sample rank-sum test was used to evaluate the controlled portion of the study. Differences resulting in $p < 0.05$ were considered significant.

Results

Oxytocin concentration in maternal blood did not increase significantly at any time from the time of intraumbilical injection of the 100 IU of oxytocin to the

From the Department of Gynecology and Obstetrics and Department of Clinical Physiology, Glostrup University Hospital. *Received for publication July 11, 1988; revised January 11, 1989; accepted February 23, 1989.*

Supported by a grant from the Danish Hospital Foundation for Medical Research, Region of Copenhagen, The Faroe Islands, and Greenland.

Received for publication July 11, 1988; revised January 11, 1989; accepted February 23, 1989.

Reprint requests: Bo Rosenkilde-Gram, MD, Smakkegaardsvej 24, DK-2820 Gentofte, Denmark.

delivery of the placenta in any of the seven patients in the open portion of the study. The median concentration after injection was 11 (range, 7 to 19) μU/ml. The median time passed from injection to delivery of the placenta was 5 minutes (range, 1 to 7).

In the controlled portion of the study, 19 women received the placebo injection and 18 women received oxytocin. The time from injection until the placenta was delivered was significantly shorter in the oxytocin-treated group than in the control group: 12 minutes (range, 4 to 40) versus 40 minutes (range, 29 to 40) (median and interquartile range, $p < 0.05$). Five patients in the oxytocin group and 11 in the control group had the placenta removed manually. In the oxytocin group hemorrhage was 330 ml compared with 370 ml in the control group; this difference was not significant. No differences in blood pressure or pulse rate were observed.

Comment

Because of concerns of side effects after 100 IU of oxytocin was injected into the umbilical vein, we measured the maternal concentration of oxytocin in seven patients and found no significant increase. To our knowledge no other studies are available for comparison. Previous studies of the effect of intraumbilical oxytocin on a retained placenta have not been double-blinded, the dose of oxytocin has only been 10 IU, or the injection has been given only 5 minutes after delivery.

Our study appears to demonstrate that a 100 IU injection of oxytocin into the umbilical cord for the delivery of a retained placenta is safe and has a high success rate.

REFERENCES

1. Golan A, (Baruch) Lidor AL, Wexler S, David MP. A new method for the management of the retained placenta. AM J OBSTET GYNECOL 1983;146:708-9.
2. Kristiansen FV, Frost L, Kaspersen P, Møller BR. The effect of oxytocin injection into the umbilical vein for the management of retained placenta. AM J OBSTET GYNECOL 1987;156:979-80.

Make researching easier with the Ten-Year Cumulative Index (1978-1987) to the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY

The Ten-Year Cumulative Index is a complete guide to more than 30,000 pages of original articles, case reports, letters, and editorials published in the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY from 1978 through 1987 (volumes 130 to 157). Numbering more than 800 pages, the Cumulative Index includes both a subject and an author index. This hard-cover volume is $64.50 ($69.00 international). Prices include shipping. *Payment must accompany all orders and must be in U.S. funds, drawn on a U.S. bank.*

Contact The C.V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146 USA; phone (314) 872-8370, ext. 394. In the U.S., call toll-free: 1-800-325-4177, ext. 394. Please allow six weeks for delivery.

The use of transvaginal ultrasonography in the diagnosis of ectopic pregnancy

Ilan E. Timor-Tritsch, MD, Ming N. Yeh, MD, David B. Peisner, MD, Karen B. Lesser, MD, and Theresa A. Slavik, BA, RDMS

New York, New York

Despite advances in diagnosis made by the introduction of serum β-subunit of human chorionic gonadotropin determinations and transabdominal ultrasonography, ectopic gestations still present a major diagnostic challenge. The increased resolution of the transvaginally introduced high-frequency ultrasound transducer probes seems to solve this diagnostic problem. In this study 145 patients were referred for ultrasonographic workup because of a suspected ectopic gestation. In 38 patients a diagnosis could be made with classical transabdominal scanning. One hundred seventeen patients required additional transvaginal scanning with a 5.0 and a 6.5 MHz probe. In 98 patients a diagnosis was made during the first transvaginal scan; nine patients were rescanned within 3 days for the final diagnosis. In 56 patients, ectopic pregnancy was successfully ruled out by transvaginal scanning. Thirty-nine ectopic pregnancies were diagnosed. Only one false-positive identification was made. The sensitivity of diagnosing ectopic pregnancy by high-frequency transvaginal sonography was 100%; the specificity was 98.2%. The positive predictive value of this method was 98%, and the negative predictive value was 100%. The rate of the beating fetal heart was seen in the tube (23%). The high number of unruptured tubal pregnancies in this series (66%) suggests the possibility of an early diagnosis that may have therapeutic implications. The use of higher-frequency transvaginal transducer probes improves the diagnosis of the ectopic gestation. (Am J Obstet Gynecol 1989;161:157-61.)

Key words: Pregnancy, ectopic pregnancy, ultrasonographic studies, transvaginal ultrasonography, endovaginal ultrasonography

Ectopic pregnancy remains a major clinical diagnostic challenge. The combined use of transabdominal sonography and serum β-sub unit of human chorionic gonadotropin (β-hCG) determinations has resulted in a relatively high accuracy (70% to 95%) in predicting ectopic pregnancy.[1-6] The gestational age at which this "accuracy" of diagnosis is reached and the ratio of ruptured versus unruptured fallopian tubes is seldom reported. In 1980 Levi and Lebliq[7] reported a false-positive rate of 3% to 30% (mean 16%) and a false-negative rate of 2% to 35% (mean 5%) in making the correct diagnosis of ectopic gestation with transabdominal sonography. Romero et al.[4] used the "discriminatory β-hCG" level to predict the presence or the absence of ectopic pregnancy. By using the definition of the "discriminatory β-hCG" level, the absence of a normal intrauterine gestational sac with a titer of 6500 mIU/ml had a sensitivity of 100% and a specificity of 96%. The positive and negative predictive values in establishing the diagnosis of ectopic pregnancy were 86% and 100%, respectively. However, in most reports the specificity and the sensitivity for making the correct diagnosis of ectopic pregnancy by transabdominal sonography were 97% and 77% to 88%, respectively.[1-6]

The introduction of transvaginal transducer probes, particularly those with scanning frequencies as high as 7.0 MHz, presents a new opportunity to review pelvic organ morphologic features.[8,9] The high resolution of the picture enables the sonographer to focus on the fallopian tube. Attention has been directed to the early and reliable imaging of tubal gestations.[10,11]

The purpose of this study was to determine the effectiveness of transvaginal sonography in the management of ectopic pregnancy to make an early diagnosis. Both are a result of the technique of producing high-resolution pictures of the pelvic organs involved.

Material and methods

One hundred forty-five patients were referred for ultrasonographic examination over 1-year period (beginning in March 1987) because of suspected ectopic pregnancy. In 38 patients an unequivocal diagnosis was reached with transabdominal sonography. In 117 cases in which a definite ultrasonographic diagnosis could not be reached by transabdominal sonography, im-

From the Department of Obstetrics and Gynecology, Columbia-Presbyterian Medical Center.

Received for publication April 13, 1988; revised August 17, 1988; accepted January 10, 1989.

Reprint requests: Ilan E. Timor-Tritsch, MD, Director of Ultrasound, Department of Obstetrics/Gynecology, Columbia-Presbyterian Medical Center, 630 West 168th St., New York, NY 10032.

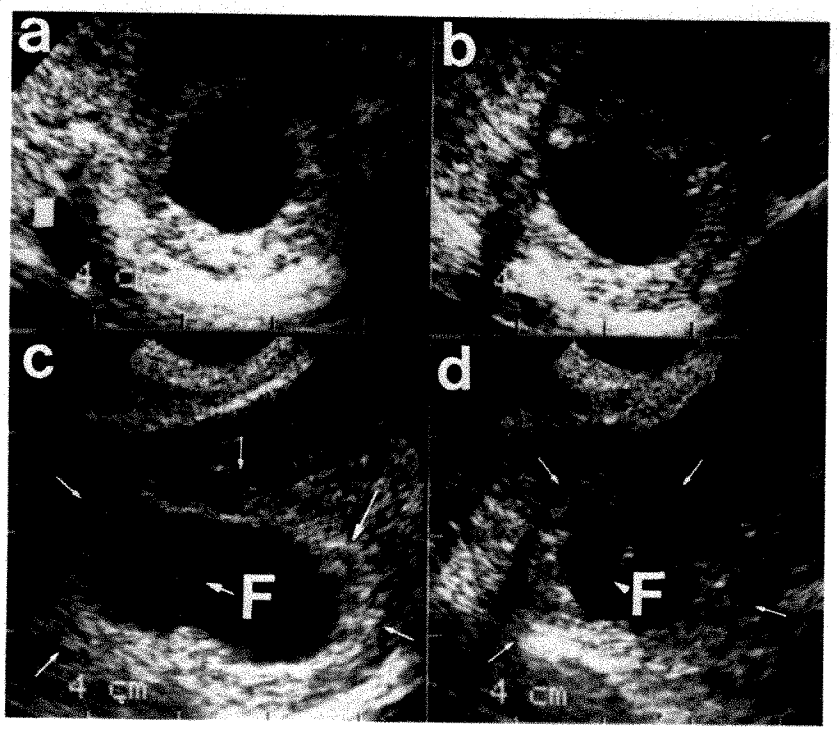


Fig. 1. Serial cross sections (**a, b,** and **d**) and a longitudinal section (**c**) of a thick-walled tube, containing fetal echoes *(F)*. The echogenic "tubal ring" is outstanding in the adnexa. This was a 7-week-old, unruptured tubal gestation with no fetal heartbeats at the time of the scanning.

mediate additional scanning was performed via the transvaginal route. A serum β-hCG test was performed in all patients. In most cases the test results were known by the sonographers. The transabdominal sonography and transvaginal sonography were performed by different persons.

The equipment used in the study included a General Electric 3000 scanner to which a 5.0 MHz 90-degree fixed-angle phased-array vaginal probe was attached; an Elscint ESI-1000 scanner with a variable angle, 6.5 MHz mechanical sector scanner transvaginal probe was used. After coupling gel was applied to the tip of the probe, it was inserted into one of the digits of a surgical rubber glove. The detailed scanning technique has been described in earlier reports.[10]

A tubal gestation was diagnosed if the uterus did not show signs of a normal or abnormal pregnancy and one or more of the following structures were observed: (1) a gestational sac or a yolk sac, or both, or fetal heartbeats within a thickened "tubal ring" (Fig. 1); (2) free pelvic fluid containing particulate matter (i.e., blood) with or without a coexisting thickened tube or parts of it (sometimes discrete blood clots could be seen and diagnosed). A positive result of the qualitative or quantitative serum β-hCG pregnancy test indicated that a pregnancy was present. In a minority of cases, the result of the pregnancy test was still pending at the time of the scan.

The outcomes of surgical procedures such as laparoscopy, laparotomy, curettage, culdocentesis, or clinical follow-up were recorded.

Results

In 38 of the 145 patients referred for ultrasonographic studies, the conclusive diagnosis of intrauterine pregnancy (normal or abnormal) or ectopic pregnancy could be made by transabdominal scanning. This group is referred to as the *transabdominal sonography group*. In 37 patients the diagnosis was made on the basis of the detection or absence of fetal heartbeat, indicating a normal or abnormal intrauterine gestation. In one additional patient, a tubal gestation was diagnosed when the fetal heartbeat was clearly visible outside the uterine cavity. Therefore, these 38 patients did not require additional transvaginal imaging. In the remaining 117 patients, the transabdominal sonography showed indirect ultrasonographic signs of an "empty uterus," "adnexal masses," or "free fluid" but was not conclusive enough to warrant an unequivocally positive or negative diagnosis of ectopic pregnancy. These patients had immediate transvaginal sonography. This group is referred to as the *transvaginal sonography group*. Two subgroups, categorized by the results, were created and are defined below.

Subgroup 1. In 98 of the 117 vaginally scanned patients a final diagnosis could be reached at the time of

Table I. Results of scanning 114 patients for suspected ectopic pregnancy

	Transabdominal sonography	*Transvaginal sonography*		*Total*
		Subgroup 1	*Subgroup 2*	
No ectopic pregnancy	37			37
Normal intrauterine pregnancy		28	3	31
Abnormal intrauterine pregnancy		10	2	12
Not pregnant		11	2	13
Total	37	49	7	93
Ectopic pregnancy	1			1
Unruptured tubal ectopic pregnancy		27	2	29
Ruptured tubal ectopic pregnancy/tubal abortion		16	0	16
Ovarian ectopic pregnancy		1	0	1
"Chronic" ectopic pregnancy		4	0	4
False-positive ectopic pregnancy		1	0	1
Subtotal	1	49	2	52
TOTAL	38	98	9	145

the first scan. No ectopic pregnancy was found in 49 of these 98 patients. A live intrauterine pregnancy was visible in 28 patients, and an abnormal gestation (no heartbeats) was found in 10. In the remaining 11 patients other pelvic pathologic conditions were found. In these patients the pending serum β-hCG level was obtained after the completion of the vaginal scan, excluding the presence of any pregnancy. The reasons for not excluding these patients from the study are discussed in the Comment section. All but one of the normal intrauterine gestations had a menstrual age of ≤7 weeks; one patient had a 9½-week intrauterine pregnancy but her extreme obesity prevented transabdominal sonography from making this diagnosis.

Ectopic pregnancy was diagnosed in 49 of the 98 patients. Four patients with distinct "tubal rings," a minimal amount of free pelvic fluid, and no visible fetal heartbeats were followed up until serially determined serum β-hCG dropped to nonpregnant levels and transvaginal sonography failed to image the previously present tubal pathologic condition. Forty-five of these 49 patients underwent laparotomy. In 43, tubal ectopic pregnancy was found. One patient had a ruptured ovarian pregnancy. In one patient no ectopic pregnancy was found. This last patient had a previous ectopic pregnancy and was first seen with an extremely tender adnexal mass. She had a positive urine pregnancy test in the emergency room. The transabdominal sonography showed an adnexal mass. A "tubal ring" was diagnosed by transvaginal sonography. Some blood was also seen. Because of the clinical signs of an acute lower abdominal disease, she was taken to the operating room before the serum β-hCG result was obtained. The next day the serum β-hCG titer was reported to be below pregnancy levels. Further review of the films revealed that the corpus luteum was misinterpreted for a "tubal ring."

Subgroup 2. Nine of the 117 vaginally scanned patients were rescanned within 3 days because the initial scan was not conclusive. No ultrasonographic or clinical signs of impending danger existed in these patients; therefore a subsequent scan was planned within 3 days. After rescanning the following results were found: tubal gestation without heartbeats (confirmed at surgery) in two patients, a very early normal intrauterine pregnancy in three patients, and an abnormal gestation in two patients. Two additional patients in this group had a corpus luteum cyst with no pregnancy. Although this group consisted of only nine patients, it seemed to pose the most problems for the ultrasonographers. The scanning time was almost double the approximate mean scanning time (23 minutes) of the previous groups. Because neither ectopic pregnancy nor intrauterine pregnancy could be visualized, the report relied heavily on the result of the quantitative serum β-hCG test, which often was unavailable at the time of the scan. Thirty-three of the 50 true ectopic pregnancies diagnosed by transvaginal sonography were unruptured (66%). In 17 patients a ruptured tube or a tubal abortion was found (34%). In 11 of the 50 ectopic pregnancies that were correctly diagnosed in this series, fetal heartbeats were detected (22%). In 18 cases, various amounts of blood were aspirated from the pelvis; in two patients the amount of blood exceeded 200 ml. The results of scanning are presented in Table I.

The statistical analysis regarding the diagnostic precision of the 117 vaginally scanned patients had the following results: No false-negative but one false-positive identification was made. Therefore the diagnostic sensitivity was 100%, whereas the specificity was 98.2%. The positive and negative predictive values were 98% and 100%, respectively. When all 145 patients in the group referred for ectopic pregnancy workup were analyzed, the specificity of the combined

transabdominal sonography and transvaginal sonography diagnostic procedures rose to 98.9%, and their positive predictive value increased to 98.1%.

Comment

Because of their physical properties, the 5.0 and the 6.5 MHz transducer probes produce images with higher resolution. The "unobstructed view" of the pelvic organs as a result of the vaginally introduced probe becomes obvious to the ultrasonographer early in the experience with this technique. The increased accuracy of transvaginal sonography in the diagnosis of ectopic pregnancy is the result of a combination of two components: first, its effective use in the diagnosis of the early intrauterine pregnancy, and second, its capability for imaging the abnormal (and sometimes the normal) fallopian tube. When transvaginal sonography is compared with transabdominal sonography, the normal intrauterine pregnancy, early embryonic structures, and the abnormal pregnancy may be detected about 1 week earlier with transvaginal sonography.[12, 13] A normal 4½-week intrauterine pregnancy is consistently detected by transvaginal sonography.[12]

One effective way to rule out ectopic pregnancy is by diagnosing an intrauterine pregnancy. Every ultrasonography laboratory should be aware of its own "discriminatory β-hCG zone" at which a certain transducer probe detects the earliest normal intrauterine pregnancy. This term, introduced by Kadar et al.[14] is now widely used but has to be determined in each ultrasonography laboratory for each of the different transducers.

Discriminatory β-hCG zones for vaginal probes are now being reported by authors.[15] As mentioned before, these zones should be determined for each vaginal probe and for each laboratory.

Despite the fact that the "discriminatory zone" for our probes was determined, we elected not to use them because occasionally only the qualitative β-hCG result was available. The only criterion regarding this laboratory test was whether it was indicative of a pregnancy.

The second component of the diagnostic algorithm is the direct examination of the fallopian tube.[9] The normal tube may be seen by transvaginal sonography if fluid surrounds it. If fluid is detected in the pouch of Douglas, every effort should be made to image the tube. When the tubal lumen is filled with fluid, a gestational sac, or a blood clot, imaging is easier. The "adnexal mass" detected with transabdominal sonography may be better evaluated with the higher resolution vaginal probe, and the diagnosis of "adnexal ring" can now be replaced with the more specific term "tubal ring."

The fact that fetal heartbeats were seen in 22% of the cases in this series, compared with the 5% to 10% detection by transabdominal sonography,[3] supports the assumption that transvaginal sonography provides the opportunity for detecting ectopic pregnancy at an earlier stage when tubal abortion or even tubal rupture has not yet occurred. The ratio of unruptured to ruptured tubal gestations and tubal abortions in this series (66% to 34%) at the time of the diagnosis adds additional support to the fact that the correct diagnosis is made at a relatively early stage when the gestational sac is still intact. The review of the literature supports the fact that with the improvement of the diagnosis over time, the percentage of intact tubal gestations at the time of diagnosis increases.[16]

Even the smallest amounts of free pelvic fluid may easily and consistently be detected by transvaginal sonography.[17] All ruptured ectopic gestations had some amount of free pelvic fluid at the time of the vaginal scan. Only two of the patients had >200 ml of blood in the abdominal cavity. However, in our cases in which the tubes were found to be intact, there were variable but small amounts of free fluid in the pelvis. Easy evaluation of the presence or absence of fluid in the cul-de-sac in cases of suspected ectopic pregnancy has important and practical clinical implications. The correct diagnosis in these cases may require clinical experience. However, even a relatively inexperienced operator during a busy night call in the emergency ward can make important decisions based on very simple but reliable findings, such as the presence or absence of life-threatening bleeding originating from a ruptured ectopic pregnancy. The exclusion of such a clinical situation may save the patient (and the physician) from unnecessary surgery. The final diagnosis may be left to a subsequent scan by a more experienced ultrasonographer.

We mention the various transvaginal images that may cause differential diagnostic problems for the ultrasonographer. Four structures should be carefully examined and correctly recognized:

1. The corpus luteum, which is eccentrically located within the ovary, surrounded by ovarian tissue, may create the impression of a "tubal ring."
2. A graafian follicle of the ovary has a wall that is usually thinner than the corpus luteum and the "tubal ring."
3. Small bowel—Difficulty in differentiating this from a tubal structure arises only if a static film is read. During real-time scanning, the peristalsis of the bowel is obvious.
4. Tubal pathologic conditions, such as a hydrosalpinx, which tends to be larger in size, is pear-shaped, and contains a homogeneous (mostly sonolucent) fluid.

Generally, the diagnosis of a tubal gestation should be made after a meticulous examination, with different and multiple scanning planes that prove the reproducibility of the structure. The diagnosis must be made during real-time scanning.

In our review of the false-positive case, an alternate

method for awaiting the final hormonal assay, such as laparoscopy preceding the planned laparotomy, would have avoided the surgical procedure.

The 13 cases referred to us to rule out ectopic pregnancy that subsequently had nonpregnant levels of β-hCG merit explanation. These cases were intentionally included in the study because they were part of the "rule out ectopic pregnancy" population with which every ultrasonography laboratory deals daily. It would be ideal to scan only those patients with an available β-hCG report. Unfortunately, the practice of emergency medicine does not always allow this. This was also true for this group of 13 patients. At the time of referral, results of their hormonal tests were pending. The patients were scanned before the results were known; a variety of pelvic pathologic conditions other than ectopic pregnancy were detected. Among the findings were corpus luteum cysts, simple cysts, and a hydrosalpinx.

In four cases, the diagnosis of a tubal pregnancy was not proved by surgical means. Therefore it is difficult to accept the validity of a diagnosis of ectopic pregnancy without pathologic confirmation. However, the reliability of the positive findings obtained by transvaginal sonography, such as the empty uterus, a "tubal ring" with an embryonic pole, absence of pelvic bleeding in the presence of falling levels of β-hCG in a patient who never passed blood, or tissue through the cervix, complies with the diagnosis of "missed ectopic pregnancy" even by the strictest standards. The clearly visualized "tubal ring" regressed in all three cases within 10 to 15 days, along with the falling hormone levels. More experience with conservatively managed cases may lead to acceptable clinical management of certain small-size tubal gestations with absent fetal heartbeats and a declining titer of β-hCG. Subsequent tubal patency in these cases should be further investigated.

We emphasize the importance of first documenting the presence of a pregnancy with qualitative β-hCG determinations before transvaginal sonography is performed. Furthermore, it is important to obtain serial quantitative serum β-hCG levels in patients with suspected ectopic pregnancies, (or early intrauterine gestations) who have a stable clinical condition.

We believe the transvaginal route, preferably with a higher-frequency probe, is the test of choice in the reliable and early ultrasonographic workup of the patient with suspected ectopic pregnancy.

Addendum

At the time of this publication the total number of patients scanned vaginally was 202. Eighty-three true ectopic gestations were found. One false-positive case and one false-negative case were also seen. The specificity and sensitivity of the test did not change significantly (99.1% and 98.8%, respectively). The positive and negative predictive values of the test were 98.8% and 99.1%, respectively.

REFERENCES

1. Piironen O, Punnonen R. The use of ultrasonography in the diagnosis of ectopic pregnancy. Clin Radiol 1981; 32:331.
2. Gleicher N, Giglia RV, Deppe R, Elrad H, Friberg J. Direct diagnosis of unruptured ectopic pregnancy by realtime ultrasonography. Obstet Gynecol 1983;61:425.
3. Robinson HP, de Crespigny LCH. Ectopic pregnancy. Clin Obstet Gynecol 1983;10:407.
4. Romero R, Kadar N, Jeanty P. Diagnosis of ectopic pregnancy: value of the discriminatory human chorionic gonadotropin zone. Obstet Gynecol 1985;66:357.
5. Robinson HP, de Crespigny LCH, Harvey J, Hay DL. Ectopic pregnancy—potentials for diagnosis using ultrasound and urine serum pregnancy tests. Aust NZ J Obstet Gynaecol 1985;25:49.
6. Mahoney BS, Filly RA, Nyberg DA, Callen PW. Sonographic evaluation of ectopic pregnancy. J Ultrasound Med 1985;4:221.
7. Levi S, Lebliq P. The diagnostic value of ultrasonography in 342 cases of suspected ectopic pregnancy. Acta Obstet Gynecol Scand 1980;59:29.
8. Timor-Tritsch IE, Rottem S, eds. Transvaginal sonography. New York: Elsevier Publishing, 1987.
9. Timor-Tritsch IE, Bar-Yam Y, Elgali S, Rottem S. The technique of transvaginal sonography with the use of a 6.5 MHz probe. AM J OBSTET GYNECOL 1988;158:1019-24.
10. Timor-Tritsch IE, Rottem S. Transvaginal sonographic study of the Fallopian tube. Obstet Gynecol 1987;70: 424.
11. Rottem S, Timor-Tritsch IE. Think ectopic. In: Timor-Tritsch IE, Rottem S, eds. Transvaginal sonography. New York: Elsevier Publishing, 1987:125-41.
12. Blumenfeld Z, Rottem S, Elgali S, Timor-Tritsch IE. Transvaginal sonographic assessment of early embryonic development. In: Timor-Tritsch IE, Rottem S, eds. Transvaginal sonography. New York: Elsevier Publishing, 1987: 87-108.
13. Timor-Tritsch IE, Rottem S, Blumenfeld Z. Pathology of the early intrauterine pregnancy. In: Timor-Tritsch IE, Rottem S, eds. Transvaginal sonography. New York: Elsevier Publishing, 1987:109-23.
15. Goldstein SR, Snyder JR, Watson C, Danon M. Very early pregnancy detection with endovaginal ultrasound. Obstet Gynecol 1988;72:200.
16. Caspi E, Sherman D, Tubal abortion and infundibular ectopic pregnancy. Clin Obstet Gynecol 1987;20:155.
17. Timor-Tritsch IE, Rottem S, Thaler I. Review of transvaginal ultrasonography: a description with clinical application. Ultrasound 1988;6(1):1.

Ileocecal vaginal construction

Rainer A. Bürger, MD,[a] Hubertus Riedmiller, MD,[1] Paul G. Knapstein, MD,[b] Volker Friedberg, MD,[b] and Rudolf Hohenfellner, MD[a]

Mainz, West Germany

In 10 patients, six with previously failed vaginal reconstruction of Mayer-Rokitansky syndrome, two with male pseudohermaphroditism, one with a history of Wertheim-Meigs operation followed by irradiation of the true pelvis, and one with vaginectomy caused by trauma, a neovagina was created out of cecal or ileocecal segments. Advantages of this technique are the excellent blood supply to the ileocecal region and the long vascular pedicle. The supplemental application of terminal ileum for prolongation of the cecal vaginoplasty allows for tensionless anastomosis between the neovagina and the vulva, even in patients with difficult anatomic conditions. A detailed description of the operative technique and indications for ileocecal and cecal vaginoplasty are presented, and the techniques in current use for vaginal replacement are reviewed and discussed. (Am J Obstet Gynecol 1989;161:162-7.)

Key words: Vaginal aplasia, reconstruction, ileocecal segments

Vaginal reconstruction may become necessary in cases of congenital vaginal aplasia and functional or traumatic vaginal loss. Of the variety of operative techniques currently available, only skin transplants[1-3] and sigmoid vaginoplasty[4, 5] have found widespread application. Until now, the use of cecum vaginal replacement has been described by Markland and Hastings[6]; in the current literature there is no mention of the use of ileocecal segments.

Two factors prompted the use of cecal and ileocecal segments for vaginal replacement: the extremely low morbidity rate observed in more than 120 patients in whom urinary bladder replacement was done with cecum, ascending colon, and terminal ileum[7] and the close topographic proximity of the cecum and terminal ileum to the vagina and thus the prospect of good acceptance of the material. The following report presents our technique of vaginal reconstruction, clinical experience in a small number of patients, and a comparison of this method with other techniques of vaginal reconstruction.

Material and methods

We have used cecum and ileocecal segments since April 1986 to create a neovagina in 10 patients, ages 16 to 34 years. Six of these patients had vaginal aplasia (Mayer-Rokitansky syndrome), with fibrotic shrinkage and total obliteration of the reconstructed vaginal tube, which occurred after creation of a peritoneal neovagina or after vaginal reconstruction with a free skin graft.

Hysterectomy and vaginectomy were performed in the seventh patient after a polytrauma with multiple pelvic injuries (rupture of the vagina, uterus, rectum, urethra, etc.). The eighth patient had a history of Wertheim-Meigs operation followed by irradiation of the true pelvis 5 years ago. The patient later developed a 2 cm deep vaginal groove. The remaining two patients had male pseudohermaphroditism.

Operative technique

The preoperative intestinal preparation of the patient was performed via colonic irrigation by means of a gastric tube with approximately 8 to 10 L of Ringer's lactated solution plus potassium chloride. Antibiotic prophylaxis was carried out perioperatively. To enable a combined abdominal and vaginal approach, the patient was placed in the supine position with lower extremities resting on adjustable leg supports. Access to the abdomen was provided via a lower medial abdominal incision approximately 3 inches above the navel. A description of the operative procedure, along with illustrations to visually demonstrate it, follows.

Fig. 1. Cecal pole and ascending colon are mobilized after a right-sided paracolic incision of the dorsal parietal peritoneum. Complete mobilization of the right colonic flexure is very important to ensure subsequent tension-free ileocolostomy. In obese patients, one must take special care to safeguard the right retroperitoneal ureter. Precise localization of the ileocolic artery via cold light diaphanoscopy must be accomplished before selection of the ileocecal segment to be isolated. A 12 to 15 cm segment of cecum and ascending colon with good

From the Departments of Urology[a] and Obstetrics and Gynecology,[b] Johannes Gutenberg University Mainz, Medical School.
Received for publication May 20, 1988; revised December 20, 1988; accepted January 11, 1989.
Reprint requests: Rainer H. Bürger, Department of Urology, Johannes Gutenberg University Mainz, Medical School, Langenbeckstr. 1, 6500 Mainz, West Germany.

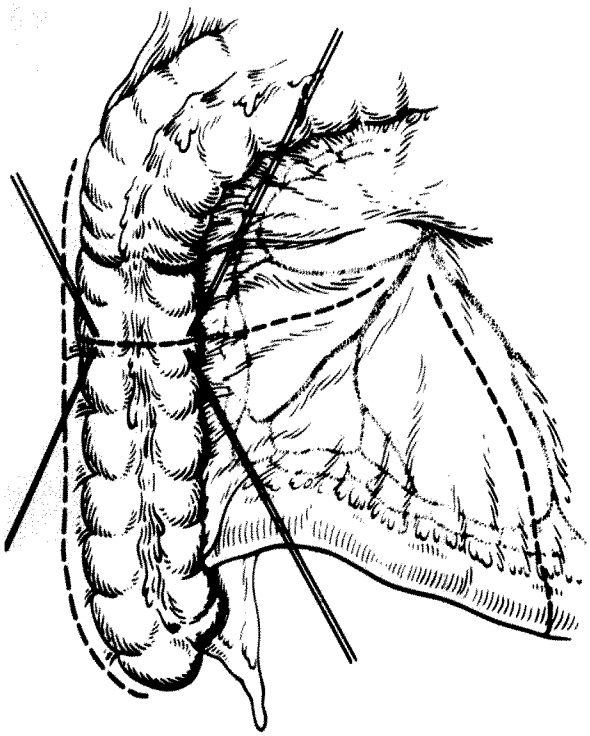


Fig. 1. See text for description.

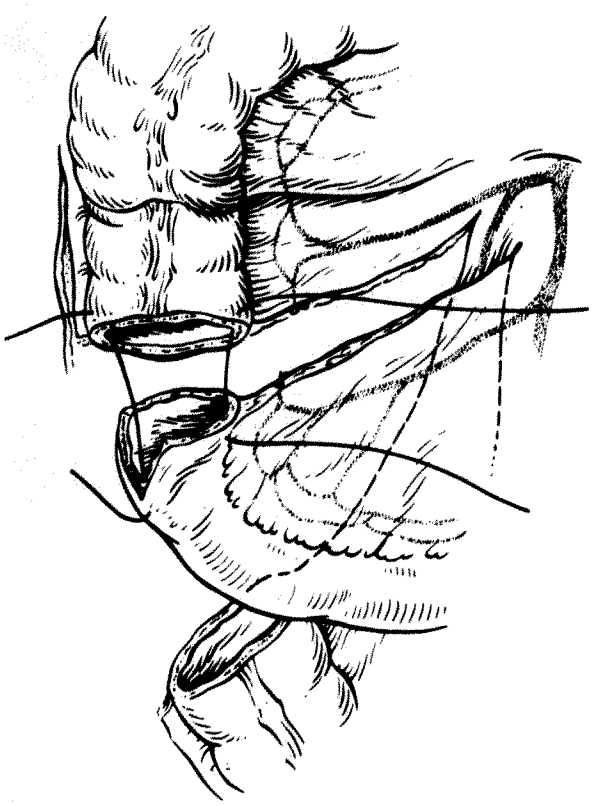


Fig. 2. See text for description.

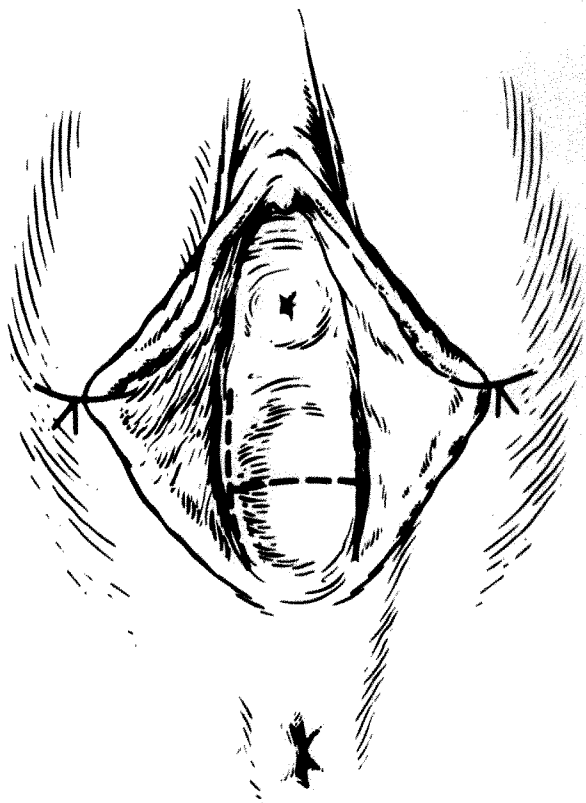


Fig. 3. See text for description.

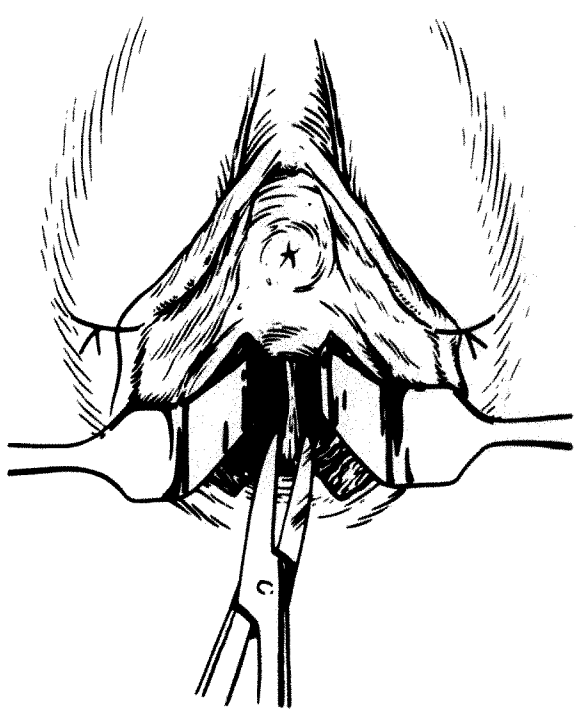


Fig. 4. See text for description.

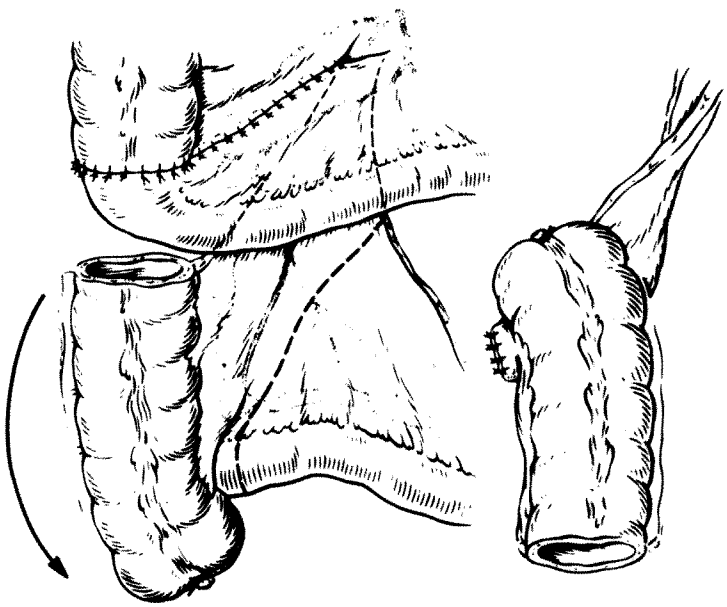


Fig. 5. See text for description.

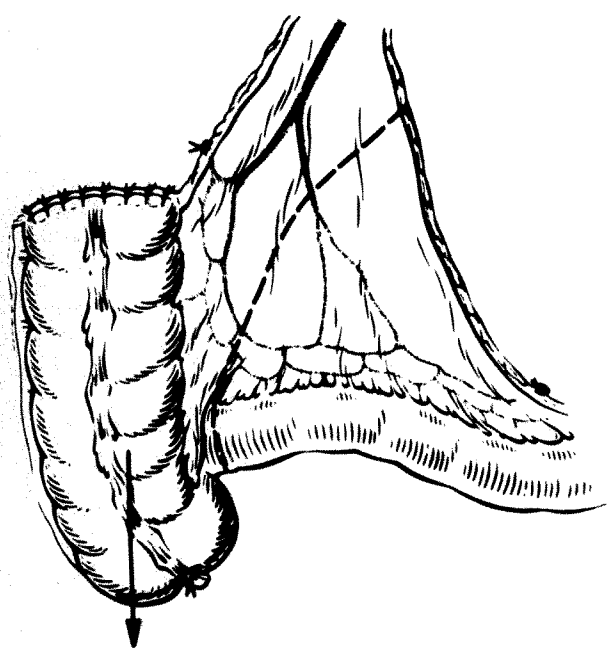


Fig. 6. See text for description.

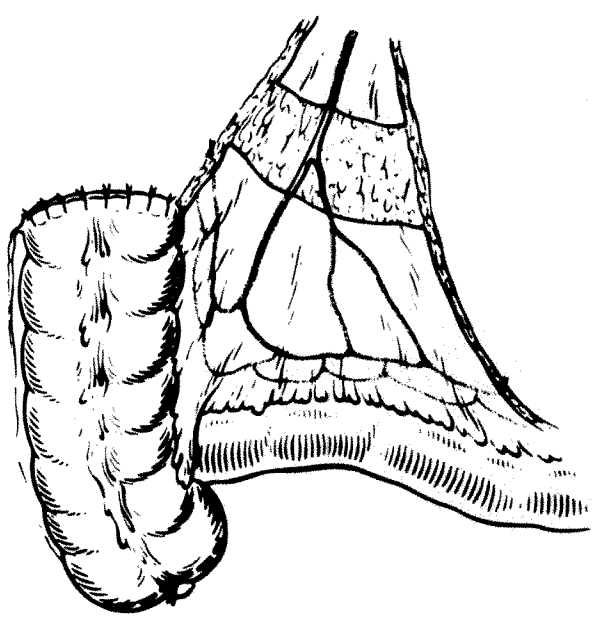


Fig. 7. See text for description.

vessel supply is selected. The point of subsequent intestinal dissection is marked by four stay sutures.

Only the vessel arcade located between the ligatures next to the intestine will be dissected in this region. After appendectomy, the cecum should be moved caudally to ensure tension-free anastomosis to the vulva. If possible, the ileum is dissected close to Bauhin's valve.

Fig. 2. Bowel continuity is achieved by ileocolostomy with single-layered seromuscular sutures (PDS or Maxon 4/0) in which the narrow ileum and the wide colon ascendens are anastomosed in a fish-mouthed manner to compensate for the difference in diameter. After completion of the intestinal anastomosis, the mesenteric incision is closed by sutures. The dissected intestinal segment is cleansed with physiologic saline solution via an 18F balloon catheter in the ileum, and fecal remains are removed by suction through the lumen of the ascending colon.

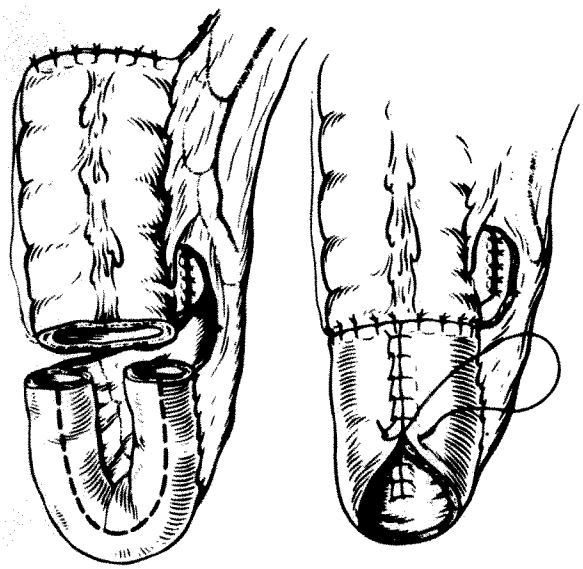


Fig. 8. See text for description.

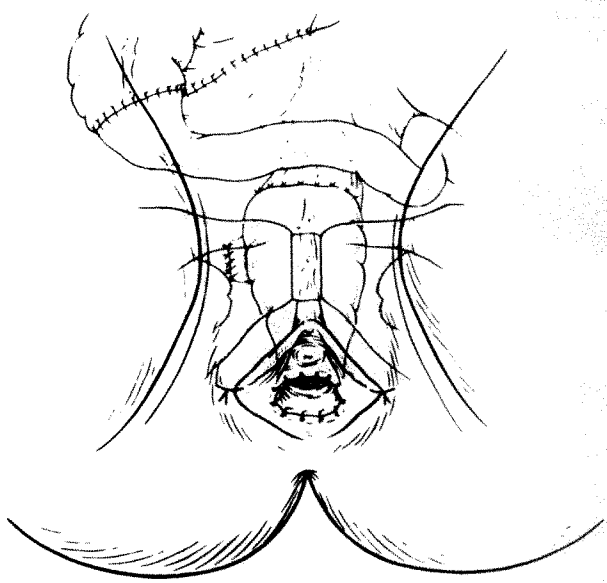


Fig. 10. See text for description.

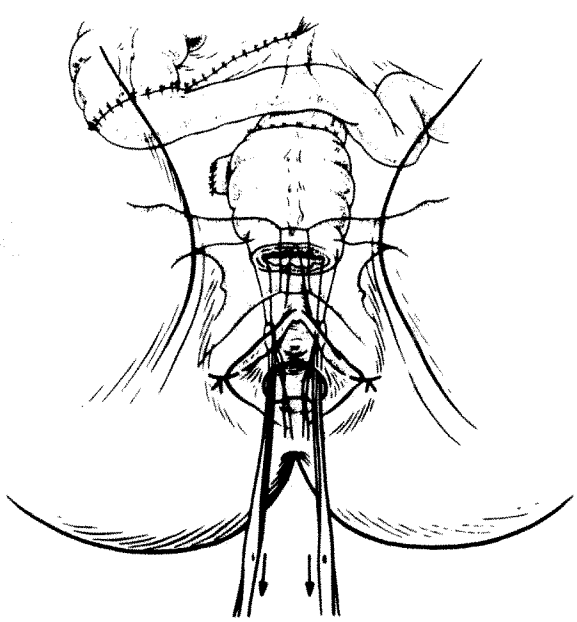


Fig. 9. See text for description.

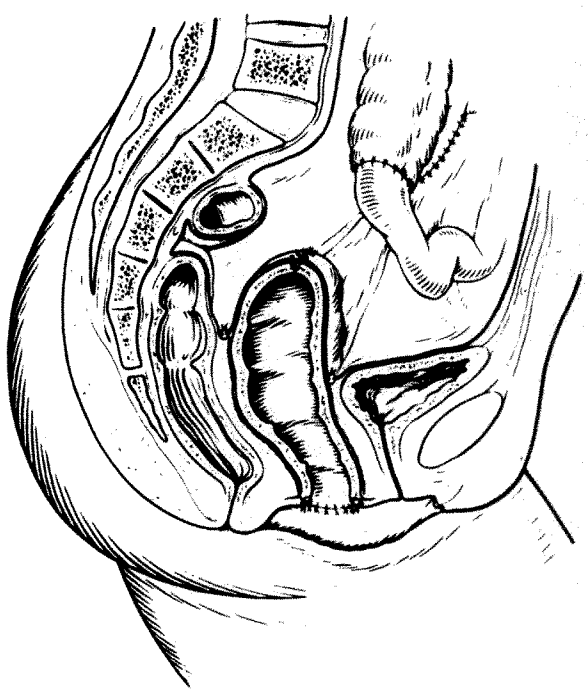


Fig. 11. See text for description.

Fig. 3. Simultaneously, a second operating team performs the vaginal approach. A pouch for the insertion of the intestine through the pelvic floor is created by an H-shaped incision in the vaginal groove between the urethral orifice and the posterior commissure. A canal is formed by spreading the tissue with the fingers and with scissors until the Douglas peritoneum has been reached.

Fig. 4. The taut tissue between the urethra and bladder on the one side and the rectum on the other side is cut in two with the scissors. After completion of this preparation, the canal is wide enough for three fingers up to the pelvic floor, which widens out cranially.

Depending on the length of the mesentery of the ileocecal segment that is to be used to form a neovagina, the following operative strategies should be observed.

Fig. 5. With normal insertion of the ileocolic artery, the cecum or ascending colon is rotated 180 degrees

Table I. Methods of treating vaginal aplasia or atresia[8,9]

1. Nonsurgical dilation according to Frank[10]
2. Surgical methods that use skin transplantation
   - Pedicle skin flaps or musculocutaneous flaps
   - Free skin cleavage grafts
   - Free skin cleavage grafts with use of a meshgraft technique
   - Free skin transplantation
3. Surgical methods that use epithelialization from outer skin
   - Simple formation of a vaginal tube with prothesis
   - Joining of labia majora
   - Lining of vaginal tube with peritoneum
   - Vecchietti method[11]
4. Surgical methods that use intestinal transplantations
   - Sigmoid vaginoplasty
   - Ileal vaginoplasty
   - Lining of vaginal tube with free mucosa–muscularis transplant made from small intestine

counterclockwise so that the margin of the ascending colon can be positioned without tension through the newly formed canal in the pelvic floor down to the vulva.

Fig. 6. High insertion of the ileocolic artery does not allow rotation. In this case the cecal pole is moved caudally through the canal in the pelvic floor down to the vulva. The ascending colon margin is closed, and the cecal pole at the vulva is opened by a horizontal incision.

Fig. 7. If the mesentery is 2 to 3 cm too short for positioning the cecal pole at the vulva, a horizontal incision of the parietal peritoneum above the supplying vessel arcade can usually lengthen the mesentery enough to attain a tensionless anastomosis with the vulval region.

Fig. 8. If the mesentery of the cecum is still too short, a 15 cm long ileal segment attached to the cecum is used for formation of the neovagina. In contrast to the mesentery of the ascending colon, the ileal mesentery must be dissected as far as possible toward the mesenteric end between ligatures to achieve a tensionless ileocolostomy. Dissection of the intestine is performed between the suture markers along the ileal margin and the margin in the colon ascendens region. Although it protects the vessel arcade near the bowel, the ileal segment is mobilized and positioned at the opening of the cecum. The U-shaped ileal segment is then positioned at the horizontally incised lower cecal pole and its mesentery incised lengthwise. By suturing the two intestinal edges with a running suture, a small pouch is formed and then anastomosed to the cecal pole with interrupted sutures to create a 5 to 7 cm extension of the neovagina.

Fig. 9. The completed neovagina is pulled through the canal in the pelvic floor.

Fig. 10. While the second operating team performs an anastomosis of the neovagina to the remaining vulva using interrupted sutures, the first operating team closes the abdomen after the neovagina has been attached to the lateral abdominal wall and a vaginal drain has been inserted.

Fig. 11. The sagittal cut through the pelvis shows the position of the neovagina as related to the other pelvic organs. By attaching the Douglas peritoneum to the wall of the neovagina, further extraperitonealization of the neovagina is accomplished.

The nasogastric tube should be left in place for 3 to 5 days after surgery; nutriments are administered intravenously until the first spontaneous stool passage, and coitus is expressly forbidden for 4 to 6 weeks.

Comment

Most methods for the treatment of aplasia or atresia of the vagina can be categorized as nonsurgical, such as dilatation, or surgical, such as skin transplants, intestinal transplants, or epithelialization from the outer skin layer (Table I).[8-11] Currently, the most popular surgical method is the formation of an artificial vaginal lumen lined with free transplanted split-thickness graft as described by Kirscher and Wagner[12] and improved on by McIndoe and Barnister.[3] Positive results brought about the wide use of this relatively simple and safe technique for more than 50 years. However, later examinations by Cali and Pratt[13] and Counseller[1] demonstrated a high shrinkage tendency in the upper third of the neovagina, with partial or complete obliteration in 21% to 41% of patients. This problem could not be corrected satisfactorily by meshgraft techniques, free bowel mucosa transplants, or lining the vaginal tube with peritoneum.[8, 14-16] In addition to the necessary dilatation of the vaginal canal or the lengthy wearing of a mold, these patients very often developed extended scarring at the skin transplant site, which in some cases led to keloid formation.[1, 3, 8, 15, 17] Various authors have described urethrovaginal fistulas and necroses of the urethra caused by the implanted mold.[1, 3] Bruck[14] believed that the main problem in the construction of an artificial vagina was not so much the formation of a hollow area but rather finding an appropriate lining that guarantees lasting patency of the neovagina.

At the turn of the century, the idea of using nonshrinking bowel as a vaginal replacement was proposed. Küstner, Snegireff, Baldwin, Albrecht, and Hochenegg were the first to prepare the way for ileal, rectal, and sigmoid transplants between 1892 and 1912.[17] The patients very often suffered from stool incontinence when the rectum was transplanted and experienced a high rate of mucous secretion (as much as 250 ml/day) when ileum was used.[9, 17] The sigmoid is topographically situated in a convenient position, possesses enough flexibility, and has an optimal blood supply with a sufficiently long and well-vascularized blood arcade. Further advantages of the sigmoid are a wide lumen and

only a slight tendency to traumatization involving postcoital bleeding. Occasionally lethal complications encountered in the first series of sigmoid vaginoplasties, such as thrombosis, strangulation ileus, and implant necrosis with ascending pelvioperitonitis caused by vessel distention, could be overcome by the modifications of Schmidt[4] and Zängl.[5]

The advantages of the sigmoid segment also apply to the cecum, which is particularly useful as vaginal replacement material in patients with insufficient size of the sigmoid segment, sigmoiddiverticulitis, extended sigmoid adhesions, or previous sigmoid resections. Until now, only Markland and Hastings[6] have published their experiences with the cecum as a vaginal substitute in five transsexual patients. Good results were achieved in these patients after clockwise rotation of the cecum on its axis and anastomosis of the distal end with a newly created vaginal orifice. We were also able to use this operative technique successfully in two patients. Because the ileocolic artery insertion was higher in the other two patients, we constructed a neovagina using cecum without rotation of the bowel segment to create a free anastomosis to the vulva. Despite complete mobilization of the right colonic flexure, it may not always be possible to achieve tension-free positioning of the cecum to the vaginal orifice. This problem has been described repeatedly in sigmoid transplants and by Markland and Hastings[6] in a cecal transplant. Sigmoid transplant rejection or necrosis must be related to tension between the anastomosed sigmoid and vaginal orifice.[4, 5] When the mesentery cannot be extended sufficiently as a result of horizontal incision of the peritoneum across the supplying vessel arcade (Fig. 7), the extension of the cecal vagina can be achieved by inclusion of the terminal ileum.

The 10 patients operated here had a complication-free postoperative course, minimal mucous secretion in the neovagina, and satisfactory function with no shrinkage tendency. In the polytraumatized patient, it was necessary to undertake vaginal reconstruction 2 months after surgery to correct a stenosis of the vaginal orifice with massive scar tissue formation.

In addition to vaginal aplasia or atresia, the operative technique described can be applied in cases of radical carcinoma surgery (total vaginectomy or anterior exenteration with simultaneous vaginal reconstruction[18, 19]), rectovaginal fistulas, radiogenic vaginal shrinkage (case No. 5), and in rare cases of vaginal agenesia with a functional uterus.[20]

The decision to attempt nonsurgical dilatation, surgery with skin transplants or epithelialization from the outer skin, or intestinal transplants must be based on the patient's anatomy, the number of previously failed operations, the surgeon's operative experience, and the psychologic well-being of the patient. On the basis of initial results in a small group of patients, it is possible to say that the previously described operative technique allows the treatment of various types of vaginal aplasia or atresia. Follow-up of 6 to 30 months has shown good cosmetic and functional results. No complications were observed within this time period.

The major advantages of a neovagina created from cecum or ileocecum can be summarized as follows: (1) excellent blood supply of the ileocecal region; (2) a longer vessel arcade of the anastomosed segment, which, when used in combination with the colon ascendens, can be extended as long as needed; (3) the possibility of rotating this segment 180 degrees without endangering the blood supply; (4) inclusion of the terminal ileum to form the neovagina when a higher insertion of the ileocolic artery is present; and (5) rapid decrease and almost total loss of mucous secretion.

REFERENCES

1. Counseller VS. Congenital absence of the vagina. JAMA 1948;136:861.
2. Farber M, Mitchell GW. Surgery for congenital absence of the vagina. Obstet Gynecol 1978;51:364.
3. McIndoe AH, Barnister JB. An operation for the cure of congenital absence of the vagina. J Obstet Gynaecol Br Emp 1938;45:490.
4. Schmid HH, ed. Scheidenbildung aus dem S-förmigen Dickdarm. Jena: G Fischer Verlag, 1956.
5. Zängl A. Konstruktion der Vagina durch Sigmatransposition. Langenbecks Arch Chir 1955;339:413.
6. Markland C, Hastings D. Vaginal reconstruction using caecal and sigmoid bowel segments in transsexual patients. J Urol 1974;111:217.
7. Thüroff JW, Alken P, Riedmiller H, et al. The Mainz pouch (mixed augmentation ileum and cecum) for bladder augmentation and continent diversion. J Urol 1986; 136:17.
8. Lang N. Operationen zur Wiederherstellung der Funktion bei angeborenem oder erworbenem Verschluss oder Stenose der Vagina. Gynäkologe 1972;15:639.
9. Pratt JH. Vaginal atresia corrected by use of small and large bowel. AM J OBSTET GYNECOL 1971;15:639.
10. Frank RT. The formation of an artificial vagina without operation. AM J OBSTET GYNECOL 1938;35:1053.
11. Vecchieti G. Die Neovagina beim Rokitansky-Küster-Hauser-Syndrom. Gynäkologe 1980;13:112.
12. Kirschner M, Wagner GA. Ein Neues Verfahren der künstlichen Scheidenbildung. Zentralbl Gynakol 1930;54: 2690.
13. Cali RW, Pratt JH. Congenital absence of the vagina. Long-term results of vaginal reconstruction in 175 cases. AM J OBSTET GYNECOL 1968;100:752.
14. Bruck HG. Erfahrungen mit der Dermatoscheidenplastik. Wien Klin Wochenschr 1970;82:562.
15. Knapstein P, Friedberg G. Plastische Eingriffe an Vulva und Vagina. Gynäkologe 1981;14:42.
16. Wilfingseder P. Konstruktion der Vagina durch Haut- und Schleimhaut-Transplantation. Langenbecks Arch Chir 1975;339:403.
17. Antoine T. Plastische Korrekturoperationen bei Scheidenatresie. Wien Klin Wochenschr 1970;82:559.
18. Berek JS. Hacker NF, Lagasse LD. Vaginal reconstruction performed simultaneously with pelvic exenteration. Obstet Gynecol 1984;63:318.
19. Hatch KD. Construction of a neovagina after exenteration using the vulvobulbocavernosus mycocutaneous graft. Obstet Gynecol 1984;63:110.
20. Pilkington JW. Pregnancy and spontaneous delivery following operation for congenital atresia of the vagina. AM J OBSTET GYNECOL 1960;78:804.

Renal artery flow-velocity waveforms in normal and hypoxemic fetuses

S. Vyas, MD, K. H. Nicolaides, MD, and S. Campbell, MD
London, England

Color flow mapping was used to identify the fetal renal artery and to obtain flow-velocity waveforms. A reference range of the fetal renal artery pulsatility index with gestation was constructed from a cross-sectional study of 114 appropriate for gestational age fetuses of 17 to 43 weeks' gestation. The pulsatility index decreased linearly with gestation, indicating a fall in impedance to flow, and presumably an increase in renal perfusion. The renal artery pulsatility index was also determined in 18 small-for-gestational-age fetuses and was higher than the appropriate-for-gestational-age group. The small-for-gestational-age fetuses also had fetal blood sampling performed by cordocentesis. A significant, direct correlation was found between blood oxygen deficit and increased renal artery pulsatility index. (AM J OBSTET GYNECOL 1989;161:168-72.)

Key words: Fetal renal artery, color flow mapping, Doppler, cordocentesis, fetal hypoxemia

Fetal Doppler velocimetry in association with fetal blood sampling by cordocentesis allows examination of the effects of hypoxemia on the cardiovascular system of the human fetus.[1, 2] Fetal blood sampling by cordocentesis has provided evidence that some small-for-gestational-age (SGA) fetuses are hypoxemic, and Doppler studies have demonstrated that in fetal hypoxemia there is a redistribution of cardiac output that results in a brain-sparing effect.[3-6] Thus in fetal hypoxemia there is an increase in the mean blood velocity and decrease in pulsatility index (PI) in the flow-velocity waveform from the common carotid artery, with a concomitant reduction in the mean blood velocity and increase in pulsatility index of flow velocity waveforms from the fetal descending thoracic aorta.[1, 5, 6] However, the characteristics of flow-velocity waveforms in the fetal aorta represent the summation of flow to the kidneys, other abdominal organs, the femoral arteries, and the placenta. Indeed the umbilical arteries, which are not under the control of the fetal homeostatic mechanisms, receive 50% of aortic blood flow.[7] Therefore study of the flow-velocity waveforms from branches of the abdominal aorta such as the renal arteries is likely to provide a more sensitive method for the prediction of fetal oxygenation than examination of aortic flow-velocity waveforms.

Study of the renal arteries has been limited by the inability of conventional B-mode ultrasonography to resolve small fetal vessels. This problem has been overcome by the introduction of color flow mapping. This article describes the method of obtaining flow-velocity waveforms from the renal artery and establishes references ranges for the pulsatility index of flow-velocity waveforms derived from this vessel.[8] Furthermore, it examines the relationship between renal artery pulsatility index Po_2 in blood samples obtained by cordocentesis from 18 SGA fetuses.

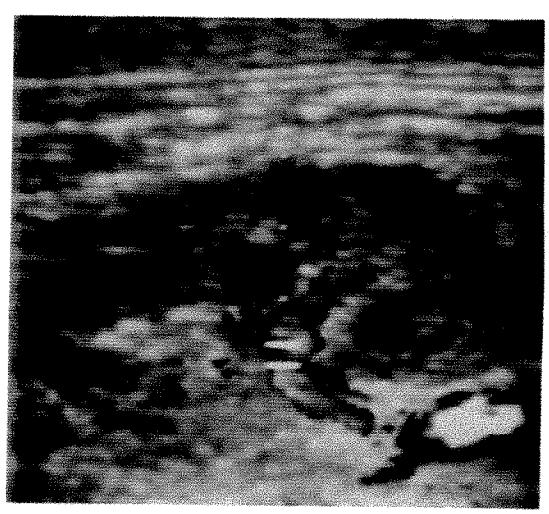


Fig. 1. Longitudinal axis of fetal kidney with renal artery *(red)* demonstrated by color flow mapping.

Patients and methods

A reference range for the fetal renal artery pulsatility index was derived from a single examination of 114 women with uncomplicated singleton pregnancies between 17 and 43 weeks' gestation. Gestational age was

From the Harris Birthright Research Centre for Fetal Medicine, Department of Obstetrics and Gynaecology, King's College Hospital.
Received for publication September 11, 1988; revised December 21, 1988; accepted December 31, 1988.
Reprint requests: S. Vyas, MD, Harris Birthright Research Centre for Fetal Medicine, Department of Obstetrics and Gynaecology, King's College Hospital, Denmark Hill, London SE5 8RX, United Kingdom.

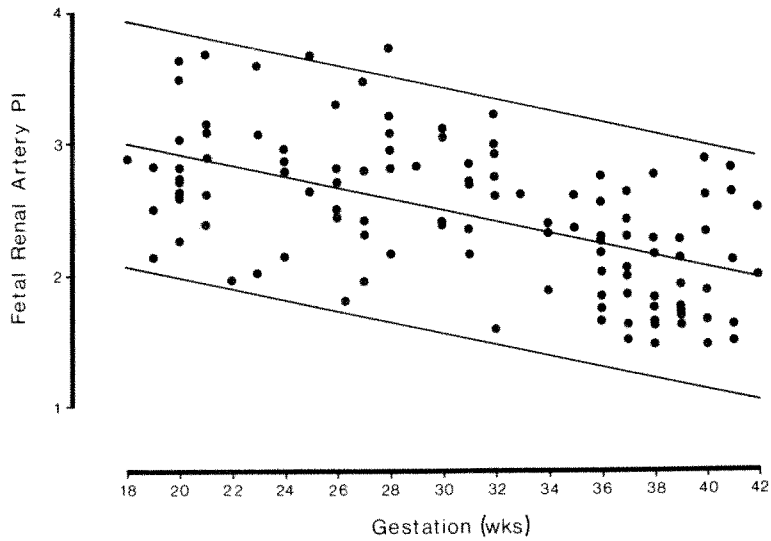


Fig. 2. Reference range (mean and individual 95% confidence intervals) of fetal renal artery pulsatility index with gestation constructed from study of 114 AGA fetuses (●).

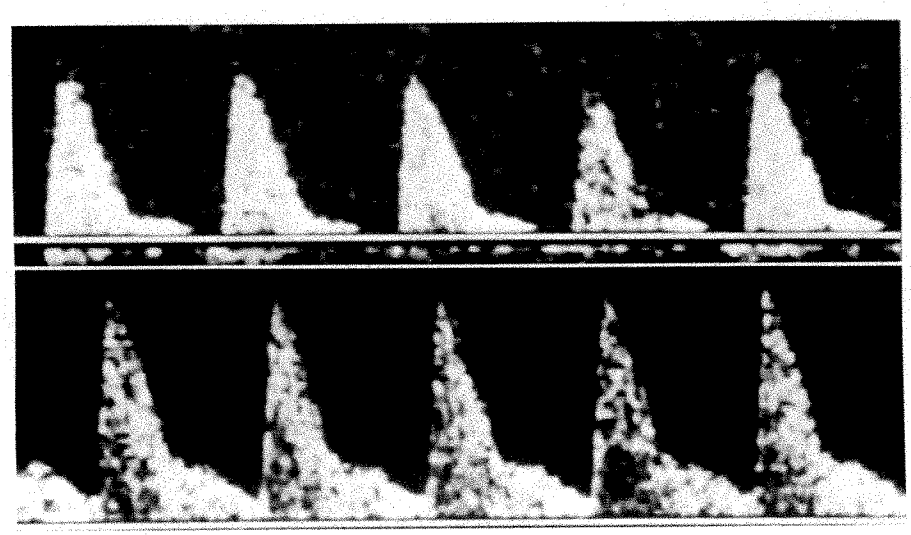


Fig. 3. Flow-velocity waveforms from the fetal renal artery at 26 *(top)* and 38 *(bottom)* weeks' gestation with absence of end-diastolic frequencies in the former and presence in the latter.

calculated by Naegele's rule and was confirmed by an ultrasonographic scan performed at 18 to 22 weeks of amenorrhea. The women were recruited from the antenatal clinic during their routine visit and had no pregnancy complications. Ultrasonographic examination at the time of the Doppler studies showed all the fetuses to be structurally normal and of appropriate size for gestational age (AGA). Furthermore, neither oligohydramnios nor polyhydramnios was present.

Studies of the fetal renal artery were performed with a color flow mapping system with a 5 MHz transducer (Acuson 128, Acuson) and a high-pass filter setting of 125 Hz. The patient was placed in a semirecumbent position and a longitudinal view of the fetal kidneys was obtained by real-time ultrasonography. All examinations were performed during fetal apnea because fetal breathing movements are known to have marked effects on fetal blood flow.[9] Fetal breathing movements are recognized by the rhythmic movement of the fetal kidneys independently of fetal trunk movements. The image of the kidney was magnified and the color flow mapping element of the transducer was started. Thus Doppler-shifted frequencies in the scan plane were colored either red (movement toward the transducer) or blue (movement away from the transducer). In the longitudinal plane the renal artery runs directly toward the transducer from its origin as a lateral branch of the abdominal aorta to the hilum of the kidney (Fig. 1).

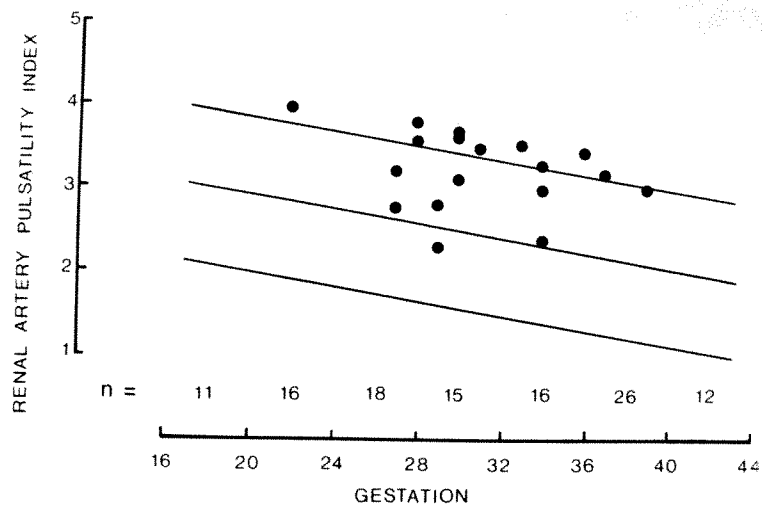


Fig. 4. Renal artery pulsatility index of 18 SGA fetuses (●) plotted on reference range.

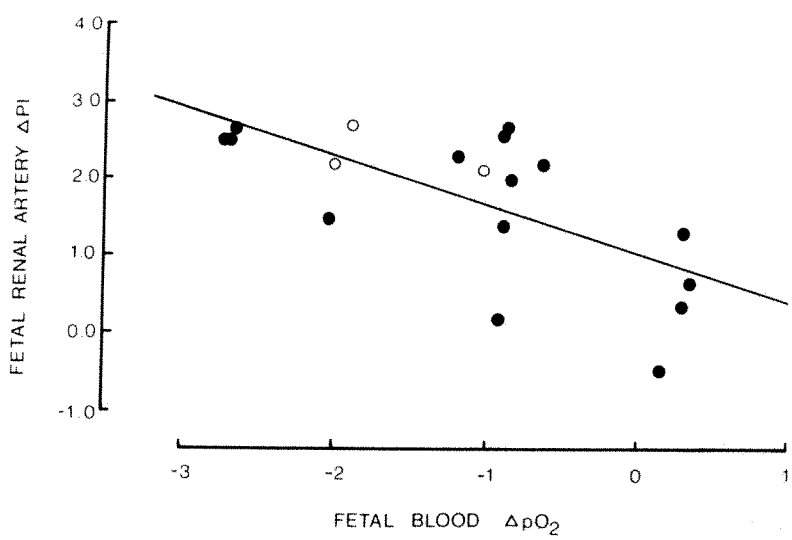


Fig. 5. Relationship between fetal hypoxemia and renal artery pulsatility index, both expressed as number of SDs by which observed blood Po_2 and renal artery pulsatility index differed from respective mean for gestational age. Pregnancies in which oligohydramnios was noted are indicated by ○.

The abdominal aorta lies parallel to the transducer. In this orientation the beam-vessel angle for the renal artery is close to 0 degrees, whereas the angle for the abdominal aorta is approximately 90 degrees. This ensures that the signals from the renal artery are optimized, with no contamination from the aorta. The Doppler range gate was placed over the renal artery instead of one of its branches. Flow-velocity waveforms were recorded and the pulsatility index was determined by an on-line spectrum analyzer (Doptek Ltd.).

The intraobserver coefficient of variation for the pulsatility index of flow-velocity waveforms for the renal artery was determined by examining three fetuses; 10 measurements were taken from each fetus during a 1-hour period. The intraobserver variation was 5.4%. Measurements were made from both the left and right fetal renal artery in 12 cases; no significant difference was found (Student's paired t test, $t_{11} = 0.21, p = 0.85$).

The fetal renal artery pulsatility index was also determined in 18 SGA fetuses who were referred to our unit for blood gas analysis and rapid karyotyping at gestational ages between 22 and 39 weeks. The fetal abdominal circumference was 2 to 7.5 SDs below the mean for gestation of our reference range, which was constructed from the study of 1610 pregnancies. The Doppler studies were performed up to 30 minutes before cordocentesis, which was performed without maternal sedation or fetal paralysis.[2] Blood vessels were identified by the intravascular injection of 0.4 ml normal saline solution at the time of cordocentesis.[2] The

umbilical vein was sampled in 14 cases and the umbilical artery was sampled in four cases. Fetal blood gases were measured by a Radiometer ABL 330 (Radiometer).

Linear regression analysis was used to calculate the mean and individual 95% confidence intervals of the relationship between renal artery pulsatility index and gestation. In the SGA fetuses the individual observed renal artery pulsatility index and blood Po_2 values were expressed as the number of SDs from the respective normal means for gestation (ΔPo_2, Δpulsatility index).[10] One-sample Student's t test was used to compare blood Po_2 and renal artery pulsatility index between the SGA and AGA groups.

Results

Fetal renal artery flow-velocity waveforms were successfully obtained from 114 of the 131 examinations attempted (87%). Failure to obtain flow-velocity waveforms was always the result of the relatively short depth of field imposed by a 5 MHz transducer and occurred in association with an extensive anterior placenta (eight cases) or when the fetal spine was posterior (nine cases).

The renal artery pulsatility index decreases linearly with gestation ($r = -0.56$, $p < 0.0001$, constant = 3.77, slope = −0.04, SD = 0.46; (Fig. 2). The flow-velocity waveforms are highly pulsatile; the presence of end-diastolic frequencies becomes more common with advancing gestation (Fig. 3). Thus end-diastolic frequencies were present in 3 of 27 cases (11%) at 18 to 25 weeks' gestation, 6 of 37 cases (19%) at 26 to 34 weeks, and 43 of 50 cases (86%) at 35 to 43 weeks' gestation.

The renal artery pulsatility index is significantly higher (Fig. 4) and the blood Po_2 is significantly lower in the SGA than in the AGA fetuses ($t_{17} = 6.86$, $p < 0.0001$ and $t_{17} = -3.40$, $p < 0.01$, respectively). Furthermore, in the SGA fetuses there is a significant correlation between Δpulsatility index and ΔPo_2 (Fig. 5, $r = -0.66$, $p < 0.005$, slope = −0.63, constant = 1.04, SD = 0.75). Oligohydramnios was noted in three of the SGA fetuses.[11]

Comment

Fetal renal artery flow-velocity waveforms can be obtained by the use of color flow mapping. Since end-diastolic frequencies are absent in 54% of cases, the pulsatility index was selected for analysis of the flow-velocity waveforms because this index is not dependent on end-diastolic frequencies for its calculation. Impedance to flow in the renal circulation decreases with advancing gestation.

If it is assumed that the arterial pressure remains constant, then fetal renal perfusion increases. This may offer an explanation for the increase in fetal urine production rate that occurs with advancing gestation.[12] This is in agreement with studies on chronically catheterized fetal lambs in which total renal blood flow (in milliliters per minute) and filtration fraction are significantly higher near term when compared with values earlier in the third trimester.[13]

Although the hourly fetal urine production rate does not continue to increase at gestations beyond 40 weeks, the renal artery pulsatility index continues to fall.[12] This finding suggests that the change in urine production rate at this gestation may not be a result of changes in renal perfusion. Robillard et al.[13] have shown in the chronically catheterized sheep model that near-term fetal lambs may be more sensitive to circulating vasopressin than younger fetuses.[13] It is possible that the cause of reduction in urine production rate near term in the human fetus represents a maturation of the renal rsponse to circulating vasopressin and therefore increased tubular reabsorption.

In SGA fetuses there is a direct relationship between hypoxia and the renal artery pulsatility index (Fig. 5). This relationship would be part of the redistribution of cardiac output that has been reported in hypoxic human fetuses to produce the brain-sparing effect. When the content of inspired oxygen is reduced in the pregnant ewe, circulation in the fetus is redistributed so that perfusion of the brain, heart, and adrenals is increased, whereas perfusion of the carcass, pulmonary, splenic, gut, and renal vascular beds is decreased.[14] Previous studies with Doppler ultrasonography have alluded to these changes in the human fetus. However, exact information about the perfusion of individual fetal organs has not been obtained because of the inability to visualize the arterial supply.[15]

Although reduced renal perfusion may result in a reduction in fetal urine production and therefore amniotic fluid volume, examination of the latter reflects the chronic state whereas the former represents the acute state. Thus oligohydramnios was noted in three of the SGA pregnancies, presumably as a result of chronic fetal hypoxemia (Fig. 5).

The use of color flow mapping to identify the fetal renal artery and to correlate changes in flow impedance with measurements of fetal blood Po_2 has enabled the demonstration of a direct relationship between fetal oxygenation and impedance to flow in the fetal renal circulation.

REFERENCES

1. Soothill PW, Nicolaides KH, Bilardo CM, Campbell S. Relation of fetal hypoxia in growth retardation to mean blood velocity in the fetal aorta. Lancet 1986;1:1118-9.
2. Nicolaides KH, Soothill PW, Rodeck CH, Campbell S. Ultrasound-guided sampling of umbilical cord and placental blood to assess fetal well-being. Lancet 1986; 1:1065-7.
3. Soothill PW, Nicolaides KH, Campbell S. Prenatal as-

phyxia, hyperlacticaemia, hypoglycaemia, and erythroblastosis in growth retarded fetuses. Br Med J 1987; 1:1051-3.
4. Wladimiroff JW, Wijngaard JAGW, Degani S, Noordam J, van Eyck J, Tonge HM. Cerebral and umbilical arterial flow velocity waveforms in normal and growth retarded pregnancies. Obstet Gynecol 1987;69:705-9.
5. Bilardo CM, Nicolaides KH, Campbell S. Doppler measurement of fetal and uteroplacental circulation: relationship with umbilical venous blood gases measured at cordocentesis. [In press].
6. Arabin B, Bergmann PL, Saling E. Simultaneous assessment of blood flow velocity waveforms in uteroplacental vessels, the umbilical artery, the fetal aorta and the fetal common carotid artery. Fetal Ther 1987;2:17-26.
7. Griffin D, Cohen-Overbeek T, Campbell S. Fetal and utero-placental blood flow. Clin Obstet Gynaecol 1983; 10:565-602.
8. Gosling RG, King DH. Ultrasound angiology. In: Marcus AW, Adamson L, eds. Arteries and veins. Edinburgh: Churchill Livingstone, 1975:61-98.
9. Marsal K, Lindblad A, Lingman G, Eik-Nes SH. Bloodflow in the descending aorta: intrinsic factors affecting fetal bloodflow, i.e. fetal breathing movements and cardiac arrhythmia. Ultrasound Med Biol 1984;10:339-48.
10. Soothill PW, Nicolaides KH, Rodeck CH, Campbell S. Effect of gestational age on fetal and intervillous blood gases and acid-base values in human pregnancy. Fetal Ther 1986;1:168-75.
11. Manning F, Platt L, Sipos L. Antepartum fetal evaluation: development of a fetal biophysical profile. Am J Obstet Gynecol 1980;136:787-91.
12. Cambell S, Wladimiroff JW, Dewhurst CJ. The ante-natal measurement of fetal urine production. J Obstet Gynaecol Br Commonw 1973;80:680-6.
13. Robillard JE, Weitzman RE, Burmeister L, Smith FG. Developmental aspects of the renal response to hypoxemia in the lamb fetus. Circ Res 1981;48:128-38.
14. Cohen HE, Sacks EJ, Heymann MA, Rudolph AM. Cardiovascular responses to hypoxemia and acidemia in fetal lambs. Am J Obstet Gynecol 1974;120:817-24.
15. Hackett GA, Campbell S, Gamsu H, Cohen-Overbeek T, Pearce JMF. Doppler studies in the growth retarded fetus and prediction of neonatal necrotising enterocolitis, haemorrhage, and neonatal morbidity. Br Med J 1987; 1:13-6.

Twin pregnancy in premature ovarian failure after estrogen treatment: A case report

Lawrence Tang, MD, and Robert S. Sawers, MD
Birmingham, England

Spontaneous or pharmacologically induced reactivation of follicular development in patients with premature ovarian failure may occasionally result in successful singleton pregnancy. We report the first case of a dizygotic twin pregnancy after cyclic estrogen-progestin treatment in this condition. (Am J Obstet Gynecol 1989;161:172-3.)

Key words: Twin pregnancy, premature ovarian failure, estrogen treatment

Premature ovarian failure is diagnosed when hypergonadotropic amenorrhea occurs before the age of 40. Although it carries a poor fertility prognosis, spontaneous ovulation and pregnancy have been observed in few cases. Occasionally, rebound ovulation after withdrawal of cyclic estrogen-progestin treatment may produce a singleton pregnancy. To our knowledge, dizygotic twin pregnancy from such treatment has not been recorded.

From the Academic Department of Obstetrics and Gynecology, University of Birmingham, and the Department of Gynecology, Birmingham and Midland Hospital for Women.
Received for publication January 4, 1988; revised January 1, 1989; accepted February 15, 1989.
Reprint requests: Lawrence Tang, MD, MRCOG, Academic Department of Obstetrics and Gynecology, Birmingham Maternity Hospital, Queen Elizabeth Medical Centre, Edgbaston, Birmingham, England B15 2TG.

Case report

The patient, 32 years old, was first seen because of secondary amenorrhea and primary infertility. Her menarche was at the age of 12 with regular 28-day cycles until the age of 18 when she started to take oral contraceptives and continued use for the next 6 years. When she married no spontaneous menstrual period occurred after discontinuation of oral contraceptives for 1 year. Her past medical history and family history were unremarkable.

The patient weighed 47.5 kg and was 156 cm in height. She had normal secondary sexual development. Pelvic examination was normal except for an atrophic vagina and a small uterus.

She had no withdrawal bleeding after Provera challenge. Levels of both follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were high when measured on three separate occasions (FSH >50 IU/L, normal 1 to 9 IU/L [Medical Research Council

78/549]; LH >120 IU/L, normal 1 to 12 IU/L [Medical Research Council 68/40]). Serum prolactin and thyroid function test results were normal. Complete blood count, urinalysis, liver function test, serum electrolytes, serum cholesterol, serum calcium and phosphate, and serum protein determinations were within normal limits. Chromosomal study showed a normal karyotype of 46,XX. Laparoscopy revealed a hypoplastic uterus, patent tubes, and small ovaries. Bilateral ovarian punch biopsies failed to demonstrate any primordial follicles. Seminal fluid analyses of her 33-year-old husband were normal.

After discussion with the couple about the poor fertility prognosis, the patient agreed to a 3-week trial course of ethinyl estradiol treatment at 50 μg daily. Weekly endocrine assessment showed gradual suppression of gonadotropin levels. The patient had withdrawal bleeding but no rebound ovulation after treatment for 6 months. She was then given buserelin (Hoechst U.K. Ltd.), a gonadotropin-releasing hormone analog, at a dose of 6 × 100 μg daily for 21 days followed by a 28-day drug-free period to observe for rebound ovulation. The gonadotropins were suppressed to normal levels but the serum estradiol level remained low; no rebound ovulation occurred after three treatment cycles. Subsequently adequate suppression of gonadotropins was achieved with ethinyl estradiol, which was followed by ovarian stimulation with clomiphene citrate (100 mg daily for 5 days) for three cycles and then human menopausal gonadotropin (2925 IU) for one cycle. All treatments failed to induce any follicular development.

The couple was counseled after 22 months of intensive treatment. The patient was started on a regimen of ethinyl estradiol, 100 μg daily for 28 days, with medroxyprogesterone acetate, 5 mg daily, for the last 10 days followed by a 28-day treatment-free period to await rebound ovulation. She had regular withdrawal bleeding for 6 months. In the seventh month, she had no withdrawal bleeding. Ultrasonographic scan showed a twin pregnancy. At 28 weeks' gestation, cesarean section was performed for severe proteinuric preeclampsia and no growth in the second twin. A male infant weighing 1160 gm and a female infant weighing 820 gm were delivered.

Comment

Accurate diagnosis of the cause of premature ovarian failure is the best guide to the likelihood of spontaneous ovulation and the aptness of the ovaries to respond to estrogen treatment or ovulation induction. Patients with ovarian failure after chemotherapy may respond readily, whereas autoimmune ovarian failure and resistant ovary syndrome are quite refractory to treatment. In idiopathic premature ovarian failure, a history of secondary amenorrhea or oligomenorrhea seems to have a better outcome than primary amenorrhea.[1] Patients with serum estradiol concentrations above menopausal range and/or serum LH levels greater than FSH levels are more likely to respond because the syndrome reflects the presence of intermittent ovarian follicular activity and appropriate feedback mechanism.[2] As illustrated in our patient, laparoscopic ovarian biopsy is not useful in the prediction of disease outcome because of inadequate sampling. Ovarian biopsy via laparotomy may help to diagnose autoimmune oophoritis or resistant ovary syndrome; however, postoperative adhesion formation may compromise fertility.

We believe that in vitro fertilization with oocyte donation should be recommended to those patients who are considered to be poor responders to ovulation induction. Both estrogen treatment and gonadotropin-releasing hormone analog can efficiently suppress the elevated gonadotropin levels. However, estrogen therapy is more successful in terms of ovulation induction because it can promote new FSH receptor formation by decreasing the down-regulation effects of high tonic levels of FSH and also synergizes with FSH to increase the number of FSH receptors.[1] For those who do not desire pregnancy or further pregnancies, estrogen replacement therapy should be started to prevent osteoporosis. However, in view of the unpredictable outcome and the remote possibility of a pregnancy, proper contraception or sterilization should be advised.[1]

REFERENCES

1. Alper MM, Jolly EE, Garner PR. Pregnancies after premature ovarian failure. Obstet Gynecol 1986;67:59S-62S.
2. Rebar RW, Erickson GF, Yen SSC. Idiopathic premature ovarian failure: clinical and endocrine characteristics. Fertil Steril 1982;37:35-41.

Uterine development and endocrine relationships after menarche

Eleonora Porcu, MD, Stefano Venturoli, MD, Raffaella Fabbri, BSc, Luigi F. Orsini, MD, Emilia Sganga, MD, Lorena Brondelli, MD, Roberto Paradisi, MD, and Carlo Flamigni, MD

Bologna, Italy

Uterine volume and ovarian/uterine ratio during the first 6 postmenarchal years in 143 women were compared with an adult control group. Plasma gonadotropins and sex steroids were also correlated with uterine volume in adolescents. Uterine volume increased progressively and correlated positively with advancing postmenarchal ($r = 0.24$; $p < 0.05$) and chronologic ($r = 0.23$; $p < 0.05$) age. However, normal adult uterine volume was not yet reached by the highest postmenarchal (6 years) and chronologic (18 years) age. The ovarian/uterine ratio decreased gradually with increasing postmenarchal age but was always higher (0.22) than that of control subjects (0.12). Uterine volume correlated positively with plasma estradiol ($r = 0.25$; $p < 0.05$) and dehydroepiandrosterone ($r = 0.22$; $p < 0.05$) and its sulfate ($r = 0.22$; $p < 0.05$) levels. This study indicates that the uterus continues to grow after menarche in conjunction with increasing hormone levels. (Am J Obstet Gynecol 1989;161:174-7.)

Key words: Uterine development, adolescence, ovaries

In pubertal development, menarche is an intermediate step that is followed by further physical and endocrine changes. Some variables, such as height, skeletal maturation, and secondary sexual features, usually undergo minor modifications after menarche. On the other hand, the endocrine pattern shows more or less prolonged maturation toward normal ovulatory function.

As for the genital tract, maturation is not yet completed after menarche. From the onset of puberty to menarche, the ovaries gradually enlarge with an increase in the stroma and the number and size of antral follicles as documented by histologic and ultrasonographic studies.[1-4] Postmenarchal morphologic studies of the ovaries have been described by our group in previous studies.[5, 6] These studies have shown that at menarche ovarian volume is already in the adult range, often exceeding it, and that ovarian structure is frequently multifollicular. Before menarche, the uterus increases in size, and the cervix/corpus ratio decreases progressively.[4, 7]

This study defines the postmenarchal evolution of the uterus and its relationship with the reproductive endocrine background.

From the Department of Reproductive Physiology and Pathology and the Chair of Prenatal Pathophysiology, University of Bologna.
Received for publication September 18, 1988; accepted January 30, 1989.
Reprint requests: Eleonora Porcu, MD, Servizio di Fisiopatologia della Riproduzione, Università di Bologna, Via Massarenti, 13-40138, Bologna, Italy.

Material and methods

One hundred forty-three healthy teen-age girls (range, 13 to 18 years; mean, 16.2 years) were studied. The subjects ranged between 0.5 and 6 years (mean, 3.2 years) postmenarche. Each subject attended the hospital on an outpatient basis because of various nonendocrine disturbances. Mean (±standard deviation) height was 163 ± 4.6 cm, weight as 52.4 ± 7.6 kg, and body mass index was 20.9 ± 3.6 (normal range, 18 to 24). Each girl was at pubertal stages 4 and 5 in pubic hair and breast development according to Marshall and Tanner.[8] Menstrual rhythm varied between 18 and 90 days.

Thirty-three normal volunteers, normally menstruating, nulliparous, sexually mature, and 21 to 28 years of age, made up the control group. Height, weight, and body mass index were 165 ± 5.6 cm, 54.4 ± 2.3 kg, and 20.1 ± 1.7 (mean + SD), respectively. They had not used oral contraception for at least 12 months before the start of the study.

Blood samples were collected on the fifth day of the cycle and on a day between the tenth and the fourth before the next menstrual bleeding to evaluate the follicular and premenstrual endocrine pattern and the presence of ovulation. To evaluate frequency of ovulation, the girls were asked to record basal body temperature for at least 6 months.

The radioimmunoassay techniques used for hormonal measurements were previously reported.[6] In particular, follicular-stimulating hormone, luteinizing hormone, prolactin, 17-hydroxyprogesterone, proges-

Table I. Mean ± SD values (mm) of uterine diameters in adolescents during the first 6 postmenarchal years and in sexually mature control subjects

Postmenarchal yr (No. of subjects)	*Yr 1 (n = 27)*	*Yr 2 (n = 30)*	*Yr 3 (n = 32)*	*Yr 4 (n = 32)*	*Yr 5 (n = 10)*	*Yr 6 (n = 12)*	*Controls (n = 20)*
LD	62.2 ± 9.4*	66.3 ± 10.5*	70.3 ± 10.3†	66.3 ± 14.5‡	73.3 ± 7.2	70.7 ± 7.5†	75.8 ± 4.9
APD	28.1 ± 9.8	28.0 ± 4.8*	27.5 ± 6.8‡	28.3 ± 4.7*	30.2 ± 3.9	30.7 ± 3.7	33.2 ± 4.0
TD	38.6 ± 6.9‡	40.6 ± 7.4†	40.6 ± 6.3†	42.3 ± 6.7	41.6 ± 4.8	43.7 ± 4.8	44.5 ± 5.8

LD, longitudinal diameter; APD, anteroposterior diameter; TD, transverse diameter.
*$p < 0.001$ with respect to adult controls.
†$p < 0.05$ with respect to adult controls.
‡$p < 0.01$ with respect to adult controls.

terone, dehydroepiandrosterone and its sulfate, testosterone, androstenedione, 5α-dihydrotestosterone, estrone, and estradiol were assayed.

Ultrasound examinations of the uterus and ovaries were carried out by means of the full-bladder technique, with serial longitudinal and transverse scans of the pelvis in the supine position using one of three real-time ultrasound scanners. A 2.5 MHz mechanical sector scanner (Combison 100, Kretztechnik GmbH, Zipf, Austria) was used during the first period of our study. Two General Electric sector scanners (SRT with a 3 MHz transducer and RT 3000 with a 5 MHz transducer, General Electric, Milwaukee, Wis.) were subsequently used. Sonograms were performed during the early follicular phase.

Mean ovarian volume was considered representative of both ovaries in each subject. Uterine and ovarian volumes were determined by the formula for a prolate ellipsoid (volume = 0.5233 × D1 × D2 × D3, where D1, D2, and D3 are the three maximal longitudinal, anteroposterior, and traverse diameters).

Results are expressed as mean ± standard deviation, and the differences between the group means for large groups were tested with the use of the Students *t* test for unpaired data. Correlations between uterine volume and chronologic and gynecologic age (expressed as number of months from menarche) and hormone values are determined by linear regression analysis.

Results

In general, the uterus showed typical, mature, pear-shaped morphology with a corpus/cervix ratio greater than 1. Table I shows that uterine diameters slowly increased in the years after menarche. Significant differences disappeared around the third or fourth gynecologic year, but mean diameters did not reach the average control value by the sixth year.

A positive correlation between uterine volume and gynecologic age ($r = 0.24$; $p < 0.05$) was found. A positive correlation was also seen between uterine volume and chronologic age ($r = 0.23$; $p < 0.05$).

Fig. 1 shows mean values (±SD) of uterine volume grouped according to postmenarchal and chronologic ages in adolescents and sexually mature control subjects. Mean uterine volume increased gradually from the first to the sixth year after menarche when its value was still lower, albeit not significantly different from that of adult control subjects. Regarding chronologic age, uterine volume increased progressively starting from age 13 years onward. Significant differences between adolescent and control values disappeared at age 18 years.

Uterine volume increased in correlation with estradiol levels in both the follicular and premenstrual phases ($r = 0.22$; $p < 0.05$; $r = 0.25$; $p < 0.05$, respectively). Dehydroepiandrosterone ($r = 0.22$; $p < 0.05$) and dehydroepiandrosterone sulfate ($r = 0.22$; $p < 0.05$) correlated with uterine volume during the follicular phase.

No correlations were found between uterine volume, the other hormones, bone age, weight, height, and ovarian volume. Furthermore, uterine volume does not change with the frequency of ovulation (i.e., the number of ovulations in six months) nor with the frequency of menstrual bleedings. The ovarian/uterine ratio progressively decreased in the first 6 postmenarchal years, but at the sixth year it was still higher than that found in adults.

Comment

The uterus grows progressively during fetal life.[9] After birth, uterine volume is relatively high, longer, and thicker than the prepubertal uterus and shows endometrial and myometrial characteristics very similar to those of adults[10]

After the first weeks, uterus size regresses,[11] probably because of the withdrawal of maternal hormone stimulation. During childhood the uterus shows no changes in size and shape until the age of 7 years.[12] From this age onward, uterine volume increases progressively at a slow rate before the appearance of secondary sexual characteristics, whereas a sharp acceleration is seen

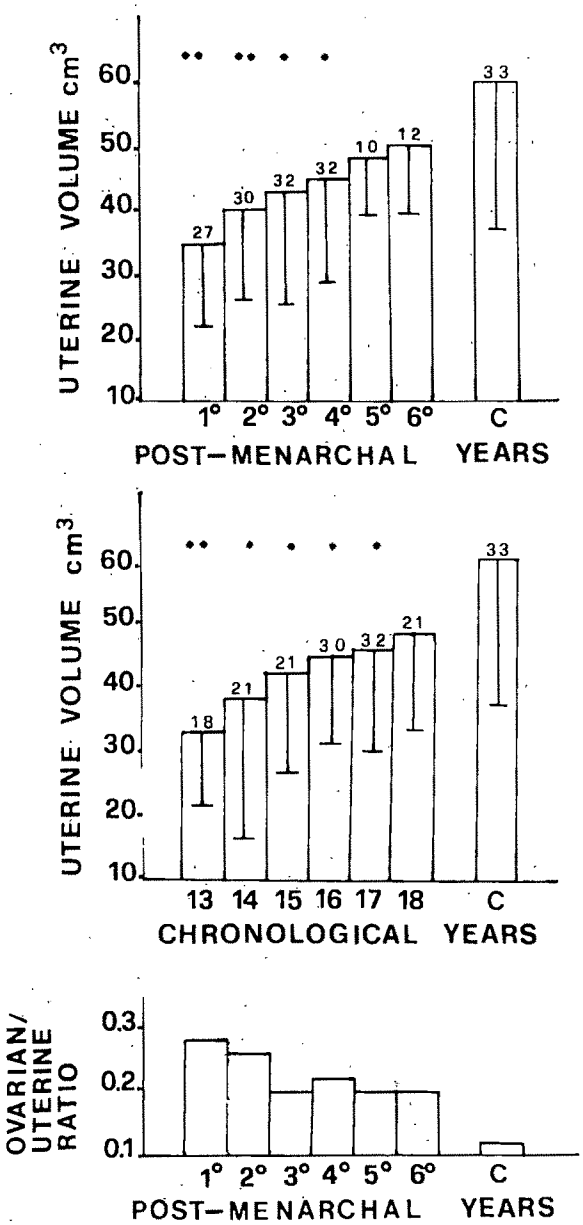


Fig. 1. Mean values (±SD) of uterine volume grouped according to gynecologic and chronologic years in adolescents. Sexually mature controls *(C)* are reported. Number of subjects is reported in each column. $*p < 0.01$; $**p < 0.001$, with respect to control uterine volume. *Bottom panel:* Mean ovarian/uterine ratio in adolescent girls during gynecologic years and sexually mature controls *(C)*.

during puberty, which has a good correlation with Tanner stages,[7] age, weight, height, and estradiol levels,[12] and the most dramatic changes between stage 3 and 4 of breast development.

This study documents thtat after menarche, the uterus continues to grow, even after the almost-complete development of the secondary sexual characteristics. Menarche is a critical step toward the development of the whole reproductive system, especially of the uterus, which assumes a new function. After menarche, uterine growth rate slows and gradually tends to take on the adult size. However, normal adult values are not attained even by the sixth gynecologic year, which shows that full development of this reproductive organ takes even more time, although adult morphology and corpus/cervix ratio are already achieved by menarche.

Comparison between our data and values reported by other authors in postpubertal girls is difficult because of the low number of subjects in those studies that did not group adolescents into postmenarchal years. However, the mean uterine length found by Sample et al.[13] in a group of 25 girls ranging in age from 13 to 20 years and the mean uterine longitudinal diameter and volume reported by Ivarsson et al.[7] at 13 and 17 years correspond fairly closely to our values.

We previously demonstrated[14] the dynamic postmenarchal behavior of many reproductive parameters. Among the increasing reproductive hormones, estradiol seems to be the main agent for uterine growth in this study. On the other hand, other reproductive parameters, such as the frequency of menstrual bleeding and that of ovulation and progesterone levels, do not appear related to uterine size and growth rate.

Height and weight correlate with uterine volume before menarche but not afterward, since at this age they have nearly exhausted the developmental changes and vary independent of growth factors. The adrenal steroids dehydroepiandrosterone and dehydroepiandrosterone sulfate, which are faithful indexes of biologic maturation, continue to increase during adolescence,[14] and interestingly, they correlated with uterine development in this study. The low correlation coefficients that we found between uterine increases and hormone and age changes show that other influences are probably mainly responsible for uterine size variations during the first postmenarchal years.

The finding of a still small uterus during adolescence may have reproductive implications. The lack of complete genital tract maturity might partly account for problems such as preterm labor, preeclampsia, and small-for–gestational age infants; these problems are not uncommon in adolescence.[15]

Our data show an ovarian/uterine ratio that decreases with increasing gynecologic age. The mean value at the first gynecologic year is very similar to that found in the polycystic ovary syndrome,[16] probably because unlike the uterus, the ovaries seem to have already reached complete growth at menarche.[6] Furthermore, their volume often exceeds the adult value and needs a reduction in size to reach the final maturational step.[6] Thus ovarian development frequently follows a biphasic process in adolescence, first increasing up to menarche and afterward decreasing in size to reach the

adult pattern. The second part of the process is associated with and caused by an increase in the frequency of ovulation.[6] We previously reported that the reproductive endocrine and ovarian pattern of the first gynecologic years is often similar to that of polycystic ovary syndrome.[6] The ovarian/uterine ratio we found in this study further documents that the reproductive maturation in adolescence often includes polycystic ovary syndrome–like features that tend to disappear with the progression of postmenarcheal development.

REFERENCES

1. Peters H. The human ovary in childhood and early maturity. Eur J Obstet Gynaecol Reprod Bio 1979;9:137.
2. Simkins CS. Development of the human ovary from birth to sexual maturity. Am J Anat 1932;51:465.
3. Merril JA. The morphology of the prepubertal ovary. South Med J 1961;56:225.
4. Orsini LF, Salardi S, Pilu G, Bovicelli L, Cacciari E. Pelvic organs in premenarcheal girls; real-time ultrasonography. Radiology 1984;153:113.
5. Venturoli S, Porcu E, Fabbri R, et al. Ovarian multifollicularity, high LH and androgen plasma levels, and anovulation are frequent and strongly linked in adolescent irregular cycles. Acta Endocrin (Copenh) 1986; 111:368.
6. Venturoli S, Porcu E, Fabbri R, et al. Menstrual irregularities in adolescents: hormonal pattern and ovarian morphology. Horm Res 1986;24:269.
7. Ivarsson SA, Nilsson KO, Persson PH. Ultrasonography of the pelvic organs in prepubertal and postpubertal girls. Arch Dis Child 1983;58:352.
8. Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. Arch Dis Child 1969;44:261.
9. Mukerje B. Measurements on the human fetal uterus and ovary. Acta Obstet Gynaecol Scand 1980;59:549.
10. Nussbaum AR, Sanders RC, Douglas Jones M. Neonatal uterine morphology as seen on real-time US. Radiology 1986;160:641.
11. Krantz KE, Atkinson JP. Gross anatomy. Ann NY Acad 1967;142:551.
12. Salardi S, Orsini LF, Cacciari E, Bovicelli L, Tassoni P, Reggiani A. Pelvic ultrasonography in premenarchal girls: relation to puberty and sex hormone concentrations. Arch Dis Child 1985;60:120.
13. Sample WF, Lippe BM, Gyepes MT. Gray-scale ultrasonography of the normal female pelvis. Radiology 1977; 125:477.
14. Venturoli S, Porcu E, Fabbri R, et al. Postmenarchal evolution of endocrine pattern and ovarian aspects in adolescents with menstrual irregularities. Fertil Steril 1987; 48:78.
15. Hollingsworth DR. Hypertension and preeclampsia during adolescent pregnancy. In: Kreutner AK, Hollingsworth DR, eds. Adolescent obstetrics and gynecology. Chicago: Year Book Medical Publishers, 1978;161.
16. Orsini LF, Venturoli S, Lorusso R, Pluchinotta, Paradisi R, Bovicelli L. Ultrasonic findings in polycystic ovarian disease. Fertil Steril 1985;43:709.

Leiomyoma of the ovary with ascites and hydrothorax

Jonathan J. Nicoll, MA, and Peter J. Cox, MS
London, England

A case is described in which a leiomyoma of the ovary was accompanied by hydrothorax. There was no reaccumulation of fluid after oophorectomy. (Am J Obstet Gynecol 1989;161:177-8.)

Key words: Ovarian leiomyoma, hydrothorax

Leiomyoma arising primarily in the ovary is rare. We describe a case in which such a tumor was associated with ascites and hydrothorax, an association that has not previously been reported.

Case report

A 33-year-old woman who had been treated for supradiaphragmatic Hodgkin's disease 4 years previously complained of lower abdominal pain, chest discomfort, and shortness of breath. Examination revealed a large pelvic mass and signs of a right-sided pleural effusion. Ultrasonography and computed tomography confirmed the presence of ascites and a pelvic mass, but failed to demonstrate the organ of origin of the tumor. A chest radiograph showed a large right-sided pleural effusion.

Two liters of straw-colored fluid was aspirated from the right chest. Cytologic examination of the fluid showed mononuclear and binuclear cells with prominent nucleoli that were highly suggestive of Hodgkin's disease. Treatment was started with standard combination chemotherapy for Hodgkin's disease but there was reaccumulation of pleural fluid.

After three cycles of chemotherapy a large right-sided pleural effusion still remained and the pelvic mass was larger. It was decided to proceed to laparotomy,

From the Stanford Cade Department of Clinical Oncology, and the Department of Surgery, Westminster Hospital.
Received for publication November 18, 1988; accepted January 30, 1989.
Reprint requests: Dr. Jonathan J. Nicoll, Stanford Cade Department of Clinical Oncology, Westminster Hospital, London SW1P 2AP, England.

which was performed with an intercostal drain in situ.

At operation a 9 cm spherical tumor was found arising from the right ovary. All other organs were normal; there was no lymphadenopathy.

Right oophorectomy was performed without difficulty. Histologic examination of the specimen showed a benign leiomyoma of the ovary that was well circumscribed and composed of spindle-shaped cells with eosinophilic cytoplasm and blunt-ended nuclei. Myofibrils were visible on staining with phosphotungstic acid–hematoxylin.

The day after operation the chest radiograph was normal and the drain was removed. No further chemotherapy was given. Two years later the patient remained well with no abnormal physical findings and a normal chest radiograph.

Comment

Leiomyoma of the ovary was originally described by Sangalli in 1862, and the literature on the subject was well reviewed in 1972.[1]; at that time 28 cases had been reported. The well-recognized association between ovarian fibroma and hydrothorax (eponymously known as Meigs' syndrome) was described in 1937.[2] All seven cases in Meigs' original report had fibromas or fibromyomas. We believe our case is the first reported case in which hydrothorax was found with a leiomyoma of the ovary.

Although our patient had a history of Hodgkin's disease, the fact that pleural fluid reaccumulated rapidly despite combination chemotherapy but disappeared completely after removal of the ovarian tumor strongly suggests that the hydrothorax was caused by the tumor. We initially thought the hydrothorax was a result of a recurrence of Hodgkin's disease, but subsequent events have led us to believe that the lymphoma has not reactivated and that the cytologic appearances were caused by the presence of reactive mesothelial cells.

We thank Dr. K. A. Newton and Mr. J. M. Thomas for permission to publish this case.

REFERENCES

1. Fallanzadeh H, Dockerty MB, Lee RA. Leiomyoma of the ovary: report of five cases and review of the literature. AM J OBSTET GYNECOL 1972;113:394.
2. Meigs JV, Cass JW. Fibroma of the ovary with ascites and hydrothorax. AM J OBSTET GYNECOL 1937;33:249.

Individual growth curve standards in twins: Prediction of third-trimester growth and birth characteristics

Theodor Stefos, MD,[a] Russell L. Deter, MD,[a] Reba M. Hill, MD,[b] and Nicolas V. Simon, MD[c]

Houston, Texas, and York, Pennsylvania

The ability of Rossavik growth models, determined from measurements obtained before 24 weeks, to predict third-trimester growth and birth characteristics in normally growing twins has been investigated. Third-trimester values for head circumference, abdominal circumference, and femur diaphysis length were predicted with an accuracy of ±6% to 9% (95% to 98% of percent deviations). For thigh circumference and estimated weight, the comparable values were ±15% and ±16%, respectively. The head circumference at birth was predicted without bias; the random error was approximately ±5% (94% of percent differences). Weight, abdominal circumference, and thigh circumference were systematically overestimated (3.1%, 14.9%, and 11.3%, respectively) as a result of differences in prenatal and postnatal measurement procedures. After correction for systematic errors, these parameters could be predicted with random errors of −11.5% to 7.2% (weight), −12.8% to 5.4% (abdominal circumference), and −15.3% to 10.0% (thigh circumference). Growth Potential Realization Index values were found to have means of approximately 100% and ranges from 91% to 118%. These results are similar to those for singletons and indicate that *individual* assessment of growth in twins can be carried out with the same methods used for singletons. (Am J Obstet Gynecol 1989;161:179-83.)

Key words: Twins, growth prediction, Rossavik model

Twin pregnancies represent a high-risk condition in which growth problems frequently occur. Previous studies have evaluated twin growth through comparisons with the growth of singletons[1-5] by establishing specific growth standards for twins[3, 4, 6] or from the assessment of differences in the growth of two twin fetuses.[4-10] Growth studies concerned with comparisons with singletons and the establishment of twin growth standards[1-6] have used cross-sectional analytic methods even though serial measurements were included in the data sets ("mixed" type of growth study). This approach does not provide valid estimates of variability and does not account for differences in fetal growth potential.[11] Assessment of differences in growth between twins has also failed to account for differences in growth potential, has frequently used arbitrary criteria to determine what represents a significant growth difference, and has not provided a means for detecting a major loss of maternal support that could adversely affect both twins equally.

As with the growth of singletons, the problems associated with the evaluation of twin growth can be eliminated by the use of *individual growth curve standards*.[11] This requires specification of Rossavik growth models based on growth curves obtained in the second trimester[11, 12] Recent studies (Stefos and Deter. Unpublished data.) have shown that second-trimester growth in twins is similar to that in singletons, and no statistically significant effect of zygocity on growth could be detected. These results indicated that during the second trimester, the growth of twins could be treated like the growth of two singletons in the same mother. Such a concept of twin growth provides justification for the use of the Rossavik growth model specification procedures previously developed for singletons.[12] This article presents the results of our study of the use of Rossavik growth models in predicting growth after 24 weeks, menstrual age, and the birth characteristics of normally growing twins.

Material and methods

Sample. This study was carried out with data collected from 16 twin fetuses in 11 pregnancies. The patients studied were primarily middle-class whites (10 of 11 patients, one Hispanic) from the Houston area. The maternal age ranged from 23 to 32 years (mean, 29 years). These patients delivered fetuses between 33 and 39 weeks, menstrual age (mean [±SD]: 36.9 [±1.7] weeks), and comprehensive pediatric assessment (within 24 hours of delivery) revealed no evidence

From the Departments of Obstetrics-Gynecology[a] and Pediatrics,[b] Baylor College of Medicine, and the Department of Obstetrics-Gynecology,[c] York Hospital.

Received for publication July 26, 1988; revised January 4, 1989; accepted July 11, 1989.

Reprint requests: Russell L. Deter, MD, Department of Obstetrics-Gynecology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030.

Table I. Prediction of growth parameters after 24 weeks (twins) and 26 weeks (singletons) in normal fetuses

	Twins			*Singletons**		
		% Deviation			*% Deviation*	
Parameter	*N*	*Mean (%)*	*Range† (%)*	*N*	*Mean (%)*	*Range† (%)*
HC	94	0.5	±6 (98%)	97	0.5	±7 (97%)
AC	99	−1.7	±9 (96%)	97	0.1	±8 (95%)
FDL	99	−2.0	±7 (95%)	97	−0.1	±7 (94%)
ThC	98	2.1	±15 (94%)	92	1.1	±15 (94%)
EWT	95	1.7	±16 (96%)	97	1.9	±22 (95%)

HC, Head circumference; *AC*, abdominal circumference; *FDL*, femur diaphysis length; *ThC*, thigh circumference; *EWT*, estimated weight.

*Data for singletons previously published[12] and presented here for comparison with those for twins.

†The values in parentheses indicate the proportion of the percent deviations after 24 weeks (twins) or 26 weeks (singletons) included in the range.

of growth abnormalities or congenital anomalies. Birth characteristics such as weight (mean [±SD]: 2904 [±350] gm) length (mean [±SD]: 48.8 ± 2.0 cm), and head circumference (mean [±SD]: 33.5 [±1.1] cm) were within the normal ranges for singletons determined by Usher and McLean.[13] In 14 twin fetuses abdominal circumference (mean [±SD]: 29.6 [±2.1] cm) and thigh circumference (mean [±SD]: 14.1 [±1.1] cm) measurements were also available and within the normal ranges for singletons.[13] Nutrition score values, a measure of subcutaneous tissue, were also available for 14 twin fetuses and were above 12 (range, 13 to 16) for all but one fetus (8.5), values associated with well-nourished infants. Growth Potential Realization Index values were within the normal ranges of singletons[14] for weight and head circumference in all but two of the twin fetuses. Ten of 14 twin fetuses had abdominal circumference Growth Potential Realization Index values within the singleton normal range, whereas comparable numbers were nine of 14 for thigh circumference. There were eight male and eight female twin fetuses in this sample.

Fetal age determination. Fetal age determination was based on the first day of the last menstrual period, which was confirmed by first-trimester and early second-trimester ultrasound examinations (crown-rump length, biparietal diameter, and femur diaphysis length measurements)[15] in 13 of 16 fetuses. In three fetuses, fetal age estimates determined by ultrasound examination and last menstrual period did not agree. The ultrasound age estimates (based on early second-trimester scans) were used in these cases. Birth ages derived from obstetric data were similar to those determined postnatally by either the Dubowitz or Ballard methods (mean differences [±SD]: 1.0 [±1.1] weeks and 1.4 [±1.4] weeks, respectively) for the 14 twin fetuses in whom estimation of birth age with obstetric and pediatric methods was carried out. However, the differences in age estimates were significantly different from zero (t test; $p < 0.05$).

Ultrasound examinations. The ultrasound examinations were carried out at 2-week intervals from 15 weeks, menstrual age (initial scan [mean ± SD]: 14.7 ± 1.7 weeks), to 36 weeks (last scan [mean ± SD]: 35.9 ± 1.5 weeks). The interval between the last scan and delivery was (mean ± SD) 1.0 ± 0.5 week. Each twin was identified at each examination by its position in the uterus, its size, or its sex. Measurements of the biparietal diameter, the long and short axes of the head and abdominal profiles, as well as measurements of the femur diaphysis length and thigh circumference, were obtained at each examination for each twin with use of procedures described in previous publications.[16, 17] The head and abdominal circumferences were calculated from the corresponding diameters,[17] and the estimated weight was determined from values of the head and abdominal cubes as previously described.[18]

Evaluation of placentas and fetal membranes. Examination[19] of the placentas and fetal membranes in these twin pregnancies indicated that eight of the fetuses were from dizygotic pregnancies and eight were from monozygotic pregnancies.

Data analysis. Values for start points and the coefficients k, c, and s of the Rossavik growth model [$P = c(t)^{k + s(t)}$] were obtained, as indicated in a previous publication,[12] for the head circumference, abdominal circumference, femur diaphysis length, thigh circumference, and head and abdominal cubes from measurements obtained before 24 weeks' menstrual age. The models specified by these data were used to predict appropriate values for each parameter during the third trimester and at birth for head circumference, abdominal circumference, thigh circumference, and estimated weight. Birth predictions for fetuses delivering after 38

Table II. Prediction of birth characteristics in normal twin and singleton fetuses

	Twins			*Singletons**		
		% Difference			*% Difference*	
Parameter	*N*	*Mean (%)*	*Range† (%)*	*N*	*Mean (%)*	*Range† (%)*
HC	16	0.0‡	−2.0- 5.9	20	1.5‡	−4.9- 6.3
AC	14	14.9§	2.7-23.8	20	17.3§	6.1-23.5
ThC	14	11.3§	−4.3-24.3	20	6.4§	−4.1-16.9
EWT	16	3.1§	−8.2-10.7	20	1.0‡	−7.7-10.1

HC, Head circumference; *AC,* abdominal circumference; *FDL,* femur diaphysis length; *ThC,* thigh circumference; *EWT,* estimated weight.

*Data for singletons published previously[14] and presented here for comparison with those for twins.

†Range contains 100% (abdominal circumference, thigh circumference: twins), 94% (head circumference, estimated weight: twins), or 95% (all parameters: singletons) of the percent differences.

‡Not significantly different from zero ($p > 0.05$).

§Significantly different from zero ($p < 0.05$).

weeks (six of 16) were those for 38 weeks, since no growth after 38 was assumed.[14]

The ability of models derived from data obtained before 24 weeks, menstrual age, to predict fetal growth in the third trimester was evaluated by comparing actual measurements at various time points to predicted values and expressing the differences as a percentage of the predicted values (percent deviations). Means (systematic prediction errors) and ranges (random prediction errors) for the percent deviations were determined.

To evaluate the ability of these models to predict birth characteristics, predicted birth head circumference, abdominal circumference, thigh circumference, and estimated weight values were compared with actual measurements at birth, and the differences were expressed as a percentage of the actual measurements (percent differences). The means and standard deviations of these percent differences were determined, and the mean values were compared with zero by Student *t* test to identify systematic errors. The relationships between the percent difference and birth age for each of the four parameters studied were evaluated by linear regression analysis.

To provide standards for assessing pregnancy outcome on an *individual* basis, Growth Potential Realization Index (GPRI) values were calculated for each parameter in each twin fetus.[14] Growth Potential Realization Index values are determined by means of the following equation:

$$\text{GPRI} = \frac{\text{Actual measurement at birth}}{\text{Predicted measurement at birth}} \times 100$$

For parameters in which a systematic difference between the predicted and actual birth measurements was found, predicted birth measurements corrected for the systematic error were used in the calculation of Growth Potential Realization Index values.[14] Growth Potential Realization Index values take into account differences in genetic growth potential.

Results

Prediction of growth in the third trimester. As shown in Table I, systematic errors in predicting third-trimester growth were small for all parameters (−2.0% to 2.1%). The random prediction errors were also small (±6% to 9%) for the one-dimensional parameters (head circumference, abdominal circumference, and femur diaphysis length) except for thigh circumference [±15%]). For estimated weight, a three-dimensional parameter, the random prediction error, was smaller (±16%) than would be expected (±21% to 22%) on the basis of random prediction errors observed for one-dimensional parameters.

Prediction of birth characteristics. Although based on models derived from data obtained 9 to 15 weeks before delivery (or more), the Rossavik growth models specified in this investigation predicted the birth characteristics of twin fetuses well. No systematic prediction errors were found for head circumference, but the weight, abdominal circumference, and thigh circumference were overestimated (3.1%, 14.7%, and 13.1%, respectively). As can be seen in Table II, such overestimations have also been observed in singleton fetuses for abdominal circumference and thigh circumference. These errors can be explained primarily on the basis of differences in the prenatal and postnatal techniques for obtaining measurements of these three parameters.[14, 18] Correction for these systematic errors (correction factors for weight, abdominal circumference, and thigh circumference: 0.969, 0.851, and 0.887, respectively[14]) resulted in random prediction errors that ranged (100% of percent differences) from −11.5% to 7.2% for weight, −12.8% to 5.4% for ab-

Table III. Growth potential realization index values in normal twin and singleton fetuses

Parameter	Twins GPRI			Singletons† GPRI		
	N	Mean (%)	Range* (%)	N	Mean (%)	Range* (%)
HC	16	100‡	94-102	20	99‡	95-105
AC	14	103‡	95-115	20	102‡	91-107
ThC	14	102‡	91-118	20	101‡	91-110
EWT	16	101‡	93-108	20	99‡	91-108

GPRI, Growth Potential Realization Index; *HC*, head circumference; *AC*, abdominal circumference; *ThC*, thigh circumference; *EWT*, estimated weight.

*Range includes 100% (abdominal circumference, thigh circumference: twins), 94% (head circumference, estimated weight: twins), or 95% (all parameters: singletons) of the GPRI values.

†Data for singletons published previously[14] and presented here for comparison with those for twins.

‡Not significantly different from 100% ($p > 0.05$).

dominal circumference, and −15.3% to 10.0% for thigh circumference.

Linear regression analysis failed to reveal any relationship between the percent difference and birth age over the 33- to 39-week interval in which delivery of these twin fetuses occurred. The respective R^2 values were 1.8% for head circumference, 0.0% for abdominal circumference, 0.0% for thigh circumference, and 0.0% for weight.

Growth Potential Realization Index. Table III presents the means and ranges of the Growth Potential Realization Index for the four parameters (head circumference, abdominal circumference, thigh circumference, and weight) evaluated postnatally in these twin fetuses. Corrected predicted values for weight, abdominal circumference, and thigh circumference were used in these calculations. The mean Growth Potential Realization Index values were not significantly different from 100%, and individual values ranged from 91% and 118% depending on the parameter being studied. The lowest variability in Growth Potential Realization Index values was seen with the head circumference (94% to 102%).

Comment

In evaluating the performance of Rossavik models in predicting growth in twins, it is essential that such assessment be carried out under conditions in which poor model performance can be detected. Because evaluation of growth predictions was based on the degree of agreement between actual measurements and predicted values, deviations caused by abnormal growth must be excluded if at all possible. This is a particular problem with twins, since twin fetuses have a much higher incidence of growth abnormalities and tend to deliver prematurely. The twins studied in this investigation were close to term in most cases (birth age: 36.9 [± 1.7 SD] weeks), and a variety of postnatal measurements were within normal ranges for singletons. In addition, most postnatal measurements were in agreement with predicted values if the criteria developed for singletons (Growth Potential Realization Index values [see Table III]) were used. These data strongly support the conclusion that growth in these twins was normal, and thus significant deviations from predicted values would have to be the result of poor model performance and not growth abnormalities.

Rossavik model performance in twins can best be judged by comparison with that seen in singletons (Tables I to III). With the exception of a small systematic overestimation of the abdominal circumference and femur diaphysis length values, the ability of Rossavik models to predict growth in the third trimester was essentially the same as that seen in singletons for all five parameters. The ability to predict birth characteristics was also similar in twins and singletons with the exception of the thigh circumference and weight, in which greater systematic overestimation was seen in twins. The reason for these differences is currently unknown, but the difference for weight was small, and specification of Rossavik models for the thigh circumference is difficult because of measurement problems in the second trimester that require the use of a fixed start point.[20] Leg position may also affect this measurement.[14]

With respect to Growth Potential Realization Index values, mean values were close to 100% for all parameters in both twins and singletons, and the ranges were similar in both groups for head circumference and weight. A small shift to higher values was seen with abdominal circumference and thigh circumference. However, these two parameters required greater correction of predicted values for systematic overestimations, and the correction factors for twins were based on fewer measurements than those for singletons. Also, the 100% range is presented for twins to compensate for the smaller number of values available, whereas the 95% range is given for singletons. In any case, there

does not appear to be major differences between twins and singletons with respect to Growth Potential Realization Index values.

Since use of Rossavik growth models *individualizes* the assessment of growth, no direct comparisons can be made with previous cross-sectional (actually "mixed") growth studies in twins. However, our results are consistent with those indicating similarities in the normal growth of twins and singletons[4, 5] and suggest that those investigations reporting differences[1-3] may have included normally and abnormally growing twins in the sample studied. The postnatal evaluation of twin fetuses in some investigations has been limited to birth weight assessment[1, 5] or not reported.[2] More extensive evaluation of the newborn twin fetuses (weight, head circumference, and length) indicated no significant differences in head circumference and length between twins and singletons, but the birth weights were significantly lower in twins.[3, 4]

The results of these investigations indicate that *individualized* assessment is as effective with twins as it is with singletons. Subsequent publications will use this approach in the detection, characterization, and establishment of relationships in abnormally growing twins.

REFERENCES

1. Leveno KJ, Santos-Ramos R, Duenholter JH, et al. Sonar cephalometry in twins. A table of biparietal diameters for normal twin fetuses and a comparison with singletons. AM J OBSTET GYNECOL 1979;135:727-30.
2. Haines CJ, Langlois SL, Jones WR. Ultrasonic measurement of fetal femoral length in singleton and twin pregnancies. AM J OBSTET GYNECOL 1986;155:838-41.
3. Socol ML, Tamura RK, Sabbagha RE, Chen T, Vaisrub N. Diminished biparietal diameter and abdominal circumference growth in twins. Obstet Gynecol 1984;64:235-8.
4. Crane JP, Tomich PG, Kopta M. Ultrasonic growth patterns in normal and discordant twins. Obstet Gynecol 1980;55:678-83.
5. Secher NJ, Kaern J, Hansen PK. Intrauterine growth in twin pregnancies: prediction of fetal growth retardation. Obstet Gynecol 1985;66:63-8.
6. Yarkoni S, Reece AE, Holford T, O'Connor TZ, Hobbins JC. Estimated fetal weight in the evaluation of growth in twin gestations: a prospective longitudinal study. Obstet Gynecol 1987;69:636-9.
7. Storlazzi EV, Vintzileos AM, Campbell WA, et al. Ultrasonic diagnosis of discordant fetal growth in twin gestations. Obstet Gynecol 1987;69:363-7.
8. Blickstein I, Shohan-Schwartz Z, Lancet M, Borenstein R. Characterization of the growth discordant twin. Obstet Gynecol 1987;70:11-5.
9. O'Brien WF, Knuppel RA, Scerbo JC, Ratton PK. Birth weight in twins: an analysis of discordancy and growth retardation. Obstet Gynecol 1986;67:483-6.
10. Erkkola R, Ala-Mello S, Piiroinen O, Kero P, Silllanpaa M. Growth discordancy in twin pregnancies: a risk factor not detected by measurements of biparetial diameter. Obstet Gynecol 1985;66:203-6.
11. Deter RL, Rossavik IK, Harrist RB, Hadlock FP. Mathematical modeling of fetal growth: development of individual growth curve standards. Obstet Gynecol 1986; 68:156-61.
12. Deter RL, Rossavik IK. A simplified method for determining individual growth curve standards. Obstet Gynecol 1987;70:801-6.
13. Usher R, McLean F. Intrauterine growth of liveborn caucasian infants at sea level: standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. J Pediatr 1969;74:901-10.
14. Deter RL, Hill RM, Tennyson LM. Predicting the birth characteristics of normal fetuses 14 weeks before delivery. J Clin Ultrasound 1989;17:89-93.
15. Hadlock FP, Deter RL, Harrist RB, et al. Estimating fetal age: computer assisted analysis of multiple fetal growth parameters. Radiology 1984;152:497-502.
16. Deter RL, Hadlock FP, Harrist RB. Evaluation of fetal growth and the detection of intrauterine growth retardation. In: Callen PW, ed. Ultrasonography in obstetrics and gynecology. Philadelphia: WB Saunders, 1983:113-40.
17. Stefos T, Deter RL. Individual growth curve standards for fetal head and abdominal circumference: effect of the type of measurement on growth prediction. J Clin Ultrasound 1989;17:33-6.
18. Deter RL, Rossavik IK, Harrist RB. Development of individual growth curve standards for estimated weight. I. Weight estimation procedure. J Clin Ultrasound 1988; 16:215-25.
19. Benirschke K. Accurate recording of twin placentation. A plea to the obstetrician. Obstet Gynecol 1981;18:334-47.
20. Deter RL, Rossavik IK, Cortissoz C, Hill RM, Hadlock FP. Longitudinal studies of thigh circumference growth in normal fetuses. J Clin Ultrasound 1987;15:388-93.

Effect of birth weight, race, and sex on survival of low-birth-weight infants in neonatal intensive care

Michael B. Resnick, EdD,[a] Randy L. Carter, PhD,[b] Mario Ariet, PhD,[c] Richard L. Bucciarelli, MD,[a] Janet H. Evans, MSPA,[d] Robert R. Furlough, PhD,[d] William W. Ausbon, MD,[d] and John S. Curran, MD[e]

Gainesville, Tallahassee, and Tampa, Florida

Survival for low-birth-weight infants has traditionally been analyzed by birth weight categories spanning considerable ranges of weight. We developed a finer description of survival rates to allow estimation of survival percentages for infants of any specific birth weight between 500 and 2500 gm. Our sample consisted of 16,183 infants treated in tertiary neonatal intensive care between 1980 and 1987. Their survival data were analyzed by 50 gm increments between 500 and 2500 gm, and a continuous survival curve was constructed by log linear regression methods. Mortality differences between males and females and blacks and whites were analyzed. Survival for females was higher than males between 500 and 1500 gm and higher for blacks than whites between 650 and 1500 gm. Between 1500 and 2500 gm, no significant effects of birth weight, race, or sex were observed, with survival remaining stable at approximately 95% across all combinations of variables. (AM J OBSTET GYNECOL 1989;161:184-7.)

Key words: Neonatal survival, neonatal mortality, low birth weight, prematurity, neonatal intensive care

For two decades research has verified that low birth weight is the major contributor to infant mortality in the United States.[1-7] Traditionally, survival for low-birth-weight infants has been analyzed using arbitrarily defined categories—most often, <1000 gm, 1000 to 1500 gm, and 1500 to 2500 gm. The creation of artificial groups spanning considerable birth weight ranges has been necessitated by the limited sample sizes available to most researchers. National statistics on low-birth-weight infants have employed even cruder birth weight categories, usually restricted to <1500 gm and 1500 to 2500 gm.[2,8]

The purpose of our research was to analyze neonatal survival statistics for low-birth-weight infants admitted to the State of Florida's Perinatal Intensive Care Program for the period 1980 to 1987 (a total of 16,183 infants weighing 500 to 2500 gm at birth). The data were analyzed by 50 gm increments to yield a more precise description of survival rates than any currently available. We developed a statistical model to estimate the probability of survival for any specific birth weight.

The mortality differences between males and females, and blacks and whites, were an additional focus of study. The survival edge of girls over boys has long been known, but its magnitude in low-birth-weight infants has not been documented. The mortality of black neonates has been reported as 20 per 1000 live births, compared to only 10 per 1000 for whites.[9] Consequently, we used the same 50-gm increments described above to analyze survival data by race and sex.

Material and methods

Patients. The low-birth-weight infants whose data were used for the study received tertiary neonatal intensive care in the State of Florida's Perinatal Intensive Care Program from July 1980 through June 1987. During this period approximately 27,000 infants were admitted to the Program's 10 neonatal intensive care units; approximately two thirds of them were inborn and the remainder were transported. Of these, 16,183 infants weighed <2500 gm at birth and were included in the study: 7231 weighed 500 to 1500 gm and 8952 weighed 1500 to 2500 gm. To be counted as a survivor, an infant had to survive the period of neonatal intensive care and subsequent hospitalization in a step-down facility or in the referring hospital. Descriptive statistics appear in Table I.

Statistical methods. Observed survival rates were analyzed with log linear regression models. These models

From the Departments of Pediatrics,[a] Statistics,[b] and Medicine,[c] University of Florida, Gainesville, Children's Medical Services, Department of Health and Rehabilitative Services, State of Florida,[d] Tallahassee, and the University of South Florida College of Medicine,[e] Tampa.

Supported in part by Children's Medical Services, Department of Health and Rehabilitative Services, State of Florida.

Received for publication June 9, 1988; revised January 1, 1989; accepted January 23, 1989.

Reprint requests: Michael B. Resnick, EdD, Neonatology Division, Department of Pediatrics, Box J-296, JHMHC, University of Florida, Gainesville, FL 32610.

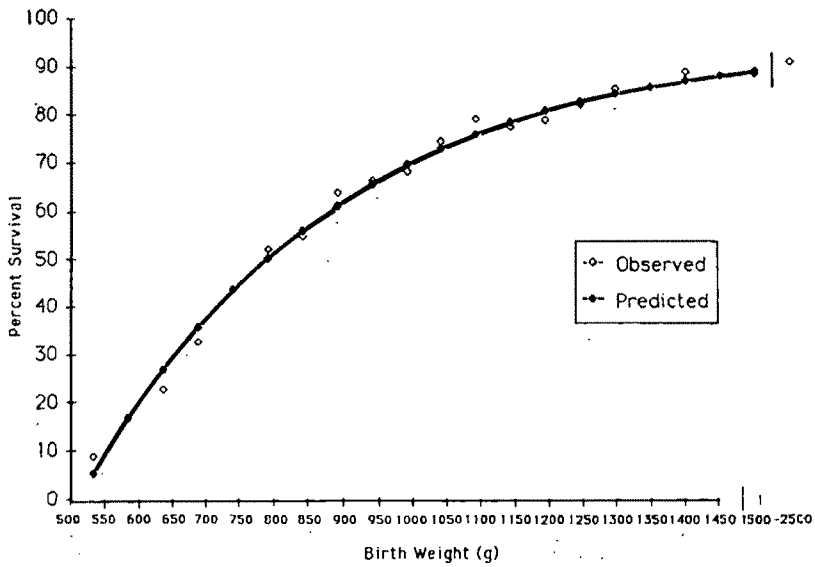


Fig. 1. Curve showing estimated probability of survival by birth weight, with 50 gm increments from 500 gm to 1500 gm. Each point is plotted midway through its birth weight interval. For example, the point for infants weighing 500 to 550 gm is plotted at 525 gm. Subsample sizes are shown in Table II.

Table I. Clinical description of low-birth-weight sample

Descriptor	*Birth weight categories*	
	<1500 gm (n = 7231) (% of sample)	*1500-2500 gm (n = 8952) (% of sample)*
Delivery information		
Male/female	51/49	55/45
Black/white	52/48	43/57
Small for gestational age	11	9
1 min Apgar score <6	69	42
5 min Apgar score <6	42	16
Vaginal/cesarean delivery	60/40	60/40
Inborn/transported	68/32	69/31
Procedures		
Major surgery	7	4
Assisted ventilation	63	33

were chosen because curves constructed with log linear regression methods have been demonstrated to fit survival data better than those constructed with logistic regression.[10] A model was used to fit curves to the observed proportions of survival for 50 gm increments from 500 to 1500 gm and another (similar) model to fit survival curves for the increments from 1500 to 2500 gm. These models were specified to yield a survival probability curve for each race-by-sex combination: black females, black males, white females, and white males.

Results

The data demonstrated significant effects of birth weight on survival from 500 to 1500 gm, with the probability of survival increasing until 1500 gm was reached (Fig. 1). Sex and race also affected survival significantly (Fig. 2). At any given birth weight up to 1500 gm, survival rates were better for females than for males, reaching a maximum difference of 10 to 14 percentage points between 500 and 1100 gm. A similar pattern emerged for blacks and whites, although white survival was greater at the lowest birth weights (500 to 650 gm). Improved survival rates for black infants emerged at 650 gm, with a maximum difference of 6 to 7 percentage points between blacks and whites reached between 800 and 1250 gm. The differences between males and females were the same for blacks and whites over all birth weights.

Analyses of data for infants who were inborn versus those who were transported showed no significant survival differences. Furthermore, no statistically

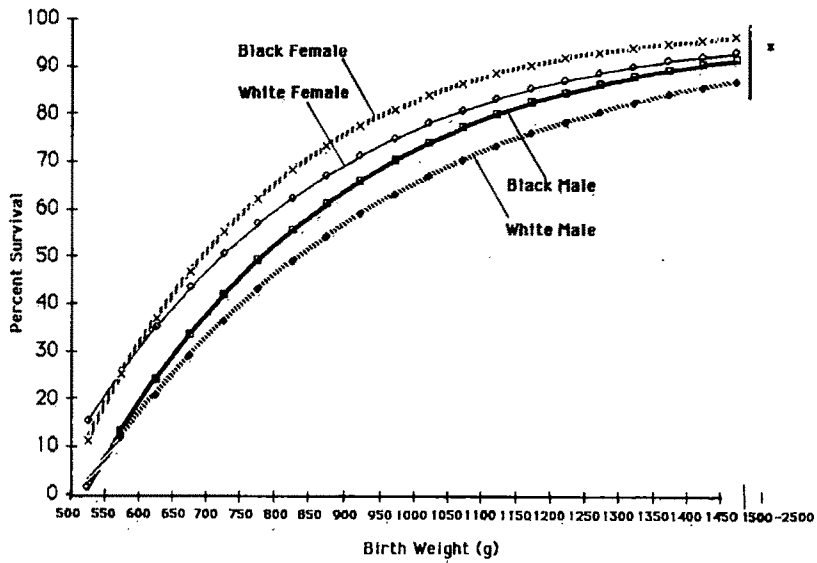


Fig. 2. Comparison of survival data for low-birth-weight infants by race, sex, and birth weight, with 50 gm increments from 500 to 1500 gm. Each point is plotted midway through its birth weight interval. For example, the point for infants weighing 500 to 550 gm is plotted at 525 gm. A measure of goodness of fit of these curves, analogous to the usual R^2 coefficient,[11] was 0.97.

Table II. Subsample size for each birth weight category, 500 to 1500 gm

Category No.	*Birth weight category (gm)*	*Sample size (N)*
1	500-550	132
2	550-600	198
3	600-650	229
4	650-700	274
5	700-750	317
6	750-800	344
7	800-850	323
8	850-900	349
9	900-950	354
10	950-1000	493
11	1000-1050	369
12	1050-1100	425
13	1100-1150	387
14	1150-1200	472
15	1200-1250	421
16	1250-1300	486
17	1300-1350	410
18	1350-1400	549
19	1400-1450	506
20	1450-1500	565

significant interactions were found between inborn/transport status and race, sex, or birth weight category.

Infants weighing 1500 to 2500 gm at birth were not significantly affected by birth weight, race, or sex, or their interactions. Survival in these infants was nearly constant (approximately 94.5%) across all combinations of variables.

Equation for probability of survival. Equations were derived for the fitted model for each race-by-sex combination to predict probability of survival for infants weighing ≤1500 gm at birth.

Black females:
$$\text{Log } (P_m) = 0.0548 - 0.1702 \text{ BWC}$$
Black males:
$$\text{Log } (P_m) = 0.1293 - 0.1348 \text{ BWC}$$
White females:
$$\text{Log } (P_m) = -0.0261 - 0.1352 \text{ BWC}$$
White males:
$$\text{Log } (P_m) = 0.0484 - 0.1098 \text{ BWC}$$

where BWC = birth weight group (for example, 1 = 500 to 550 gm, 2 = 550 to 600 gm, etc., to 20 = 1450 to 1500 gm) (Table II) and P_m = probability of death before hospital discharge.

$$\text{Probability of survival} = 1 - e^{\log(P_m)}$$

Examples. A black female weighing 1215 gm is admitted to the neonatal intensive care unit on the same day as a white male weighing 1240 gm. Their probabilities of survival are calculated below:

Black female: $\text{Log } (P_m) = 0.0548 - 0.1702 \times 15$
$= 0.0548 - 2.5531$
$= -2.4983$
Probability of survival $= 1 - e^{\log (P_m)}$
$= 1 - e^{(-2.4983)}$
$= 0.92$

White male: $\text{Log } (P_m) = 0.0484 - 0.1098 \times 15$
$= 0.0484 - 1.6464$
$= -1.5980$
Probability of survival $= 1 - e^{\log(P_m)}$
$= 1 - e^{(-1.5980)}$
$= 0.80$

Comment

Previous analyses of neonatal survival that were based on birth weight used broad, arbitrarily defined groups, such as 750 to 1000 gm, 1000 to 1500 gm, etc. Although these birth weight ranges have been dictated by some clinical rationale, their predictive value has been limited by the obvious differences in survival outlook for infants at the high and low ends of the ranges. In the 1000 to 1500 gm category, for example, an infant weighing 1000 gm has about a 75% chance of surviving, whereas one weighing 1500 gm has almost a 95% chance. The continuous curves produced by this statistical model permit estimation of the probability of survival for infants with birth weights between 500 and 2500 gm. If the curves are used to estimate survival probability prenatally with ultrasonographic data, possible measurement error must be taken into consideration.

A refinement of our model is its breakdown by sex and race. This division is critical to the accuracy of the model as a predictive tool. Our continuous curves describing neonatal survival demonstrate that race and sex effects are important. For example, at 1025 gm, 68% of white males survive compared with 84% of black females. The accuracy of survival estimates improves with the use of race-by-sex descriptors, i.e., black female, black male, white female, and white male.

The differences in infant mortality between blacks and whites have gained increasing attention.[1, 9] Data show that infant deaths are twice as high among blacks (18 deaths per 1000 live births, compared with only 9 per 1000 births for whites).[8] During the neonatal period, mortality figures are approximately 12 deaths per 1000 for blacks and 7 deaths per 1000 for whites.[12] The latter discrepancy can be explained primarily by the fact that blacks have twice the incidence of low birth weight as whites, a condition that carries a high mortality. Black infants weighing <2500 gm at birth have been found to have higher survival rates than white infants in this weight range.[12] In our study survival of black infants between 650 and 1500 gm was consistently higher than that of whites, reaching a difference of 6 to 7 percentage points between 800 and 1250 gm. Consequently, further reductions in neonatal mortality for the black population will probably depend on programs that lower the incidence of prematurity.[13, 14]

Race and sex had no significant effects on survival for infants weighing >1500 gm at birth. At this weight, the survival curves for black females, black males, white females, and white males admitted to the neonatal intensive care unit converged to the same ceiling, approximately 95% survival. Increases in birth weight >1500 gm had no significant effect on survival. These results suggest that the conventional clustering of infants weighing 1500 to 2500 gm into a single birth weight category is useful prognostically for babies in the neonatal intensive care unit. However, for infants weighing <1500 gm at birth a more detailed description by race, sex, and birth weight is desirable.

We thank Barbara G. Cox, Biomedical Editor, for her assistance in the preparation of the paper.

REFERENCES

1. McCormick MC. Contribution of low birth weight to infant mortality and childhood morbidity. N Engl J Med 1985;312:82-90.
2. Committee to study prevention of low birth weight, Institute of Medicine. Preventing Low Birth Weight. Washington, DC: National Academy Press, 1985:1-3, 53.
3. Buckwald S, Zorn WA, Egan EA. Mortality and follow-up data for neonates weighing 500 to 800 g at birth. Am J Dis Child 1984;138:779-82.
4. Raju TNK. An epidemiologic study of very low and very very low birth weight infants. Clin Perinatol 1986;13:233-50.
5. Worthington D, Davis LE, Grausz JP, Sobocinski K. Factors influencing survival and morbidity with very low birth weight delivery. Obstet Gynecol 1983;62:550-5.
6. Goldenberg RL, Koski J, Ferguson C, Wayne J, Hale CB, Nelson KG. Infant mortality: relationship between neonatal and postneonatal mortality during a period of increasing perinatal center utilization. J Pediatr 1985;106:301-3.
7. Lee K, Paneth N, Gartner LM, Pearlman MA, Gruss L. Neonatal mortality: an analysis of the recent improvement in the United States. Am J Public Health 1980;70:15.
8. Wegman ME. Annual summary of vital statistics—1985. Pediatrics 1986;78:983-94.
9. Secretary's task force on black and minority health. Infant mortality and low birthweight. Washington, DC: Department of Health and Human Services, 1986:1-2.
10. Tompkins ME, Alexander GR, Jackson KL, Hornung CA, Altekruse JM. The risk of low birth weight: alternative models of neonatal mortality. Am J Epidemiol 1985;122:1067-79.
11. Willett JB, Singer JB. Another cautionary note about R^2: its use in weighted least-squares regression analysis. Am Statistician 1988;42:236-8.
12. Infant mortality among black Americans. MMWR 1987;36:1-10.
13. Rinke CM. Infant mortality and the low-birth-weight infant. JAMA 1985;253:826.
14. Task force on infant mortality. Statement on infant mortality. Pediatrics 1986;78:1155-60.

Effects of maternal hyperoxia on the biophysical assessment of fetuses with suspected intrauterine growth retardation

David A. Ruedrich, MD, Lawrence D. Devoe, MD, and Nancy Searle, BA
Augusta, Georgia

Fifty-two third-trimester fetuses with suspected intrauterine growth retardation were studied by means of a computer-assisted biophysical assessment scheme, which used maternal hyperoxia as a diagnostic probe. Biophysical data examined included percentage of time spent in breathing, mean breath rate, and percentage of time spent in body movement during a 90-minute observation: 30-minute baseline, 15-minute hyperoxia, and 45-minute posthyperoxia. Mean baseline movement in fetuses found as neonates to have intrauterine growth retardation ($N = 18$) was significantly lower than that of normal fetuses ($N = 34$). Fetal body movements and breathing movements increased significantly after hyperoxia in the intrauterine growth retardation group but not in the normal group. Increases of more than 75% over baseline for fetal breathing and more than 250% over baseline for fetal body movements yielded a sensitivity of 56%, specificity of 94%, positive predicative value of 83%, and negative predicative value of 80%. Maternal hyperoxia during biophysical testing may improve the accuracy of detecting intrauterine growth retardation. (Am J Obstet Gynecol 1989;161:188-92.)

Key words: Intrauterine growth retardation, fetal breathing, fetal movement, maternal hyperoxia

Biophysical testing, which includes fetal breathing movements and gross body movements, has been used to assess the high-risk fetus.[1] Alterations in fetal breathing or body movement patterns may improve the detection of intrauterine compromise. Intrauterine growth retardation (IUGR) has been associated with decreased levels of fetal breathing activity, which may be related to fetal hypoxia stemming from placental insufficiency.[2] Earlier reports concerning biophysical activities in growth-retarded fetuses have provided inconsistent data. Ritchie and Lakhania[3] proposed that maternal oxygen administration, which resulted in maternal hyperoxia, might enhance breathing activity of growth-retarded fetuses. Maternal hyperoxia has been shown to increase fetal capillary P_{O_2} during the intrapartum period[4]; however, normal term fetuses do not exhibit significant alterations in fetal breathing or movement patterns after maternal hyperoxia.[5]

Because IUGR is a significant perinatal problem, accurate detection of this condition is important for antenatal management. The purpose of this study was to determine whether growth-retarded fetuses demonstrated predictable alterations in their biophysical behavior after maternal hyperoxia. If diagnostic thresholds for such alterations in breathing or body movement incidence existed, discrimination of growth-retarded fetuses from normal fetuses might then be improved.

From the Department of Obstetrics and Gynecology, the Medical College of Georgia.
Received for publication June 27, 1988; revised December 16, 1988; accepted January 23, 1989.
Reprint requests: David A. Ruedrich, MD, Department of Obstetrics and Gynecology, the Medical College of Georgia, Augusta, GA 30912-3710.

Material and methods

Fifty-two third-trimester fetuses suspected of IUGR and whose mothers received care at the Medical College of Georgia Hospital and Clinics were included in the study. Abnormal ultrasonographic growth curves, head-to-abdomen circumference ratios, or estimated fetal weight below the tenth percentile for gestational age served as a basis for inclusion in this study. All fetuses had gestational age previously established by clinical dating with ultrasonographic corroboration before the twentieth week of gestation, were singletons, and had no congenital anomalies. No pregnancy was complicated by glucose intolerance.

Informed consent was obtained from each mother before the performance of each study (investigation protocol approved by the Human Assurance Review Committee). Each study was conducted under standardized conditions that began 2 hours postprandial, between 8:30 and 10:30 AM. Smoking was not permitted for 12 hours before the study, and subjects abstained from central nervous system depressants or stimulants. Observations were made in a quiet room with the patient in a semirecumbent position. Each study lasted 90 minutes: a 30-minute baseline control period, a 15-minute period of maternal hyperoxia, and a 45-minute posthyperoxia observation period. Mater-

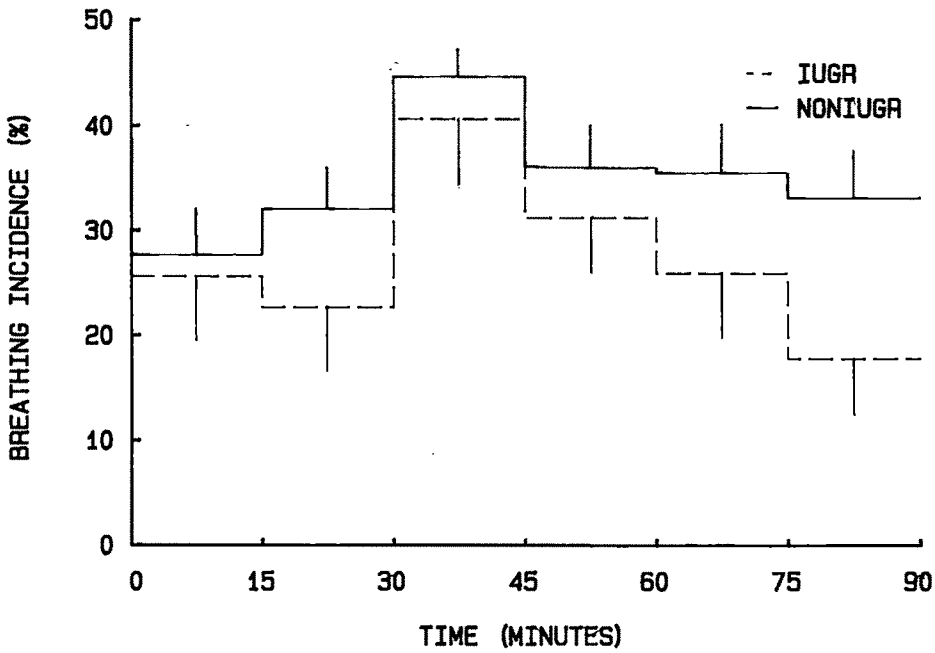


Fig. 1. Fetal breathing movements before and after maternal hyperoxia. Fetal breathing increased significantly in IUGR group during supplemental maternal oxygen administration (time = 30 minutes).

Table I. Characteristics of study population

	IUGR (n = 18)	*Normal (n = 34)*	*p value*
Fetal weight (gm)	2278 ± 370	2894 ± 514	<0.0001
Placental weight (gm)	482 ± 107	538 ± 110	NS
Fetal weight:placental weight	0.68 ± 0.03	0.70 ± 0.03	NS
EGA test (wk)	36.5 ± 1.9	36.4 ± 3.0	NS
EGA delivery (wk)	38.8 ± 2.2	38.5 ± 2.2	NS
1 min Apgar	7.67 ± 1.86	7.82 ± 1.40	NS
5 min Apgar	8.89 ± 0.31	8.94 ± 0.42	NS
$TcPO_2$ (mm Hg; 50% FiO_2)	114 ± 20.5	116 ± 17.6	NS

Results presented as means ± standard deviations.
EGA, Estimated gestational age; *$TcPO_2$*, transcutaneous oxygen pressure; *FiO_2*, fraction of inspired oxygen.

nal hyperoxia was established by means of a 50% concentration of inspired oxygen delivered by Venturi mask and documented with a Novametrix 818 continuous transcutaneous oxygen ($TcPO_2$) monitor. With this technique, maternal hyperoxia was promptly established in each case within 5 minutes of oxygen administration.

Observation of fetal breathing and movement was performed with an ATL 4000 linear array real-time ultrasound scanner operating at 3.5 MHz. A sagittal view of the fetal thorax and abdomen was obtained for optimal observation of fetal breathing and trunk flexion-extension movements. These data were entered via a remote event marker into an IBM XT microcomputer (512 K RAM) with specially designed software. This system has been previously described.[6]

Each infant was evaluated at delivery by a neonatologist for the presence of IUGR. Infants were grouped into IUGR or normal growth categories on the basis of an institutional nomogram, with the tenth percentile for weight according to gestational age as the discrimination point. Apgar scored at 1 and 5 minutes and abnormal intrapartum and neonatal outcomes were recorded.

Analysis of fetal breathing and body movement incidences (percentage of time spent in activity) and fetal breathing rates were performed off-line for each 15-minute interval of study. The two 15-minute baseline (30 minutes total) segments were grouped together for purposes of comparison. Statistical analysis used included Student *t* tests for comparing IUGR with normal fetuses and analysis of variance to examine alterations

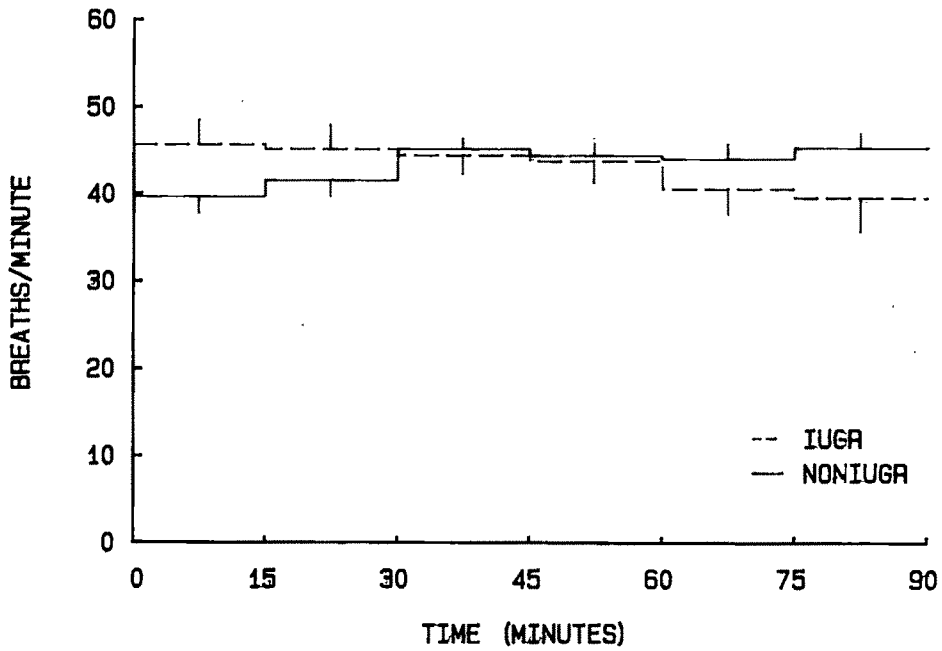


Fig. 2. Fetal breath rates before, during, and after maternal hyperoxia (given at time = 30 minutes). No significant alterations were observed.

of biophysical parameters within each group. A p value of <0.05 was considered significant.

Results

Eighteen of the 52 fetuses (34.6%) were growth retarded according to institutional criteria. Characteristics concerning gestational age at the time of study and delivery, as well as birth weight and Apgar scores for IUGR and normal fetuses, are shown in Table I. Both groups were similar in all characteristics except for mean birth weight, which was significantly lower, as expected, in the IUGR group. No perinatal deaths or significant neonatal morbidity occurred in either group.

Observation of fetal breathing movements yielded similar mean incidences of breathing activity for both groups during the baseline period. Mean fetal breathing movement incidence appeared to increase in both groups during the hyperoxia interval, but was significantly elevated over baseline values ($p < 0.05$) in the IUGR group alone (Fig. 1). Mean fetal breathing incidence decreased rapidly in the IUGR group and was significantly lower in the IUGR group during the final 15-minute period when compared with normal fetuses ($p < 0.05$). Mean fetal breathing rates were similar for both groups and were unchanged during and after hyperoxia (Fig. 2). Biophysical parameter alterations appeared to be unrelated to the transcutaneous oxygen levels achieved.

Baseline mean fetal body movement incidence was significantly lower in fetuses with IUGR when compared with the normal group ($p < 0.05$). After maternal hyperoxia, mean fetal body movement incidence increased significantly in the IUGR group ($p < 0.02$), achieving a maximal level at 30 minute after hyperoxia (Fig. 3). This alteration in the incidence of body movements was not observed in the normal group of fetuses.

Analysis of these data with receiver-operating characteristic plots yielded optimal discrimination points for both fetal breathing and body movement responses after maternal hyperoxia. Using an increase of more than 75% over mean baseline fetal breathing incidence and an increase of more than 250% over mean baseline fetal movement incidence, sensitivity was 56% (10 of 18); specificity, 94% (32 of 34); positive predictive value, 83% (10 of 12); and negative predictive value, 80% (32 of 40). Grouping the IUGR fetuses according to subtype (symmetric or asymmetric) did not improve these diagnostic values.

Comparison between the IUGR and nonIUGR groups on the basis of fetal weight showed that placental weight ratio also failed to show a difference. Assessment of amniotic fluid pockets did not show that fetuses in either group had maximal pocket dimensions of less than 2 cm by 2 cm.

Comment

The detection and antenatal evaluation of fetuses with IUGR continues to present a problem for obstetricians. Efforts at screening for IUGR, regardless of method, yield maximum sensitivities of only 60% to

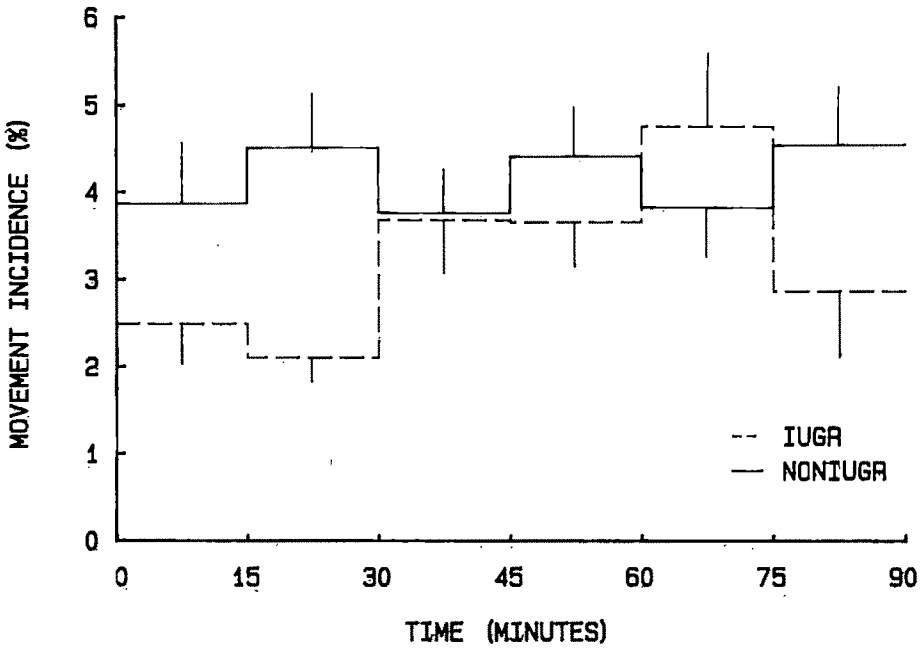


Fig. 3. Fetal body movements before, during, and after maternal hyperoxia (given at time = 30 minutes). Baseline fetal movement was significantly lower ($p < 0.05$) in IUGR group and increased significantly ($p < 0.02$) after hyperoxia period.

70% and specificities of approximately 90%.[7] Doppler flow velocimetry of umbilical artery blood flow provides improved sensitivity for the detection of IUGR.[8] However, this approach is associated with an increase in false-positive rates, thus requiring antenatal testing of many normal fetuses. The contraction stress test, regarded as a vigorous assessment of placental respiratory reserve, is usually reassuring in the presence of IUGR.[9] The nonstress test may also fail to reveal the elevated risk status of these fetuses.[10]

Biophysical testing, which provides the additional parameters of fetal breathing and movement, as well as amniotic fluid volume, has been shown to be beneficial in assessing the fetus with IUGR, particularly through its detection of oligohydramnios.[11] Investigations of fetal breathing activity in fetuses with IUGR have had conflictual results either because of small study populations or lack of standardized study conditions. Trudinger et al.[2] demonstrated that approximately 55% of fetuses with IUGR exhibited a decrease in fetal breathing activity after glucose loading, whereas the remainder showed an increase in fetal breathing. In a study of fetal breathing and behavioral states, van Vliet[12] et al. reported a decrease in breathing incidence in growth-retarded fetuses, whereas fetal movement was reported to be within or just below the expected norm. The maternal administration of oxygen as a potential diagnostic probe for placental respiratory function is based on previous observations of the biophysical effects of fetal hypoxia in chronic animal models. Bissonette[13] reported that fetal lambs exhibited more frequent apneic episodes when acute fetal hypoxia was induced. Martin et al.[14] reported similar data using rhesus monkeys. These effects probably resulted from an increase in high-voltage electrocortical activity and suppression of spinal reflexes. Clinical studies regarding the effects of maternal hyperoxia in pregnancy have demonstrated that fetal breathing movement and fetal movement are unaffected in normal third-trimester fetuses,[3, 5] whereas breathing movements may be increased in growth-retarded fetuses.[3, 13] The present study suggests that maternal hyperoxia is associated with significant increases in fetal breathing and body movement incidences in most fetuses with growth retardation. These changes in fetal activity do not occur uniformly and appear to be transient. The observed increases in these fetal activities, which follow maternal hyperoxia, are particularly dramatic when compared with those observed in normal fetuses. When IUGR results from chronic placental insufficiency, it is often associated with a redistribution of cardiac output to maintain substrate and oxygen supply to vital fetal systems, that is, brain, heart, and adrenal glands. It follows that reductions in fetal breathing and trunk movements may conserve energy and decrease oxygen requirements. Fetal responses to increased environmental oxygen may vary according to several preexisting conditions, such as the relative level of hypoxia and metabolic balance and the integrity of central regulatory mechanisms. Fetal biophysical responses to supplemental

oxygen may also be related to the severity of growth retardation, its duration, and its origin, since not all such cases result from placental respiratory insufficiency or are evident at the time of initial assessment.

Direct, noninvasive measurement of fetal oxygen levels is not presently possible; therefore interpretation of the results of this study are subject to certain limitations. Although the study sample is relatively small, the length of observation and the standardization of study conditions support the conclusion that these observed changes in biophysical activity were not likely to represent fetal state alterations. In addition, these findings are in general agreement with those previously reported by Ritchie and Lakhania[3] and Dornan and Ritchie.[15] Finally, fetal gestational ages were confined to the late third-trimester to avoid potential differences caused by maturational influences on control mechanisms of the central nervous system.

Our observation that maternal oxygen administration is associated with increased fetal breathing and body movement incidences in most fetuses with IUGR suggests that this maneuver may be a promising tool for intrauterine evaluation. A prospective clinical trial, using this approach on a larger randomized population sample, would be necessary to determine its applicability for obstetric management.

REFERENCES

1. Brar HS, Platt LD, DeVore GR. Antenatal fetal surveillance: the biophysical profile. Clin Obstet Gynecol 1987; 30:936.
2. Trudinger BJ, Lewis PJ, Pettit B. Fetal breathing patterns in intrauterine growth retardation. Br J Obstet Gynecol 1979;85:432.
3. Ritchie JWK, Lakhania K. Fetal breathing movement and maternal hyperoxia. Br J Obstet Gynecol 1980;87:1084.
4. Khazin AF, Hon EH, Frederick WH. Effects of maternal hyperoxia on the fetus. AM J OBSTET GYNECOL 1971; 109:628.
5. Devoe LD, Abduljabbar H, Carmichael L, et al. The effects of maternal hyperoxia on fetal breathing movement in third trimester fetuses. AM J OBSTET GYNECOL 1984; 148:790.
6. Devoe LD, Searle J, Searle N, et al. Computer-assisted assessment of the fetal biophysical profile. AM J OBSTET GYNECOL 1985;153:317.
7. Villar J, Belizan J. The evaluation of the methods used in the diagnosis of intrauterine growth retardation. Obstet Gynecol Surv 1986;41:187.
8. Fleischer Al, Schulman H, Farmakides G, et al. Umbilical artery velocity waveforms and intrauterine growth retardation. AM J OBSTET GYNECOL 1985;151:502.
9. Lin C, Devoe LD, River P, et al. Oxytocin challenge test and intrauterine growth retardation. AM J OBSTET GYNECOL 1981;140:282.
10. Keegan K. The nonstress test. Clin Obstet Gynecol 1987;30:921.
11. Vintzileos AM, Campbell WA, Ingardia CJ, et al. The fetal biophysical and its predictive value. Obstet Gynecol 1983;62:271.
12. van Vliet MA, Martin CB, Nijuhis JG, et al. The relationship between fetal activity and behavioral states and fetal breathing in normal and growth-retarded fetuses. AM J OBSTET GYNECOL 1985;153:582.
13. Bissonette JM, Hohimer AR, Cronan JZ, et al. Effect of oxygen and carbon dioxide tension on the incidence of apnea in fetal lambs. AM J OBSTET GYNECOL 1980; 137:575.
14. Martin CB, Murata YU, Ikenoue T, et al. Effects of alterations of Po_2 and Pco_2 on fetal breathing movements in rhesus monkeys. Gynecol Obstet Invest 1985;6:74.
15. Dornan JC, Ritchie JWK. Fetal breathing movements and materal hyperoxia in the growth retarded fetuses. Br J Obstet Gynecol 1983;90:210.

Fetal radius length: A critical evaluation of race as a factor in gestational age assessment

Lyndon M. Hill, MD, David Guzick, MD, PhD, Marion L. Thomas, RT, RDMS, and Joanne K. Fries, RDMS

Pittsburgh, Pennsylvania

Growth of the fetal radius was examined ultrasonographically in a prospective cross-sectional study of 290 white and 63 black patients between 12 and 43 weeks' gestation. The mean of radius length and 95% predictive intervals were calculated for each week. The most efficient description of the data was given by a simple log-linear model. The slopes for the white and black study groups estimated were distinctly different. Consequently, a single equation was estimated that considers race when assessing gestational age from radius length. (Am J Obstet Gynecol 1989;161:193-9.)

Key words: Fetal radius, gestational age assessment, ultrasonography, race

Queenan et al.[1] were among the first to suggest the use of long bone lengths to determine fetal age. They found the measurement of the femur to be the most readily reproducible. The radius and ulna were measured as a complex. Because the ulna extends further into the elbow than the radius, the inability to separate and measure the same long bone of the forearm would invariably lead to errors in gestational age assessment. Recently, two European studies[2, 3] reported their experience with radius length to assess gestational age.

The possible effect of race on fetal long bone length has not been properly assessed. Although a few studies[4, 5] failed to find a difference in femur length between blacks and whites, a complete evaluation extending throughout gestation has not yet been published.

The purpose of our investigation was twofold: to establish appropriate standards for fetal radius length and menstrual age in a white population within the United States, and to compare the results obtained with a similar formula devised for blacks.

Material and methods

The study group consisted of 290 white and 63 black women. Each patient had to meet the following pregnancy-dating criteria before admission into the study: regular menses, an accurate date of the last menstrual period, a positive pregnancy test by 7 weeks' gestation, and a pelvic examination by 8 weeks' gestation. Patients with diseases known to affect fetal growth (e.g., diabetes mellitus) were excluded. Patients with multiple gestations were also excluded. The gestational ages of the study population ranged from 12 to 43 weeks. All neonates were ≥10th percentile for weight at the time of delivery.[6]

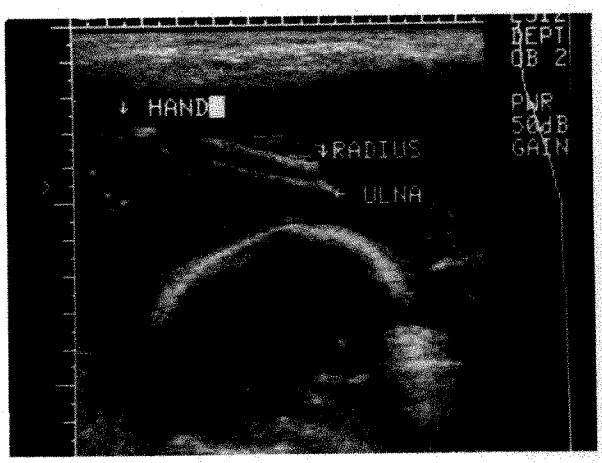


Fig. 1. Radius and ulna at 23 weeks' gestation.

The ultrasonographic measurements of the length of the fetal radius were made with a commercial linear array real-time system with a 3.5 MHz transducer at a focal zone of 5 to 12 cm. Both ends of the radius had to be identified distinctly from the ulna before an image was obtained (Fig. 1). We found the radius only slightly more difficult to image than the femur despite its greater range of motion. All measurements were taken with electronic calipers calibrated to the velocity of sound in tissue of 1540 m/sec.

Statistical analysis

Statistical analyses were conducted using the Minitab statistical software package on VAX 11/750 and DEC 20 computers. Data were evaluated in stages.

First, the means and SDs of radius length for each

From the Division of Ultrasound, Department of Obstetrics and Gynecology, University of Pittsburgh Health System, Magee-Womens Hospital.

Received for publication October 15, 1988; revised January 27, 1988; accepted December 30, 1988.

Reprint requests: Lyndon M. Hill, MD, Director of Ultrasound, Magee-Womens Hospital, Forbes Ave. at Halket St., Pittsburgh, PA 15213.

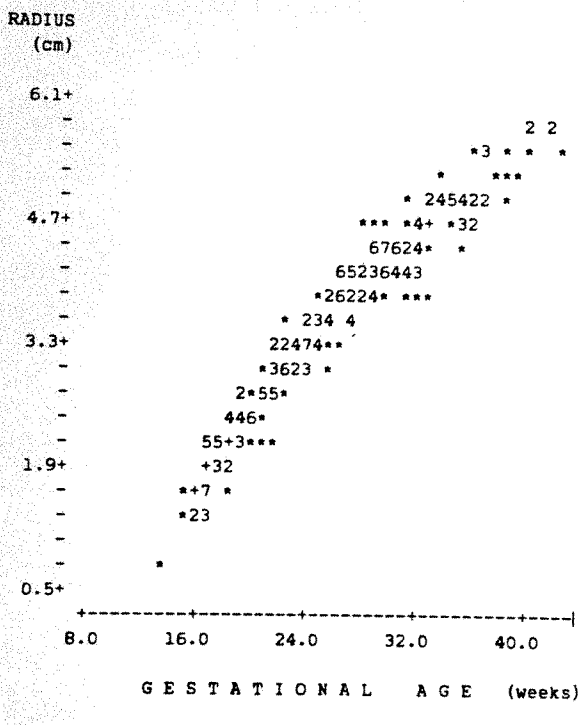


Fig. 2. Radius length (in centimeters) at specific weeks of gestation.

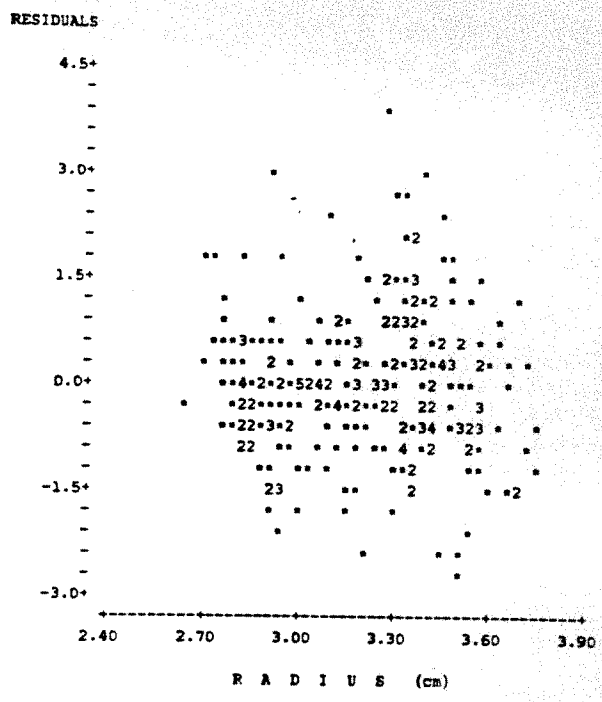


Fig. 3. Residual plot for $\log_e$ transformation of gestational age (model IV) and on $\log_e$ of radius length. The *numbers* indicate the number of observations at a specific point.

gestational age were calculated. These results were then plotted to assess the nature of the relationship (linear, curvilinear) between gestational age and radius length.

Second, alternative regression equations (linear, quadratic, log-linear) relating gestational age to radius length were estimated. In comparing these equations, we sought the simplest expression in which all estimated coefficients were significantly different from zero and in which there was the highest coefficient of determination and lowest residual SD.

Third, the best regression equation was estimated separately for whites and blacks. Differences between the estimates for whites and blacks were then systematically evaluated.

Results

The relation between gestational age and radius length for the 290 white subjects is plotted in Fig. 2. Estimates of five alternative regression equations fit to these data are presented in Table I.

Fig. 1 shows that the relation between gestational age and radius length is slightly curvilinear. Although a linear model (equation 1, Table I) explains the data well ($R^2 = 0.945$), the squared term of a quadratic model (equation 2, Table I) appears with a significant coefficient and the R^2 value improves slightly. A cubic term (equation 3) was statistically significant, but at the expense of the linear term. Its inclusion did not improve the overall explanatory power of the model.

The most efficient description of the data was provided by a simple log-linear model (equation 4). This equation maximized R^2, using only one predictor variable that was highly significant. Moreover, an increase in variability seen on residual plots for equations 1, 2, and 3 (not shown) was not evident with equation 4 (Fig. 3). The addition of a quadratic term to equation 4 (i.e., equation 5) did not improve its fit.

Thus, for the 290 white subjects, the estimated regression equation was:

$$\text{Log}_e\,(\text{Gestational age}) = 2.424 + 0.226\,(\text{Radius length})$$

When a similar analysis was conducted for the 63 black subjects, the log-linear equation was also found to provide the best fit of the data, but the coefficient estimates were somewhat different:

$$\text{Log}_e\,(\text{Gestational age}) = 2.427 + 0.219\,(\text{Radius length})$$

Four models were developed to compare the regression equations for white and black subjects:

Model I: General. The slope and the intercept for the two equations are different.

Model II: Parallel regression. In this model for $\log_e$ gestational age, the slope parameters for radius for the two populations are the same, but the intercepts are different.

Table I. Estimated regression equations for white subjects ($N = 290$)

Equation No.	*Dependent variable**	*Coefficients for independent variables†*				
		Constant	*Radius*	*Radius²*	*Radius³*	R^2
1	Age	6.16 (21.13)	5.61 (70.54)			0.945
2	Age	11.13 (15.95)	2.29 (5.26)	0.49 (7.71)		0.954
3	Age	15.23 8.72	−1.92 (−1.13)	1.79 3.48	−0.12 (−2.56)	0.955
4	Log_e(age)	2.42 247.48	0.23 (84.78)			0.961
5	Log_e(age)	2.39	0.25	−0.004		0.961

*Age is gestational age in weeks.
†t Statistics for corresponding estimated coefficients are shown in parentheses.

Table II. Summary of models that compare black and white subjects*

Variable	*Model I*	*Model II*	*Model III*	*Model IV*
Intercept, all subjects	—	—	2.424 (282.17)	2.425 (278.86)
Radius, all subjects	—	0.224 (95.88)	—	0.224 (94.88)
Intercept, whites	2.427 (126.68)	2.408 (229.14)	—	—
Intercept, blacks	2.424 (251.69)	2.429 (279.25)	—	—
Radius, whites	0.219 (42.50)	—	0.219 (78.44)	—
Radius, blacks	0.226 (86.02)	—	0.226 (95.12)	—
Error [SD]	0.052	0.052	0.052	0.053
Residual sum of squares	0.956	0.960	0.956	0.983
Degrees of freedom	349	350	350	351

**t* Statistics for the corresponding estimated coefficient are shown in parentheses.

Model III: Concurrent regression. In this model, the intercepts are equal, but the slopes are different (Fig. 4).

Model IV: Coincident regression. The regression equations for the two populations are the same. This suggests that the same equation may be used to define the study groups.

Table II summarizes the estimate for the four alternative models. The plausibility of models II, III, and IV were tested against the general model (model I) by using the F test. For our data, the F statistics were as follows:

Model II versus model I: $F = 1.46$ ($p > 0.10$)
Model III versus model I: $F = 0.09$ ($p > 0.10$)
Model IV versus model I: $F = 4.95$ ($0.005 < p < 0.01$)

From these analyses, we concluded that models II and III were as good as the least restrictive model, model I (i.e., the F statistics were not statistically significant). Because model I was statistically different from model IV (coincident regression model), the latter cannot be utilized to explain the relationship between radius length and gestational age for both populations. Models II and III were then compared with model IV:

Model IV versus model II: $F = 8.43$ ($0.001 < p < 0.005$)
Model IV versus model III: $F = 9.83$ ($0.001 < p < 0.005$)

Thus models II and III were better than model IV. Because model III not only had a larger F statistic, but also a smaller error of variance (Table I), we chose model III as the best single equation for the prediction

Table III. Gestational age and 95% prediction intervals for radius length in a white population (values for blacks in parentheses)

Radius (cm)	*5th Percentile*	*50th Percentile*	*95th Percentile*
0.6	11.7 (12.3)	12.9 (12.9)	14.3 (13.6)
0.7	11.9 (12.6)	13.2 (13.2)	14.6 (13.9)
0.8	12.2 (12.9)	13.5 (13.5)	15.0 (14.2)
0.9	12.5 (13.1)	13.8 (13.8)	15.4 (14.5)
1.0	12.7 (13.4)	14.2 (14.1)	15.7 (14.8)
1.1	13.0 (13.7)	14.5 (14.4)	16.1 (15.1)
1.2	13.3 (14.0)	14.8 (14.7)	16.4 (15.5)
1.3	13.6 (14.3)	15.1 (15.1)	16.8 (15.8)
1.4	13.9 (14.7)	15.5 (15.4)	17.2 (16.2)
1.5	14.3 (15.0)	15.8 (15.7)	17.6 (16.5)
1.6	14.6 (15.3)	16.2 (16.1)	18.0 (16.9)
1.7	14.9 (15.7)	16.6 (16.4)	18.4 (17.2)
1.8	15.3 (16.0)	17.0 (16.8)	18.8 (17.6)
1.9	15.6 (16.4)	17.3 (17.2)	19.3 (18.0)
2.0	16.0 (16.7)	17.7 (17.5)	19.7 (18.4)
2.1	16.3 (17.1)	18.1 (17.9)	20.1 (18.8)
2.2	16.7 (17.5)	18.6 (18.3)	20.6 (19.3)
2.3	17.1 (17.8)	19.0 (18.7)	21.0 (19.7)
2.4	17.5 (18.2)	19.4 (19.2)	21.5 (20.1)
2.5	17.9 (18.6)	19.9 (19.6)	22.0 (20.6)
2.6	18.3 (19.1)	20.3 (20.0)	22.5 (21.0)
2.7	18.7 (19.5)	20.8 (20.5)	23.1 (21.5)
2.8	19.1 (19.6)	21.2 (20.9)	23.5 (22.0)
2.9	19.6 (20.3)	21.7 (21.4)	24.1 (22.4)
3.0	20.0 (20.8)	22.2 (21.9)	24.7 (22.9)
3.1	20.5 (21.3)	22.7 (22.3)	25.2 (23.4)
3.2	20.9 (21.7)	23.2 (22.8)	25.8 (24.0)
3.3	21.4 (22.2)	23.8 (23.3)	26.4 (24.5)
3.4	21.9 (22.7)	24.3 (23.8)	27.0 (25.0)
3.5	22.4 (23.2)	24.9 (24.4)	27.6 (25.6)
3.6	22.9 (23.7)	25.5 (24.9)	28.3 (26.2)
3.7	23.5 (24.2)	26.0 (25.5)	28.9 (26.7)
3.8	24.0 (24.8)	26.6 (26.0)	29.6 (27.3)
3.9	24.5 (25.3)	27.2 (26.6)	30.2 (27.9)
4.0	25.1 (25.9)	27.9 (27.2)	30.9 (28.6)
4.1	25.7 (26.5)	28.5 (27.8)	31.6 (29.2)
4.2	26.3 (27.0)	29.1 (28.4)	32.4 (29.8)
4.3	26.9 (27.6)	29.8 (29.0)	33.1 (30.5)
4.4	27.5 (28.2)	30.5 (29.7)	33.9 (31.2)
4.5	28.1 (28.9)	31.2 (30.3)	34.6 (31.9)
4.6	28.7 (29.5)	31.9 (31.0)	35.4 (32.6)
4.7	29.4 (30.2)	32.6 (31.7)	36.2 (33.3)
4.8	30.1 (30.8)	33.4 (32.4)	37.1 (34.0)
4.9	30.7 (31.5)	34.1 (33.1)	37.9 (34.8)
5.0	31.4 (32.2)	34.9 (33.8)	38.8 (35.5)
5.1	32.2 (32.9)	35.7 (34.6)	39.7 (36.3)
5.2	32.9 (33.6)	36.5 (35.3)	40.5 (37.1)
5.3	33.6 (34.4)	37.4 (36.1)	41.5 (38.0)
5.4	34.4 (35.1)	38.2 (36.9)	42.5 (38.8)
5.5	35.2 (35.9)	39.1 (37.7)	43.4 (39.7)
5.6	36.0 (36.7)	40.0 (38.6)	44.4 (40.5)
5.7	36.8 (37.5)	40.9 (39.4)	45.4 (41.4)
5.8	— (38.3)	—(40.3)	—(42.4)

of gestational age from radius length for both populations:

$$\text{Log}_e\,(\text{Gestational age}) = 2.424 + 0.220\,(\text{Radius: black}) + 0.226\,(\text{Radius: white})$$

In the above equation, a zero should be used for "radius: white" when a black patient is evaluated, and vice versa. Radius length is measured in centimeters.

In model III there is concurrence of the two populations at a radius length equal to zero, which corresponds to a gestational age of 11 weeks. Before 11 weeks' gestation, the radius cannot be reliably identified and measured. The coefficient of determination for model III is 96.3%.

The lower and upper limits of the 95% prediction intervals for gestational age when radius length and

Table IV. Mean and SD of the residuals for each technician (subset of 290 white patients)

Technician	*No. of Measurements*	*Mean*	*SD*
1	22	0.28	1.45
2	67	-0.16	0.97
3	68	0.15	1.00
4	24	-0.29	0.50
5	63	-0.05	0.87
6	10	0.10	1.14
7	36	0.19	1.12

maternal race are known (values for blacks are given in parentheses) are listed in Table III.

To test for the existence of technician effect, standardized residuals from the fitted line (model IV) for the subset of 290 white patients were used. Table IV shows the number of measurements taken by each technician, the mean, and SD of the residuals. One-way analysis of variance yielded an F ratio of 1.14, which is not significant. Hence the interobserver effect on the measurements obtained was insignificant. The intraobserver difference for measuring 27 radial lengths twice a day was almost always within a range of ± 1 mm.

Comment

Several investigators have derived gestational age from long bone length.[1-3, 7-10] Initially, attention was focused on the fetal femur. Its size, absence of interfering anatomic structures, and the inherently smaller range of motion of the proximal as opposed to the distal long bones allow the femur to be measured quite easily. With improvement in the resolution of real-time equipment, finer fetal anatomic detail can now be appreciated. Hence the radius and ulna may be differentiated, whereas in the past they were measured as a "complex."[1]

Since our study began, three groups[2, 3, 11] have reported their results of gestational age assessment by radius length. Table V compares the results from these three studies and our investigation; the majority of radius lengths are within a 2-mm range.

In 1984, Jeanty et al.[7] published their results with respect to the humerus, ulna, and tibia. A linear quadratic regression was used to predict gestational age. In Table VI, the results of their study are compared with our investigation. It can be seen that the use of radius length as a predictor of fetal age leads to a better fit, even for the linear quadratic model. The variability for gestational age determination from the femur length and radius length (Table VII) is identical.

Several studies that evaluated femur length,[3, 8-10] and our assessment of radius length, concluded that variance increases with the size of the long bone measure-

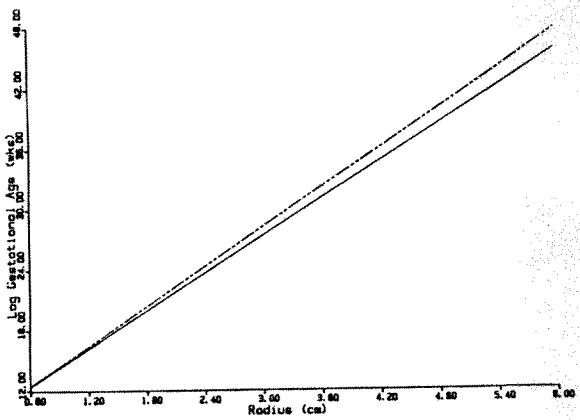


Fig. 4. The concurrent regression model has different slopes but the same intercept.

ment. However, Jeanty et al.[7] claimed that the error variance of the linear quadratic model did not increase with increasing lengths of the long bone. This conclusion was based on their use of the Bartlett test to assess significance. As emphasized by Warda et al.,[10] this was not the best test to use for their purpose because of an inadequate sample size at each week of gestation (approximately 23 measurements per week). Hence the results obtained by Jeanty et al.[7] may not have been reliable. Our results concur with the findings of Warda et al.[10] with respect to femur length; i.e., $\log_e$ transformation of gestational age should be used to reduce the systematic variability discovered on the residual plot.

Although several technicians were involved in the accumulation of the data, there were no significant interobserver differences in the results. This indicates that the landmarks for delineating the ends of the radius were readily reproducible between observers.

Scammon and Calkins[12] established the classic anthropometric interrelationships and the relationship between anthropometric variables and gestational age. Specifically, with respect to fetal skeletal maturity, Garn et al.[13] and others have shown that the female fetus reaches a given stage of development at an earlier age than the male. Black infants tend to be shorter than white infants of the same gestational age. Miller and Merritt[14] found that crown-heel lengths are affected not only by race, gestational age, and fetal sex but also by maternal height and, to a slight degree, parity. The differences noted in our investigation are merely an extension of the neonatal data accrued by others.[14]

Sabbagha et al.[15] compared the difference between the biparietal diameters from 107 white and 91 black normal pregnant women. Only minor differences (up to 2 mm after 30 weeks' gestation) were noted. When the length of the femur was assessed in racially dissimilar populations, a slight difference was found.[16] How-

Table V. Comparison of radius length at different menstrual ages

Menstrual age (wk)	*Issel*[2]	*Merz et al.*[3]	*Elejalde and de Elejalde*[11]	*Current study*
25	3.6	3.5	3.3	3.5
26	3.8	3.6	3.4	3.7
27	3.9	3.7	3.7	3.9
28	4.1	3.9	3.8	4.0
29	4.2	4.0	4.1	4.2
30	4.5	4.1	4.2	4.3
31	4.6	4.2	4.4	4.5
32	4.7	4.4	4.5	4.6
33	4.9	4.5	4.6	4.7
34	5.0	4.7	4.8	4.9
35	5.1	4.8	5.0	5.0
36	5.2	4.9	5.2	5.1
37	5.3	5.1	5.3	5.2
38	5.3	5.1	5.4	5.4
39	5.4	5.3	5.6	5.5
40	3.6	5.3	5.8	5.6
41	5.6	5.6	—	5.7

Table VI. Coefficient of determinations (r^2) and number of cases in published series of estimation of gestational age from fetal long bones

Study	*Long bone*	*Model*	r^2	*No. of cases*
Jeanty et al.[7]	Femur	Linear quadratic	0.935	557
Jeanty et al.[7]	Humerus	Linear quadratic	0.921	542
Jeanty et al.[7]	Tibia	Linear quadratic	0.937	203
Jeanty et al.[7]	Ulna	Linear quadratic	0.933	196
Current study	Radius	Linear quadratic	0.954	290
Current study	Radius	Linear $\log_e$ GA	0.961	290

GA, Gestational age.

Table VII. Comparison of variability estimates for menstrual age determined by femur and radius length

Interval studied (wk)	*Estimated variability (wk)*	
	Warda et al.[10] *(femur)*	*Current study (radius)*
12-17.9	1.4-1.9	1.6-1.9
18-23.9	1.9-2.6	1.9-2.5
24-29.9	2.6-3.2	2.5-3.2
30-35.9	3.3-3.9	3.2-3.8
36-42.0	3.9-4.4	3.8-4.3

ever, the latter study only compared fetuses up to a gestational age of 24 weeks. Similarly, Ruvolo et al.[4] could not find any differences in gestational age assessment by femur length in 61 black and 102 white patients between 19 and 32 weeks' gestation. For purposes of comparison, it is important to note that the authors did not extend their study to term. Moreover, they did not derive specific gestational age formulas for their black and white patients and then evaluate the results. They chose to compare their femur length data with a previously published chart.[9] Evaluation of their composite assessment of gestational age in a black population by Hadlock et al.[5] suffers from the same design flaw. This type of comparison may obscure small differences between populations because groups are compared with a standard instead of with each other. After deriving regression equations for each subpopulation, we noted a statistically significant difference in gestational age prediction by radius length for whites and blacks—a 10-day difference in gestational age assessment was noted in the third trimester. Although this difference lies below the estimated variability of radius length for any gestational age, we feel that this difference is significant enough to warrant the incorporation of race into the assessment of gestational age utilizing radius length.

REFERENCES

1. Queenan JT, O'Brien GD, Campbell S. Ultrasound measurement of fetal limb bones. AM J OBSTET GYNECOL 1980;138:297-302.

. Issel EP. Ultrasonic measurement of the growth of fetal limb bones in normal pregnancy. J Perinat Med 1985; 13:305-13.
. Merz E, Kim-Kern M, Pehl S. Ultrasonic mensuration of fetal limb bones in the second and third trimesters. JCU 1987;15:175-83.
. Ruvolo KA, Filly RA, Callen PW. Evaluation of fetal femur length for prediction of gestational age in a racially mixed obstetric population. J Ultrasound Med 1987;6:417-9.
. Hadlock FP, Harrist RB, Shaw YP, et al. Estimating fetal age using multiple parameters: a prospective evaluation in a racially mixed population. AM J OBSTET GYNECOL 1987;156:955-7.
. Brenner WE, Edelman DA, Hendricks CH. A standard of fetal growth for the United States of America. AM J OBSTET GYNECOL 1976;126:555-64.
7. Jeanty P, Rodesch F, Delbeke D, et al. Estimation of gestational age from measurements of fetal long bones. J Ultrasound Med 1984;3:75-9.
8. Hohler CW, Quetel TA. Fetal femur length: equations for computer calculation of gestational age from ultrasound measurements. AM J OBSTET GYNECOL 1982;143:479-81.
9. Hadlock FP, Harrist RB, Deter RL, Park SK. Fetal femur length as a predictor of menstrual age: sonographically measured. AJR 1982;138:875-8.
10. Warda AH, Deter RL, Rossavik IK, et al. Fetal femur length: a critical reevaluation of the relationship to menstrual age. Obstet Gynecol 1985;66:69-75.
11. Elejalde BR, de Elejalde MM. The prenatal growth of the human body determined by the measurement of bones and organs by ultrasonography. Am J Med Genet 1986; 24:575-98.
12. Scammon RE, Calkins LA. The development and growth of the external dimensions of the human fetus. Minneapolis: University of Minnesota Press, 1929:.
13. Garn SM, Burdi AR, Babler WJ. Male advancement in prenatal hand development. Am J Phys Anthropol 1974; 41:353-9.
14. Miller HC, Merritt TA. Fetal growth in humans. Chicago: Year Book Medical, 1979 chap. 15.
15. Sabbagha RE, Barton FB, Barton BA. Sonar biparietal diameter. I. Analysis of percentile growth differences in two normal populations using same methodology. AM J OBSTET GYNECOL 1976;126:479-84.
16. O'Brien GD, Queenan JT. Growth of the ultrasound fetal femur length during normal pregnancy. Part I. AM J OBSTET GYNECOL 1981;141:833-7.

Pregnancy after aortofemoral bypass grafting in a diabetic patient

P. J. Bradley-Watson, FRCS, FRCOG
Dyfed, Wales

A case of pregnancy after an aortofemoral bypass graft for atherosclerosis in an insulin-dependent diabetic is described. Pregnancy after this type of surgery in a diabetic patient has not been described previously. (AM J OBSTET GYNECOL 1989;161:200-1.)

Key words: Pregnancy, diabetes, aortofemoral bypass graft

Although pregnancy after an aortofemoral bypass has been described previously,[1] I could not find any reports of pregnancy with the added complication of insulin-dependent diabetes.

Case history

The patient was 32 years old and had been an insulin-dependent diabetic since the age of 9 years. At the age of 20, the patient had her first pregnancy, which was complicated by premature normal delivery of a healthy 2155 gm normal infant at 35 weeks' gestation. At the age of 30, the patient was seen with intermittent claudication in both legs, but particularly of the left calf. She smoked 10 cigarettes a day and there was a family history of arterial disease. There were also symptoms of peripheral paresthesia, and absent pulses at the femoral level and below in both lower limbs.

An aortogram showed a complete block of the aorta just below the level of the renal arteries. The lower aorta was very narrow and iliac vessels had thick walls and heavy calcification. An aortoendarterectomy and end-to-end aortobifemoral bypass graft with a knitted Dacron graft were done. Because of the extension of the disease, a left renal endarterectomy was also performed. Histologic study of the specimen confirmed atheroma. The patient made a good postoperative recovery and a scan of the abdomen revealed that the graft was functioning well. The symptoms greatly improved with relief of claudication. The femoral pulses were good but there were no pulses distal to this.

In 1988 she was seen in the tenth week of her second pregnancy; dates were confirmed by sonography. She had not had preconceptual advice. The patient was adamant that she wanted to continue the pregnancy.

From the Department of Obstetrics and Gynaecology, East Dyfed Health Authority, Carmarthen/Dinefwr Health Unit, West Wales General Hospital.
Received for publication December 21, 1988; accepted January 30, 1989.
Reprint requests: Dr. P. J. Bradley-Watson, Carmarthen/Dinefwr Health Unit, West Wales General Hospital, Carmarthen, Dyfed SA31 2AF.

Throughout the pregnancy her diabetes was monitored at the joint obstetric-medical clinic with the diabetic specialty nurse in attendance. Blood glucose levels were monitored on a home basis with a glucometer; the insulin dosage (a combination of Actrapid and Monotard twice daily) was modified accordingly. A capillary blood glucose series was done in the home on a twice-weekly basis to check the accuracy of the glucometer. Regular fructosamine levels were studied on all occasions and were within normal levels; however, the level at the inital visit was raised (2.38 nmol/L). The retinas were examined during the pregnancy and there were no adverse changes. The renal function tests were done at the initial visit and were within normal limits. The aorta and iliac vessels were examined by ultrasonography at the initial visit and throughout the pregnancy and did not alter throughout the pregnancy. The diameter of the aorta was 18 mm and the iliac vessels measured 8 mm in diameter.

The pregnancy was monitored with the usual clinical parameters. The fetal well-being was monitored both clinically and by serial ultrasonography. There was no evidence of intrauterine growth retardation or diabetic macrosomia, either clinically or on ultrasonographic scans. From the thirty-fourth week of pregnancy a fetal kick chart was kept and was always satisfactory. The patient was admitted to the antenatal ward on only one occasion, at 33 weeks, because of possible decrease in fetal movements, but all fetal investigations carried out at that time were satisfactory.

The patient was admitted at 38 weeks for a planned induction of labor because of diabetes. An ultrasonographic estimation of fetal weight carried out at this time was 3.64 kg. Labor was induced with amniotomy and intravenous Syntocinon. The first stage of labor lasted 3 hours 35 minutes, and the second stage 30 minutes with normal delivery of a healthy infant weighing 3.4 kg. During labor the patient's diabetes was controlled with an insulin and dextrose infusion with regular blood glucose estimations. The infant was observed in the special care baby unit for 48 hours and only needed early feeding to maintain adequate blood glucose levels. The patient's puerperium was uneventful, and throughout the pregnancy and puerperium there

were no detrimental symptoms or signs concerning the blood supply to the lower limbs and pelvis.

Comment

This is an unusual case of a diabetic patient with the added complication of an aortofemoral bypass. A pregnancy has been described after aortofemoral bypass, but not in a woman with diabetes.[1] The blood supply to the pelvic organs did not appear to have been compromised enough to cause any degree of intrauterine growth retardation, as seen in the previously reported case. Weir and Willocks[2] reported a case of a pregnancy after a right aortofemoral graft for thrombosis associated with intrauterine growth retardation, but that case was also complicated by malignant phase hypertension.

The problem of possible prophylactic anticoagulant therapy throughout the pregnancy was discussed at the initial antenatal visit. It was decided that no anticoagulants would be used prophylactically because the patient had already had a good response after surgery with no complications. In reviewing the literature concerning pregnancy after aortofemoral grafts, half of the patients had anticoagulant therapy. We decided that no prophylactic anticoagulants would be used because of the added complication of diabetes. Pregnancies have been previously reported after coronary artery bypass grafting,[3] and in this situation prophylactic anticoagulants were not used. In conclusion, this case demonstrates an uncomplicated outcome of a pregnancy complicated by two difficult medical problems in which the obstetrician may have advised differently at the commencement of the pregnancy.

REFERENCES

1. Valentine BH, Fenton DW, Bruce JEF, et al. Pregnancy following aortofemoral bypass grafting. J R Coll Surg Edinb 1986;26:42-3.
2. Weir RJ, Willocks J. A pregnancy following malignant phase hypertension. Br J Obstet Gynaecol 1976;83:584-6.
3. Chestnut DH, Zlatnik FJ, Pitkin RM, Varner MW. Pregnancy in a patient with a history of myocardial infarction and coronary artery bypass grafting. Am J Obstet Gynecol 1986;155:372-3.

Bound volumes available to subscribers

Bound volumes of the American Journal of Obstetrics and Gynecology are available to subscribers (only) for the 1989 issues from the Publisher, at a cost of $56.00 ($79.00 international) for Vol. 160 (January-June) and Vol. 161 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue in the volume. The binding is durable buckram with the Journal name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact The C.V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318, USA; phone (800) 325-4177, ext. 351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular Journal subscription.

Fentanyl citrate analgesia during labor

William Rayburn, MD, Anne Rathke, RN, M. Patricia Leuschen, PhD, Jan Chleborad, MD, and William Weidner, MD

Omaha, Nebraska

Fentanyl citrate is a potent short-acting narcotic reported to cause less nausea and sedation than morphine or meperidine hydrochloride. The purpose of this prospective investigation was to determine whether a safe but adequate intrapartum dosing schedule is possible. A total of 137 women with uncomplicated term pregnancies were offered a standard intravenous dose (50 mcg or 100 mcg hourly as needed) of fentanyl citrate during active labor. Temporary analgesia and mild sedation were apparent in each case. The cumulative dose varied in accordance with maternal needs (mean, 140 ± 42 μg; range, 50 mcg to 600 μg). Apart from a brief decrease in fetal heart rate variability that lasted 30 minutes, no worrisome pattern was apparent from exposure to fentanyl citrate. Pediatric examinations were performed without knowledge of analgesic therapy on infants exposed to fentanyl citrate and those not exposed to analgesics. No differences were found in frequencies of newborn depressed respirations, low Apgar scores, or neurologic and adaptive capabilities at two hours and 24 hours postnatally. With the use of the described dosing schedule, fentanyl citrate was helpful during labor and did not cause immediate or prolonged hazards to the mother and unborn infant. (Am J Obstet Gynecol 1989;161:202-6.)

Key words: Fentanyl citrate, labor, narcotics

Fentanyl citrate is a synthetic opioid used primarily during the anesthetic period for premedication, induction and maintenance, and postoperative analgesia. Compared with meperidine hydrochloride and morphine in nonpregnant adults, fentanyl citrate has a greater analgesic potency, shorter duration of action (30 to 60 minutes), and lower risk for nausea and respiratory depression.[1] Its analgesic and euphoric effects are equally antagonized by opioid antagonists, and no active metabolite is present.

Experience with intravenous fentanyl citrate during pregnancy has been limited. The manufacturer (Sublimaze, Janssen, N.J.) has written in the package insert that there are insufficient data to support its use during labor and delivery. Because of the drug's potential advantages over more traditional narcotics, we attempted to determine how much fentanyl citrate is requested during labor and whether the newborn infant is affected.

Material and methods

Data were gathered between January 1988 and April 1988 after permission was granted by our Institutional Review Board. Women in labor ≥37 weeks' gestation with no active medical or obstetric complications were eligible for enrollment. Each study participant was offered intravenous fentanyl citrate if epidural anesthesia was not desired. Those who requested fentanyl citrate were informed of its potential side effects (nausea, sedation, hypotension, and respiratory depression).

A standard 50 μg or 100 μg dose was administered intravenously as often as every hour on request during active labor (cervical dilation 3 to 10 cm). This dose range was known to be equianalgesic to 5 to 10 mg of morphine or 37.5 to 75 mg of meperidine hydrochloride.[1] The lower 50 μg dose was usually given initially unless the patient was perceived by the physician or nurse to be in severe pain. The number of requests for the drug and the total dose required during labor were recorded. A 0.02 mg (1 ml) dose of the neonatal preparation of naloxone hydrochloride (Narcan, DuPont, Wilmington, Del.) was available for intramuscular administration in the event of initial neonatal respiratory difficulties presumably from narcotic excess.

Blood was drawn from the maternal antecubital vein and umbilical vein at delivery for serum fentanyl citrate determinations. Each sample was centrifuged at 8000 *g* for 10 minutes. A 1 to 5 ml plasma specimen was drawn and stored at −20° C until the time of assay. All plasma samples were assayed simultaneously and in duplicates in accordance with the radioimmunoassay technique (Janssen Pharmaceutics, Piscataway, N.J.) described by Michiels et al.[2] The antibody used in this technique was described as being highly sensitive and specific for fentanyl citrate with no significant cross-reactivity with fentanyl citrate metabolites or close analogs.

Newborn findings were compared with a similar group of consecutively chosen term infants born during

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, and Division of Newborn Medicine, Department of Pediatrics, University of Nebraska College of Medicine.
Received for publication October 26, 1988; revised January 4, 1989; accepted January 10, 1989.
Reprint requests: William F. Rayburn, MD, Department of Obstetrics and Gynecology, University of Nebraska College of Medicine, 42nd and Dewey Ave., Omaha, NE 68105.

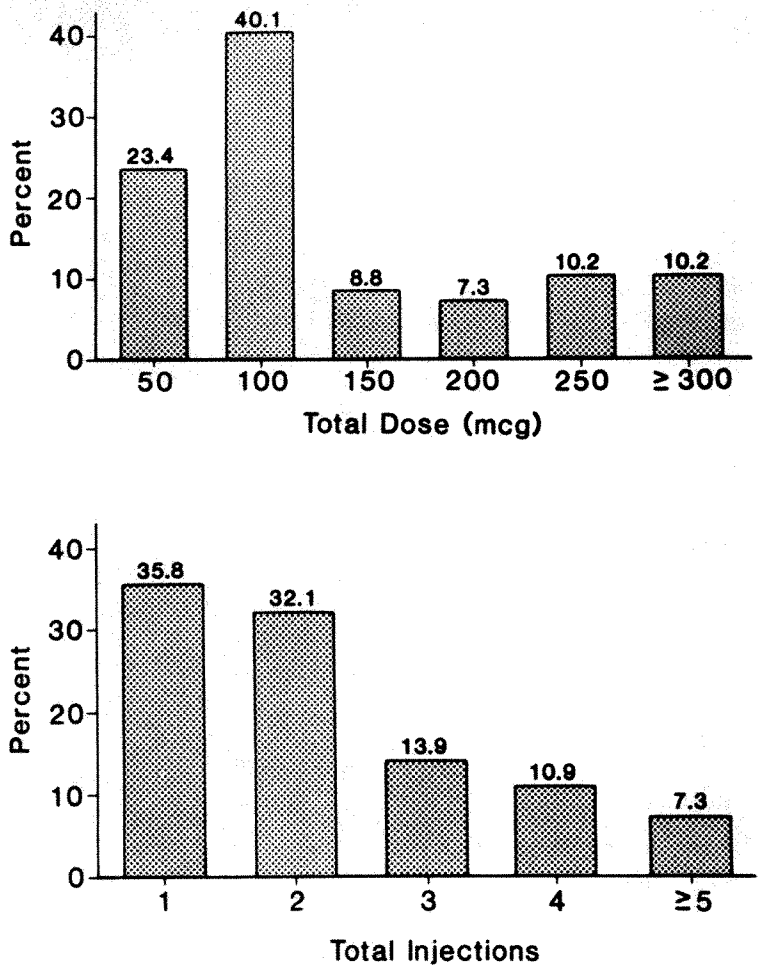


Fig. 1. Fentanyl citrate use according to the number of injections and total cumulative dose.

Table I. Profiles of study (fentanyl citrate) and control (no fentanyl citrate or other analgesic-anesthetic agent) group pregnancies

	Study group (n = 137)	*Control group (n = 112)*	*p*
Maternal age (years)	21.4 ± 0.7	25.5 ± 0.9	<0.01
Race (% white)	86 (63)	84 (75)	<0.05
Parity (% nulliparous)	82 (60)	17 (15)	<0.00001
Gestational age at delivery (wks)	39.8 ± 0.3	39.6 ± 0.4	NS
Need for oxytocin augmentation	37 (27%)	6 (5%)	<0.0001
Duration of labor (hours)			
First stage, active phase	5.3 ± 0.8	3.2 ± 0.4	<0.001
Second stage	0.41 ± 0.08	0.37 ± 0.09	NS
Route of delivery			
Vaginal	127 (93%)	105 (94%)	NS
Cesarean section	10 (7%)	7 (6%)	NS
Failure to progress	7 (5%)	5 (4%)	
Fetal distress	3 (2%)	2 (2%)	

Values are mean ± SEM.

the same period to women who also had no antepartum complication and did not require pain medication (epidural or narcotic). There were 16 pregnancies excluded (nine from the study group and seven from the control group) because the infants had evidence of birth trauma, meconium aspiration, severe hypoglycemia, or a major anomaly that required admission to the intensive care nursery. Evaluation of each infant at birth

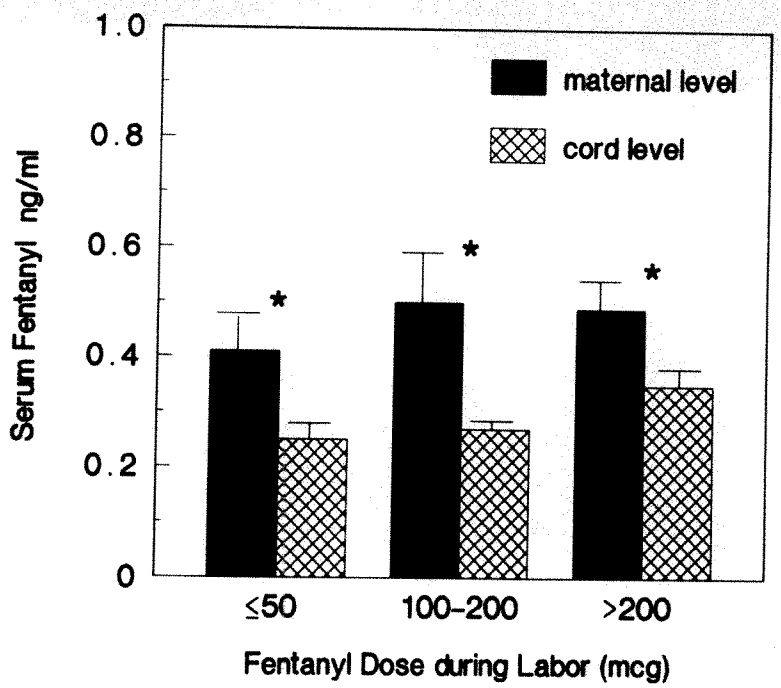


Fig. 2. Maternal and cord serum fentanyl citrate concentrations at delivery according to maternal dose during labor. (*$p < 0.03$; *bars*, SEM.)

Table II. Immediate neonatal outcomes between study and control groups

	Study group (n = 137)	*Control group (n = 112)*	*p*
Sex (Female, Male)	64, 61	60, 52	NS
Birth weight (gm)	3,263 ± 217	3,390 ± 242	NS
Depressed respirations (%)	4 (3%)	7 (6%)	NS
Apgar scores			
1 minute <7	8 (6%)	10 (9%)	NS
5 minute <7	8 (6%)	7 (6%)	NS
Need for naloxone (%)	1 (0.7%)	1 (0.9%)	NS
Neonatal vital signs			
Respiratory rate (per minute)			
At 2 to 4 hours	49 ± 3	48 ± 2	NS
At 24 hours	49 ± 2	46 ± 2	NS
Heart rate (beats/min)			
At 2 to 4 hours	141 ± 3	146 ± 2	NS
At 24 hours	140 ± 2	142 ± 2	NS
Blood pressure at 24 hours (mm Hg)			
Systolic	65 ± 2	65 ± 2	NS
Diastolic	36 ± 1	34 ± 1	NS

Values are mean ± SEM.

included Apgar score determinations at 1 and 5 minutes and selective umbilical blood gas measurements (pH, base excess) if respiratory depression of the fetus or a worrisome type of fetal heart pattern was present.

Each infant underwent complete neurologic and adaptive capacity scoring at 2 to 4 hours and 24 hours postnatally. The scoring system described by Amiel-Tison et al.[3] was used because it is less time-consuming (average, 4 minutes per examination) with presumably less interobserver bias than Scanlon scoring. The newborn evaluation included assessments of passive and active tone, primary reflexes (automatic walking, Moro reflex, and sucking), responses and habituation to sound and light stimuli, alertness, and consolability. Examinations were performed by a pediatrician (J. C.) or qualified pediatric nurse (A. R.) without knowledge of previous intrapartum drug exposure.

Averaged data were reported as means ± 1 SD value. Statistical comparisons were made with the Student *t* test and χ^2 test. A *p* value <0.05 was considered to be statistically significant.

Table III. Mean adaptive capacity and neurologic scores between infants exposed or not exposed in utero to fentanyl citrate*

	Total fentanyl citrate dose				
	50 μg (n = 32)	*100 μg (n = 55)*	*150 to 200 μg (n = 23)*	*≥200 μg (n = 27)*	*None (n = 112)*
At 2 to 4 hours					
Adaptive capacity	8.4	8.6	8.4	7.3	8.2
Neurologic	25.2	25.3	23.8	24.2	25.3
Overall assessment	33.6	33.9	32.2	31.5	33.5
At 24 hours					
Adaptive capacity	8.7	8.7	8.6	8.6	9.0
Neurologic	27.0	26.7	27.6	26.7	27.1
Overall assessment	35.7	35.5	36.3	35.3	36.1

*None of the scores for infants exposed to fentanyl citrate was different from those of the infants who received none.

Results

A total of 137 participants were prescribed fentanyl citrate during the 4-month study period. This represented 55% (137:249) of the women who underwent labor at term without previous complications. Table I compares the profiles between the study and control pregnancies. The women who received fentanyl citrate were more likely to be younger and nulliparous. They also were more likely to require oxytocin and to have longer labors after hospital admission.

Fentanyl citrate was requested 308 times. The average number of requests per case was 2.3 ± 0.8 (range, 1 to 7 doses). The number of injections per case is shown in Fig. 1. Approximately two thirds (67.9%, 93:137) required two or fewer doses. Fig. 1 also displays the total dose administered per case. The cumulative dose ranged widely (50 μg to 600 μg) according to individual patient needs, but the mean was 140 ± 42 μg. Approximately two thirds (63.5%, 87:137) received ≤100 μg, whereas 90.3% (110:137) received <300 μg.

Questioning of participants by nursing staff revealed the onset of analgesic activity to be within 5 minutes and it usually lasted approximately 45 minutes. Fentanyl citrate did not completely eradicate uterine contraction pain, especially when pain was severe in the late phase of labor, but it did "take away the edge." Mood modification, temporary relief of fears, and relaxation were present in all cases. Depressed maternal respirations (<12 respirations per minute) were not seen. If the parturient was tired, drowsiness often occurred between contractions. Transient nausea was present in 13 cases (9.5%), but never so much as to require antinauseant medication. Such episodes usually occurred in the late phase of labor.

Serum concentrations of fentanyl citrate at delivery are compared between the mother and the fetus (umbilical cord) in Fig. 2. The concentrations of fentanyl citrate were significantly less ($p < 0.03$) for the cord sera than maternal sera, regardless of the fentanyl citrate dose. There was a significant linear relationship between the fentanyl citrate dose and cord levels ($p < 0.05$). In contrast, there was not a linear relationship between fentanyl citrate dose and maternal levels. This may be explained by the longer time from last dose until delivery between the >200 μg cumulative dose and the 100 to 200 μg dose groups (82.3 ± 47.2 minutes versus 97.7 ± 53.6 minutes).

Most infants exposed to fentanyl citrate in utero did well immediately after delivery with routine observation (Table II). The frequencies were low for depressed respirations by 90 seconds after delivery, and Apgar scores <7 were no more common than those for the control group. None of the study infants had an umbilical blood pH <7.25 or base excess < −6.0. Narcan was required in one case in which a single 50 μg dose of fentanyl citrate had been prescribed to the mother. The presence of three loops of cord wrapped around the infant's neck likely contributed to the depressed respirations. Despite the fentanyl citrate and cord entanglement, the 1- and 5-minute Apgar scores were 6 and 8, respectively, and the infant's depressed respirations improved.

Infants exposed to fentanyl citrate had respiratory rates equal to those in the control group during the first 2 to 4 hours (Table II). Apneic spells were not evident. Mean blood pressures recorded from the right arm and mean heart rates were not significantly lower than the control group.

Findings on neuroadaptive examinations compared favorably between infants in the study and control groups (Table III). Adaptive and neurologic scores determined at approximately 2 and 24 hours postnatally were reassuring, similar to the control groups, and dose dependent. A total score <30 was no more common ($p < 0.05$) if the total fentanyl citrate dose was >200 μg at 2 hours than for a lesser dose (5:38, 13.2% versus 6:99, 6.1%) and 24 hours (2:38, 5.3% versus 1:99,

1.0%). The last dose was usually given 2 hours before delivery (mean 112 ± 29 minutes; range, 27 to 183 minutes) and did not appear to influence the unlikely finding of depressed neonatal respirations or delayed neuroadaptive adjustments at the examination after 2 hours.

Comment

This study was undertaken to assess the usefulness of fentanyl citrate during labor because of its known rapid and short-acting analgesic effects with less anticipated depression in nonpregnant persons.[1] With the use of the chronic maternal-fetal sheep preparation, Craft et al.[4, 5] reported that fentanyl citrate administered intravenously or epidurally in standard doses crossed the placenta easily without adverse effects to the mother or fetus. No significant deleterious changes were found in uterine blood flow, uterine tone, and maternal or fetal cardiovascular and acid-base parameters. Results from this prospective investigation indicated that fentanyl citrate used intermittently during active labor is well tolerated by the participants. The more prolonged labors in the study group would be attributed more to the hospital admissions being in the earlier phase of labor and to the differences in pregnancy profiles (primigravida, unprepared childbirth, and need for oxytocin administration) than to the effects of the drug.

A preliminary report in humans by Eisele et al.[6] revealed that neonates were not impaired when the drug was given to the mother within 10 minutes before delivery during an elective cesarean section. The few neonates who required supplemental oxygen or naloxone in our experience often had accompanying complications such as meconium passage in utero or cord entanglements. This need for oxygen was less common than that of the control group.

Food and Drug Administration guidelines recommend neurobehavioral studies for any drugs used during labor. Findings in this study also provide reassurance that the drug was not associated with any increased risk of impaired neurologic or adaptive adjustments during the first 24 hours postnatally. This was true even when relatively higher total doses (>200 μg) were administered to the participants, although the number of study cases is not large. Infants exposed to fentanyl citrate were not more likely to have diminished passive or active muscle tone or impaired responses to light or sound stimuli.

With the use of the described dosing regimen in this investigation, an accumulation of fentanyl citrate was not considered to be harmful. Serum concentrations of fentanyl citrate remained consistently less in umbilical cord than in maternal circulation at delivery, regardless of the dose. More confidence with frequent intravenous dosing and less prolonged effects were positive findings with this narcotic. Despite these reassurances, the respiratory depressant effect of fentanyl citrate may be more prolonged than the analgesic effect, necessitating close observation of the parturient. We consider our observations to be encouraging and suggest a need for further intrapartum studies to compare fentanyl citrate with more conventionally used narcotics.

REFERENCES

1. Gilman AG, Goodman LS, Gilman A, eds. The pharmacological basis of therapeutics. 6th ed. New York: MacMillan Publishing, 1985:517.
2. Michiels M, Hendriks R, Heykants J. A sensitive radioimmunoassay for fentanyl. Eur J Clin Pharmacol 1977; 12:153-5.
3. Amiel-Tison C, Barrier G, Shnider S, et al. A new neurologic and adaptive capacity scoring system for evaluating obstetric medications in full-term newborns. Anesthesiology 1982;56:340-50.
4. Craft JB, Coaldrake LA, Bolan JC, et al. Placental passage and uterine effects of fentanyl. Anesth Analg 1983;62: 894-7.
5. Craft JB, Robichaux AG, Kim Haeng-sik, et al. The maternal and fetal cardiovascular effects of epidural fentanyl in the sheep model. Am J Obstet Gynecol 1984;148:1098-103.
6. Eisele JH, Wright R, Rogge P. Newborn and maternal fentanyl levels at cesarean section. Anesth Analg 1982;61:179-83.

Significance of D-mannose as a sperm receptor site on the zona pellucida in human fertilization

Kazumasa Mori, MD, Toshifumi Daitoh, MD, DMSc, Minoru Irahara, MD, DMSc, Masaharu Kamada, MD, DMSc, and Toshihiro Aono, MD, DMSc

Tokushima, Japan

The role of monosaccharides in human fertilization was studied by testing their effects on penetration of spermatozoa into mature human oocytes (zona penetration test). When oocytes were pretreated with concanavalin A, wheat germ agglutinin, or *Ricinus communis* agglutinin-I at a concentration of 100 μg/ml, no spermatozoa were found to bind to or penetrate through the zona pellucida. Penetration of spermatozoa was restored when the zona pellucida pretreated with wheat germ agglutinin and *Ricinus communis* agglutinin-I were rinsed with *N*-acetyl-D-glucosamine (wheat germ agglutinin inhibitor) and D-galactose (*Ricinus communis* agglutinin inhibitor), respectively. Conversely, the blocking effect of concanavalin A on sperm penetration was not reversed by treatment with D-mannose (concanavalin A inhibitor). Furthermore, pretreatment of spermatozoa with D-mannose (50 mmol/L) completely inhibited sperm penetration through the zona pellucida. However, sperm penetration was clearly demonstrated when the zona pellucida was pretreated with D-mannose. These data suggest that D-mannose residues are essential in, or sterically closely related to, the sperm receptor site on the human zona pellucida. (AM J OBSTET GYNECOL 1989;161:207-11.)

Key words: Sperm-ovum interactions, carbohydrates, D-mannose, human zona pellucida, spermatozoa receptor

As a first step in fertilization, spermatozoa bind to and penetrate through the zona pellucida that surrounds mammalian eggs. The zona pellucida is thought to have a receptor site for species-specific binding of spermatozoa. We previously demonstrated that monoclonal antibodies against common antigens of human and porcine zona pellucida and alloantibodies to human spermatozoa inhibited fertilization of human eggs in vitro. These data suggest that antigens on both gametes are essential in human sperm-egg interaction.[1,2] As shown by many investigators in a wide variety of species,[3-12] including mammals, carbohydrate determinants on the zona pellucida are involved in sperm-egg interaction. However, there have been no detailed studies on the role of carbohydrate determinants on the zona pellucida in fertilization of human eggs because of the limited availability of human oocytes. Our preliminary experiments with fluorescein-conjugated lectins demonstrated the presence of monosaccharide chains with terminal D-mannose, D-galactose, and *N*-acetyl-D-glucosamine, but not L-fucose residues in the human zona pellucida. In this study, we investigated the role of monosaccharide chains in the human zona pellucida in gamete recognition.

From the Department of Obstetrics and Gynecology, School of Medicine, The University of Tokushima.

Supported in part by a Grant-in-Aid for Scientific Research (No. 62570759) from the Ministry of Education, Science and Culture of Japan.

Received for publication March 27, 1988; revised September 14, 1988; accepted January 30, 1989.

Reprint requests: Kazumasa Mori, MD, Department of Obstetrics and Gynecology, University of Tokushima, Kuramoto-Cho, Tokushima 770, Japan.

Material and methods

Reagents. Concanavalin A (Con A), soybean agglutinin (SBA), wheat germ agglutinin (WGA), peanut agglutinin (PNA), *Dolichos biflorus* agglutinin (DBA), *Ricinus communis* agglutinin-I (RCA-I), and *Ulex europaeus* agglutinin-I (UEA-I) were purchased from Vector Laboratories, Inc. (Burlingame, Calif.). *Limulus polyphemus* agglutinin (LPA) was from Polysciences, Inc. (Warrington, Penn.). *N*-acetyl-D-glucosamine, D-galactose, D-mannose, and L-fucose were from E-Y Laboratories, Inc. (San Mateo, Calif.).

Collection of oocytes. Follicular oocytes were recovered from ovaries of patients who underwent laparotomy for gynecologic disorders. The oocytes were cultured as described previously.[1] They were allowed to mature for 48 hours at 37° C in Whittingham's T6 medium[13] containing 10% normal human serum under 5% carbon dioxide in air. Mature oocytes showing a first polar body were then stored in highly concentrated salt solution[14] (0.5 mol/L ammonium sulfate, 1.0 mol/L magnesium chloride, and 0.1% dextran) at 4° C and used for zona penetration tests within 3 months.

Zona penetration test. Before use, pooled oocytes were desalted in Dulbecco's phosphate-buffered saline solution overnight at 4° C. For insemination, freshly ejaculated spermatozoa from healthy men with consis-

Table I. Effects of exposure of human oocytes to various lectins on sperm binding to and penetration through the zona pellucida in vitro

	Experiment 1		*Experiment 2*		*Experiment 3*	
	No. of sperm bound	*No. of sperm penetrating*	*No. of sperm bound*	*No. of sperm penetrating*	*No. of sperm bound*	*No. of sperm penetrating*
Control	5	3	16	9	20	6
Con A	0	0	0	0	0	0
WGA	0	0	0	0	0	0
RCA-I	0	0	0	0	0	0
DBA	15	20				
LPA	9	3				
PNA	15	15				
SBA	10	11				
UEA-I	13	8				

The zona pellucida was treated with the indicated lectin (100 μg/ml) for 1 hour at room temperature. When oocytes were exposed to Con A, WGA, and RCA-I, washed, and incubated in vitro with motile spermatozoa, spermatozoa were unable to bind to and penetrate through the zona pellucida. Experiments were repeated three times.

Table II. Effects of treatments of the zona pellucida with lectins and their inhibitory sugars

	*Experiment 1**		*Experiment 2*		*Experiment 3*	
	No. of sperm bound	*No. of sperm penetrating*	*No. of sperm bound*	*No. of sperm penetrating*	*No. of sperm bound*	*No. of sperm penetrating*
Control†					22	5
Con A	10	0	10	0	18	0
WGA	13	0	10	5	32	5
RCA-I	5	10	9	0	13	2

The zona pellucida was pretreated with Con A, WGA, or RCA-I and then incubated for 1 hour in Whittingham's T6 medium containing 0.1 mol/L specific inhibitory sugars for each lectin (D-mannose, *N*-acetyl-D-glucosamine, and D-galactose, respectively). The blocking effect of Con A on sperm penetration was not reversed by treatment with D-mannose, whereas penetration of spermatozoa was restored when the zona pellucida pretreated with WGA and RCA-I were rinsed with *N*-acetyl-D-glucosamine and D-galactose, respectively. Experiments were repeated three times.

*The oocytes subjected to this experiment were shown to be incapable of sperm binding and penetration when exposed to Con A, WGA, or RCA-I in experiment 1 of Table I.

†Oocyte was incubated in T6 medium only.

tently high semen quality were washed three times with T6 medium by centrifugation at 200 *g* for 5 minutes. T6 medium containing 10% normal human serum was then layered onto the highly concentrated sperm suspension and the preparation was incubated for 1 hour at 37° C under 5% carbon dioxide in air. The motile sperm swimming up into the upper T6 medium were collected and resuspended in T6 medium containing 10% normal human serum at a concentration of 1.0×10^6/ml. One oocyte was placed in 0.3 ml of the suspension covered with warm paraffin oil in a plastic culture dish (35 × 11 mm; Nunc, Roskilde, Denmark) and incubated for 6 hours at 37° C under 5% carbon dioxide in air. Then the oocyte was washed by pipetting and was examined with a phase-contrast microscope (Olympus, IMT, Tokyo) for sperm binding to and penetration through the zona pellucida.[1, 2] In this study, a single semen donor was used for one experiment. We could use only one oocyte for each regimen because it was impossible to prepare certain numbers of oocytes in one experiment because of the shortage of human oocytes available. Therefore we repeated the experiments to confirm the data.

First, the effects of lectins at concentrations of 100 μg/ml in Dulbecco's phosphate-buffered saline solution were examined. Oocytes were treated with 0.2 ml of various lectins (Con A, SBA, WGA, PNA, DBA, RCA-I, UEA-I, and LPA) for 1 hour at room temperature, were rinsed three times in T6 medium, and then were subjected to the zona penetration test.

For determination of the reversibility of the effects of the lectins, after treatment with the lectins the oocytes were rinsed for 1 hour in T6 medium containing 0.1 mol/L concentrations of specific inhibitory sugars for each lectin and were then subjected to the zona penetration test. In the first experiment, we tested the oocytes that had been incapable of sperm binding and penetration in the above study.

Second, the effects of sugars (*N*-acetyl-D-glucosamine, D-galactose, D-mannose and L-fucose)

Table III. Effects of treatments of spermatozoa with sugars

	Experiment 1		*Experiment 2*		*Experiment 3*		*Experiment 4*	
	No. of sperm bound	*No. of sperm penetrating*	*No. of sperm bound*	*No. of sperm penetrating*	*No. of sperm bound*	*No. of sperm penetrating*	*No. of sperm bound*	*No. of sperm penetrating*
Control	17	2	16	15	16	8	8	3
D-mannose	4	0	0	0	9	0	2	0
N-acetyl-D glucos-amine	10	6						
D-galactose	9	4						
L-fucose	13	6	19	11	13	4		

Spermatozoa were incubated with 50 mmol/L concentrations of the indicated sugars for 30 minutes before the zona penetration test. Pretreatment with 50 mmol/L D-mannose completely inhibited penetration of spermatozoa through the zona pellucida, but pretreatment with *N*-acetyl-D-glucosamine, D-galactose, or L-fucose had no effect on their penetration. Experiments were repeated four times.

Table IV. Effects of treatments of the zona pellucida with D-mannose

	Experiment 1		*Experiment 2*		*Experiment 3*		*Experiment 4*	
	No. of sperm bound	*No. of sperm penetrating*	*No. of sperm bound*	*No. of sperm penetrating*	*No. of sperm bound*	*No. of sperm penetrating*	*No. of sperm bound*	*No. of sperm penetrating*
Control	8	3	14	4	17	4	13	8
D-mannose	23	8	30	5	10	1	14	8

The zona pellucida was incubated with or without 0.1 mol/L D-mannose for 30 minutes at room temperature before the zona penetration test. Pretreatment of the zona pellucida with D-mannose did not block penetration of untreated spermatozoa. Experiments were repeated four times.

were examined by the zona penetration test. Motile spermatozoa collecting by the swimming up method at a concentration of 1.0×10^6/ml were incubated in T6 medium (0.3 ml) containing 50 mmol/L sugar for 30 minutes at 37° C under 5% carbon dioxide in air (osmolality of sugar mixtures, 326 to 338 mOsm). Then an oocyte was incubated in an aliquot of the suspension for 6 hours. In the case of D-mannose, zona penetration tests were also performed after spermatozoa and eggs had been treated separately with the sugar to study its effect on each gamete. In this study only concentrations of lectin and sugar of 100 μg/ml and 50 mmol/L, respectively, were used because of the limited availability of mature oocytes.

Results

Effects of lectins. Table I summarizes results in the zona penetration test with zona pellucida treated with various lectins. When oocytes were exposed to Con A, RCA-I, and WGA, washed, and incubated in vitro with motile spermatozoa, spermatozoa were unable to bind to and penetrate through the zona pellucida, although many came in contact with its surface. No blocking of sperm penetration was observed with untreated oocyte or oocytes treated with five other lectins (DBA, LPA, PNA, SBA, and UEA-I). In a second zona penetration test, the zona pellucida were rinsed with the inhibitory sugars of the respective lectins. Penetration of spermatozoa was restored when the zona pellucida pretreated with RCA-I and WGA were rinsed with D-galactose and *N*-acetyl-D-glucosamine, respectively. Conversely, the blocking effect of Con A on sperm penetration was not reversed by treatment with D-mannose, although the spermatozoa bound to the zona pellucida (Table II).

Effects of sugars. Table III summarizes results in the zona penetration test using spermatozoa treated with various sugars. Pretreatment with 50 mmol/L D-mannose completely inhibited penetration of spermatozoa through the zona pellucida, but pretreatment with *N*-acetyl-D-glucosamine, D-galactose, or L-fucose had no effect on their penetration. Although many spermatozoa pretreated with D-mannose came in contact with the zona surface, they did not penetrate through the zona pellucida. Furthermore, pretreatment of the spermatozoa with D-mannose blocked sperm penetration through the untreated zona pellucida, but pretreatment of the zona pellucida with D-mannose for 30 minutes did not block penetration of untreated spermatozoa (Table IV).

Comment

In this study, the zona penetration test was used to determine whether monosaccharides are involved in

human sperm-egg interaction. We used oocytes matured in vitro and stored in highly concentrated salt solution because storage under these conditions has been shown to preserve the bioactivity of the zona pellucida of oocytes.[14] The zona penetration test facilitates studies on the mechanism of human fertilization. Such studies are difficult because large numbers of mature oocytes are not available.

There are many reports on the blocking effects of various lectins on fertilization in a wide variety of species. Results have shown that Con A, RCA-I, and fucose-binding protein have blocking effects in brown algae,[3, 4] Con A, and fucose-binding protein in ascidians,[5] asparagus pea lectin (specific for L-fucose) in *Limulus polyphemus*,[6] and Con A, WGA, RCA-I, and DBA in the hamster.[7, 8] In our study spermatozoa failed to bind to and penetrate through human zona pellucida that had been pretreated with any of the lectins capable of binding to it (Con A, WGA, and RCA-I; preliminary data).

Four possible mechanisms for the blocking effects of lectins may be considered. (1) The lectin-binding sites may be essential components of the sperm receptor sites. Lectins may block fertilization by causing selective masking or conformational change of the sperm receptors. (2) The lectins may bind to other receptors in the vicinity of the sperm receptors, causing steric inhibition of sperm binding. (3) The lectins may bind to nonspecific membrane receptors, causing modulation of the membrane structure, including biochemical alterations in the nature of the zona pellucida to be resistant to proteinase dissolution,[7] which in turn causes nonspecific inhibition of fertilization. (4) The lectins bound to the zona pellucida may also bind to spermatozoa and thus prevent their penetration through the zona pellucida, because the surface of human spermatozoa and the zona pellucida share binding sites for Con A, WGA, and RCA-I (unpublished data). However, the mechanism of the blocking effect of Con A appears to be different from that of WGA or RCA-I, because the effect of Con A was not reversed by washing Con A–treated zona pellucida with D-mannose, whereas the effects of WGA and RCA-I were reversed by rinsing the zona pellucida with the respective inhibitory sugars. Thus treatment of the zona pellucida with Con A induces critical change of the molecules including D-mannose residues on the zona pellucida. Furthermore, the finding that pretreatment of spermatozoa but not oocytes with D-mannose blocked penetration of the spermatozoa indicated that D-mannose–binding structures are present on the sperm surface.

Preliminary experiments showed that none of the sugars tested, even at the highest concentrations used (0.1 mol/L) affected sperm mobility. Therefore, this blocking effect of D-mannose was not caused by a reduction of sperm mobility. Another possibility is that spermatozoal proteinases necessary for sperm penetration are inhibited by D-mannose. Anderson et al.[15] reported that human spermatozoal acrosin was inhibited by a variety of monosaccharides, especially D-fructose and D-mannose. However, they also showed inhibition of acrosin activity by D-galactose, although the inhibiting activity is somewhat low (approximately 60% of D-mannose). If the blocking effect of D-mannose on sperm penetration is caused by inhibition of acrosin activity, D-galactose should also show the blocking effect. However, this did not occur.

Blocking effects of saccharides on fertilization in other species have been reported: L-fucose in ascidians[9] and *Limulus polyphemus*,[6] L-fucose and fucoidin in the hamster and guinea pig,[10] α-methyl mannoside and D-mannose in the rat,[11] and α-methyl-mannose and sialic acid in the mouse.[12] However, results on which gamete was affected by saccharide were controversial. Consistent with our results, blocking of sperm-egg interaction by binding of saccharide residues to spermatozoa has been demonstrated in *Limulus polyphemus*,[6] the rat,[11] and guinea pig,[10] but saccharides are reported to affect the egg in the mouse[12] and both the sperm and egg in ascidians.[9] Huang et al.[10] found that in humans fucoidin blocked sperm binding to the zona pellucida and concluded that L-fucose may be part of a recognition signal between spermatozoa and eggs. We did not observe any inhibitory effect of 50 mmol/L L-fucose in the zona penetration test, but at a higher concentration L-fucose may block fertilization because fucoidin was found to be a more potent inhibitor of hamster fertilization than L-fucose.

On the basis of our results, we propose that a mandatory step in human fertilization is binding of a D-mannose–binding constituent of the sperm surface to a D-mannose residue in the sperm receptor site on the zona pellucida. Further studies at the molecular level are necessary to elucidate details of the mechanism of sperm-egg interaction.

We thank Ms. M. Ikeda for her technical assistance.

REFERENCES

1. Kamada M, Daitoh T, Hasebe H, Irahara M, Yamano S, Mori T. Blocking of human fertilization in vitro by sera with sperm immobilizing antibodies. AM J OBSTET GYNECOL 1985;153:328.
2. Mori T, Kamada M, Yamano S, Kinoshita T, Kano K, Mori T. Production of monoclonal antibodies to porcine zona pellucida and their inhibition of sperm penetration through human zona pellucida in vitro. J Reprod Immunol 1985;8:1.
3. Catt JW, Vithanage HIMV, Callow JA, Callow ME, Evans LV. Fertilization in brown algae: further investigations of lectins as surface probes. Exp Cell Res 1983;147:127.
4. Callow JA. Sexual recognition and fertilization in brown algae. J Cell Sci 1985;2(suppl):219.
5. Rosati F, DeSantis R, Monroy A. Studies on fertilization in the ascidians: lectin binding to the gametes of *Ciona intestinalis*. Exp Cell Res 1978;116:419.

6. Barnum SR, Brown GG. Effect of lectins and sugars on primary sperm attachment in the horseshoe crab, *Limulus polyphemus L.* Dev Biol 1983;95:352.
7. Oikawa T, Yanagimachi R, Nicolson GL. Wheat germ agglutinin blocks mammalian fertilization. Nature 1973; 241:256.
8. Oikawa T, Nicolson GL, Yanagimachi R. Inhibition of hamster fertilization by phytoagglutinins. Exp Cell Res 1974;83:239.
9. Rosati F, DeSantis R. Role of the surface carbohydrates in sperm-egg interaction in *Ciona intestinalis*. Nature 1980;283:762.
10. Huang TTF Jr, Ohzu E, Yanagimachi R. Evidence suggesting that L-fucose is part of a recognition signal for sperm-zona pellucida attachment in mammals. Gamete Res 1982;5:355.
11. Shalgi R, Matityahu A, Nebel L. The role of carbohydrates in sperm-egg interaction in rats. Biol Reprod 1986;34: 446.
12. Lambert H. Role of sperm-surface glycoproteins in gamete recognition in two mouse species. J Reprod Fertil 1984;70:281.
13. Quinn P, Barros C, Whittingham DG. Preservation of hamster oocytes to assay the fertilizing capacity of human spermatozoa. J Reprod Fertil 1982;66:161.
14. Yanagimachi R, Lopata A, Odom CB, Bronson RA, Mahi CA, Nicolson GL. Retention of biologic characteristics of zona pellucida in highly concentrated salt solution: the use of salt-stored eggs for assessing the fertilizing capacity of spermatozoa. Fertil Steril 1979;31:562.
15. Anderson RA Jr, Oswald C, Leto S, Zaneveld LJD. Inhibition of human acrosin by fructose and other monosaccharides. Biol Reprod 1980;22:1079.

The importance of ultrasonography in infertile women with "forgotten" intrauterine contraceptive devices

Raphael Ron-El, MD, Zvi Weinraub, MD, Rami Langer, MD, Ian Bukovsky, MD, and Eliahu Caspi, MD

Zerifin and Tel Aviv, Israel

Seven cases in which an unknown intrauterine contraceptive device in situ was the cause of infertility were referred to our Infertility Clinic after each patient had been told by her physician that she no longer had an intrauterine contraceptive device. In some cases, there were lapses in the infertility workup that might explain how the cause of infertility of an intrauterine contraceptive device in situ was missed. In other cases, a thorough investigation was made and infertility treatment was started without the existence of an intrauterine contraceptive device in situ being diagnosed. (AM J OBSTET GYNECOL 1989;161: 211-2.)

Key words: Infertility, female; intrauterine devices; ultrasonic diagnosis

Reported here are seven cases of "forgotten" intrauterine contraceptive devices (IUDs) that caused infertility.

Case reports

Case 1. A 32-year-old woman was fitted with an IUD after her second delivery. Examination after 2 years showed no IUD thread through the cervix and x-ray examination did not reveal the presence of an IUD in situ. On a later occasion, an IUD was inserted, resulting in two IUDs in the uterus for 3 years. Because the woman did not conceive after the removal of the second device, she was referred to our Infertility Clinic. In the workup an ultrasonographic examination for leading follicle measurement revealed the presence of a multi-load IUD that was removed. Two months later she conceived.

Case 2. A 26-year-old woman with a 2-year history of infertility had an induced abortion at age 18. She did not recall any procedures or instructions that were given to her after the abortion. The infertility workup included a hysterosalpingogram with normal results. Because of excessive menstrual bleeding, a curettage was performed. A "homemade" IUD of nylon thread was extracted (Fig. 1). This thread was invisible on x-ray examination yet was detectable by ultrasonography. The patient conceived 6 months later.

Case 3. A 30-year-old patient had secondary infertility for 6 years. Results of a hysterosalpingogram and laparoscopy findings were normal; however, the patient reported heavier menstrual bleeding since delivery. When an endometrial curettage was performed a "homemade" nylon thread was extracted; the woman conceived 4 months later.

From the Department of Obstetrics and Gynecology, Assaf Harofeh Medical Center, Zerifin, and Sackler School of Medicine, Tel Aviv.
Received for publication October 23, 1988; revised February 11, 1989; accepted February 24, 1989.
Reprint requests: R. Ron-El, MD, Department of Obstetrics and Gynecology, Assaf Harofeh Medical Center, Zerifin, 70300, Israel.

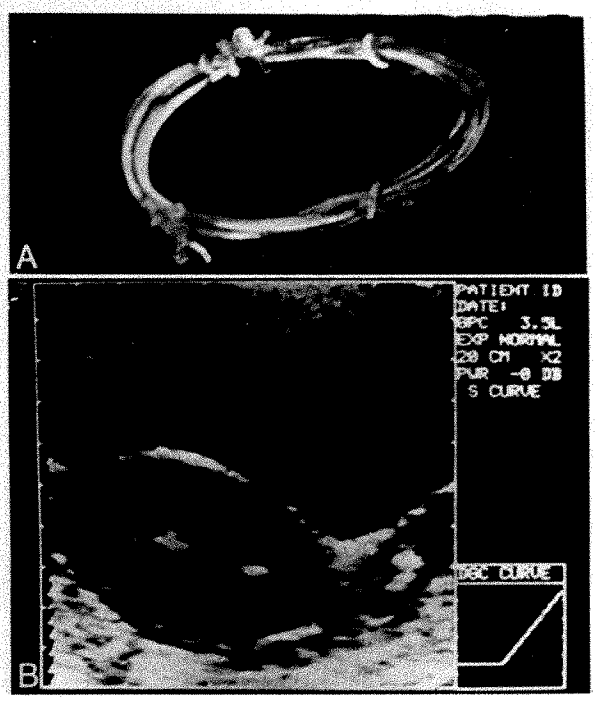


Fig. 1. Nylon thread self-made IUD (**A**) and its ultrasonographic overview in the uterus (**B**).

Case 4. A 26-year-old woman was treated for intrauterine adhesions by curettage and temporary insertion of an Ipsilon IUD. The patient recalled a second visit 2 months later for removal of the IUD. Repeated hysterosalpingogram examination did not reveal any pathologic condition. She then conceived but the pregnancy terminated in an early spontaneous abortion. At the subsequent curettage, an Ipsilon IUD (without the membrane) was found and removed. The patient conceived 2 months later.

Case 5. A 32-year-old patient had an IUD inserted after a vaginal delivery. A year later, she conceived with an IUD in situ. The physician who performed the abortion did not find the IUD. It was assumed that it had been expelled spontaneously. The patient took oral contraceptives, stopped the medication, and then did not use contraception for 2 years. A thorough investigation including hysterosalpingogram and laparoscopy had normal findings. The patient was being treated with menotropins for superovulation when an ultrasonographic examination for follicle size revealed the IUD in situ. A Cu-7 device was removed. On reexamination of the hysterosalpingogram x-ray film, the branches of the Cu-7 device were detected. Five months later she conceived.

Cases 6 and 7. Two patients, 31 and 35 years old, primiparous and secundiparous, respectively, each had an IUD inserted after delivery. When they requested removal of the devices, the thread was not seen through the cervical canal and x-ray examination gave no indication of an IUD in situ. Both patients had ultrasonographic examinations that showed an IUD in the uterine cavity; a "homemade" device and a Cu-7 device, respectively, were removed. Neither patient has yet conceived.

Comment

With widespread use of IUDs, a "forgotten" IUD in situ has emerged as a cause for infertility. This situation causes an iatrogenic infertility, prompting unnecessary noninvasive and invasive examinations and unjustified treatments. Therefore a seemingly infertile patient with a history suggestive of IUD insertion could have a "forgotten" IUD in situ. An accurate diagnosis is more often obtained by a preliminary x-ray examination rather than by hysterosalpingogram.[1] Many cases reveal devices not currently available on the market and are initially unrecognizable on x-ray films. The accuracy of ultrasonography in the diagnosis of an IUD in the uterine cavity[2] indicates its justification in the case of any infertile woman whose past history shows use of an IUD or any gynecologic intervention. Frustration, discomfort, and expense to the patient are thus minimized.

REFERENCES

1. Rowe T, McComb P. Unknown intrauterine devices and infertility. Fertil Steril 1987;47:1038-9.
2. Najavian KE, Kurtz AB. New observations in the sonographic evaluation of intrauterine contraceptive devices. J Ultrasound Med 1986;5:205-10.

Acid-base status at birth and neurodevelopmental outcome at four and one-half years

Jennifer Dennis, DM, Ann Johnson, MD, Lesley Mutch, MD, Patricia Yudkin, MA, and Paul Johnson, MB, ChB

Oxford, England

Two hundred thirty term infants with measured acid-base status in umbilical arterial blood at birth were selected from 1210 consecutive deliveries for detailed neurodevelopmental follow-up at age 4½ years; 203 were examined. Cutoff points approximately 1 SD from the mean (pH ≤ 7.10; base deficit >12 mmol/L) were used to define acidosis. No statistically significant associations between acidosis and developmental outcome were found. The highest proportion of unimpaired children was found among those who were most severely acidotic at birth (pH ≤ 7.04; 2 SD below mean), but this finding was not statistically significant. These findings suggest that the ability of the fetus to produce an acidosis in response to the stress of labor may be beneficial to long-term outcome. The 10 nonacidotic babies with 1-minute Apgar scores of ≤3 showed statistically significant deficits in some areas. Coincident acidosis was not associated with a worse outcome for infants with low Apgar scores. (AM J OBSTET GYNECOL 1989;161:213-20.)

Key words: Umbilical arterial acidosis, neurodevelopmental outcome, Apgar score, perinatal asphyxia

Much perinatal intervention is directed toward the prevention of fetal and neonatal asphyxia in the belief that such insults may result in death or later disablement. Morbid anatomic studies have demonstrated unequivocally that events occurring during labor and delivery can lead to cerebral damage and death in the infant through the effects of trauma and asphyxia.[1] Asphyxial signs in those babies who die range from diffuse cerebral edema to cortical necrosis, boundary zone infarction, and basal nuclei damage. It is assumed that similar lesions may be present in those who survive perinatal asphyxial insults and that these lesions may give rise to a variety of neurodevelopmental problems.

There is no generally accepted definition of asphyxia. There are a number of commonly used, clinical indirect markers of asphyxia including Apgar score, neonatal neurologic state, and acid-base status at birth. None of these can be regarded as "diagnostic" of preceding hypoxia. Indeed, they may reflect other intrapartum events or even underlying and more fundamental problems of prenatal origin.[2] Historically, it should be recalled that the Apgar score was devised as an objective measurement of the condition of the neonate at birth[3] and not as a marker of asphyxia.

The notion of a simple relationship between hypoxia, acid-base status, and cerebral damage attributed to asphyxia is open to challenge.[4] Acidosis, as measured in umbilical artery blood at birth, is widely assumed to reflect fetal asphyxia. Thus it might be expected to have a relationship with the condition of the infant at delivery, neonatal neurologic status, or later neurodevelopmental integrity. Despite a remarkable lack of evidence, the strength of this belief is such that a major obstetric goal in some centers is to deliver babies with "normal" cord blood acid-base status.[5]

Sykes et al.[6] set out to measure acid-base status and Apgar scores in the umbilical cord blood of 1210 consecutively born infants delivered in the John Radcliffe Hospital, Oxford, between April and July 1981. Full data were obtained for 895 infants. About half of the missing cases arose because no member of the team responsible for collecting the blood samples was available at the time of delivery, and the remainder because blood could not be drawn from the umbilical artery. A detailed comparison between the deliveries with and without full blood gas values confirmed that the cases with full blood gas measurements were an unbiased sample of the whole.

These 895 children provided an opportunity to determine whether a single measure of acid-base status at birth in term infants would be predictive of neuro-

From the Human Development Research Unit, Park Hospital for Children, National Perinatal Epidemiology Unit, and Nuffield Department of Obstetrics and Gynaecology, Oxford, and the Oxford Region Child Development Project.

Supported by the locally organized research scheme of the Oxford District Health Authority and the Spastics Society.

Received for publication August 12, 1988; revised December 13, 1988; accepted January 11, 1989.

Reprint requests: Paul Johnson, MBChB, Nuffield Department of Obstetrics and Gynaecology, Level 3 Maternity Department, John Radcliffe Hospital, Headington, Oxford OX3 9DU, England.

Table I. Study population

	A	*B*	*C*	*D*	*E*	*Total*
Eligible	72	22	42	689	14	825
Selected	72	22	42	80	14	230
1 min Apgar score ≤3	(8)	(2)	(0)	(0)	(14)	(24)
Losses before assessment						
Refusals	2	2	2	14	2	22
Abroad	2	1	1	—*	1	5
Nos. assessed	68	19	39	66	11	203
Exclusions after assessment						
Other factors	1	—	1	1	—	3
English not first language	4	1	1	1	1	8
Final Nos. analyzed	63	18	37	64†	10	192
1 min Apgar score ≤3	(7)	(1)	(0)	(0)	(10)	(18)

*Losses abroad replaced—see text.

†This constitutes group Ds.

developmental outcome at age 4 to 5 years and form the basis of this study.

Methods

Categorization of infants by acid-base status and Apgar score. Children were assigned to study groups according to acid-base status and Apgar score at birth.

Acid-base status. From the original group of 1210 infants, 895 had full data on umbilical artery pH, base deficit, and Apgar score. From these all singleton infants born at or after 37 weeks' gestation and surviving at age 4½ years were selected ($N = 825$).

They were divided into the following four groups: (1) group A: pH ≤ 7.10, base deficit >12 mmol/L ($n = 72$); (2) group B: pH ≤ 7.10, base deficit ≤12 mmol/L ($n = 22$); (3) group C: pH > 7.10, base deficit >12 mmol/L ($n = 42$); and (4) group D: pH > 7.10, base deficit ≤12 mmol/L ($n = 689$). The cutoff levels were those used in the 1981 study and were approximately 1 SD from the mean obtained in that study.[6] Group A contained the most acidotic infants and group D the least acidotic.

All children in groups A, B, and C and a random sample of 80 from group D (group Ds) were selected for study (see "Statistical Methods"). Social class and sex are known to influence child development.[7] Thus the social class composition of group Ds was selected to match that of groups A, B, and C combined; the sample was chosen randomly from within each social class group. Social class was based on the occupation of the head of household, and five social class groups were defined: (1) professional, (2) managerial and lower professional, (3) skilled, manual, and nonmanual, (4) partly skilled, and (5) unskilled.[8] Members of the armed forces, students, and the unemployed formed a sixth group. Because the sex breakdown in group Ds matched that in the other three groups, no adjustment for sex was necessary.

Apgar score at 1 minute. Of the 24 children with a 1-minute Apgar score of ≤3, eight were in group A and two were in group B. The remainder ($n = 14$) formed group E, the nonacidotic, low Apgar score group.

Thus 230 children were selected for study (Table I); all were traced. Nine had gone abroad; four of these were in group Ds and therefore could be replaced from the total group D.

Number assessed. The general practitioners of all the children were contacted. With their permission, the parents were approached by letter, explaining the study and offering the choice of an appointment at home or in-hospital. Five physicians refused us access to their patients because of social problems in the family (one in group Ds). In addition, 17 parents (one adoptive) did not want their children assessed (13 in group Ds). Thus there were 22 refusals (14 in group Ds). We are unable to explain this excess in group Ds and acknowledge it may introduce a bias in either direction. These children were not replaced because families who refused access may constitute a special group. This left 203 children who were actually assessed (Table I).

Exclusions after assessment. After assessment there were further exclusions before analysis. Two children had suffered undoubted postnatal neurologic insults that affected their neurodevelopmental status. One was profoundly deaf after meningitis at the age of 2 years, and the other had achondroplasia and had developed hydrocephalus requiring insertion of a ventriculoatrial shunt. A third child had many dysmorphic features, suggesting organic brain disorder of prenatal origin.

The eight children whose first language was not English were also excluded, since it was clear that they were at a considerable disadvantage during testing.

Findings for 192 children were analyzed.

Assessment. A protocol was devised covering a wide range of areas of development, including perceptual, cognitive, and language skills, neurologic status, and behavioral attributes. The standardized tests used were as follows. As a basic global instrument, five of the six

Table II. Social characteristics in the study population

	A (*n* = 63)	*B* (*n* = 18)	*C* (*n* = 37)	*D* (*n* = 64)	*E* (*n* = 10)
Male (%)	52	44	38	52	80
Social class (%)					
I and II (high)	32	39	46	41	30
III	37	28	27	33	50
IV and V (low)	16	17	19	17	—
Other	16	17	8	9	20
≥5 preschool session/wk (%)	59	33	49	41	30
Age at testing (mo)					
Mean	56.8	56.8	56.3	56.7	56.1
SD	2.1	2.4	2.4	2.0	2.5

subscales of the Griffiths Developmental Scales were used.[9] These were locomotor, personal/social, hearing/speech, eye-hand coordination, and performance. The test is designed for children, ages 0 to 8 years. The subscale scores are expressed in terms of developmental quotient (DQ = mental age/chronologic age × 100). The Test for the Reception of Grammar (TROG)[10] was used to assess the children's ability to understand different and increasingly complex grammatic constructions. This test is standardized in age percentiles for children ages 3 to 10 years. As a measure of behavior disorder, we used a questionnaire based on the Rutter behavior screening questionnaires.[11] These were originally standardized for children 9 to 13 years. In this study we used a modification of the parents' scale that has been developed for 5-year-old children.[12] This 15-item–rated questionnaire is filled in by the parents. Behavior disorders can be divided into conduct-type problems and neurotic-type problems.

The nonstandardized tests were as follows. For auditory discrimination, we used the Renfrew picture-pointing test,[13] which is appropriate for children from age 3½ years. Discrimination errors made during the test reflect either conductive hearing problems (secretory or acute otitis media) or centrally mediated impaired discrimination. For further testing of fine motor and visuo-motor skills, two tests were used. The Keyhole pegboard test[14] consists of a 10 by 10 cm board with 25 small, keyhole-shaped holes pointing in different directions. Into these can be slotted closely fitting pegs. The test has been standardized only for adults, and for these young children we used only the central nine holes. The task requires good fingertip and visuo-motor skills to orientate the pegs correctly and good targeting to slot them in the holes. It is a timed test performed with both preferred and nonpreferred hands. The second extra test of fine motor and visuo-motor skills required the child to draw a line between tramlines arranged to form a 4-inch square.This is a modification of one of the Frostig tests.[15] The tramlines were wide (20 mm apart) and narrow (6.5 mm apart), and the test was performed with preferred and nonpreferred hands. Scores were based on the number of errors, which were defined as touching or crossing the boundary tramlines, and on the continuity of the line drawn. For neurologic evaluation we devised a checklist. This covered specific qualitative deviations and asymmetries observed in carrying out gross and fine motor tasks used in the schedule, an evaluation of articulatory dyspraxia, and some additional neurologic "soft" signs.

Growth parameters were recorded and set against Tanner et al.[16] and Ounsted et al.[17] charts. Information was sought from the parents about the child's medical history and other social details including family structure, mother's work, and the child's preschool, playgroup, or nursery experience. Information about vision and hearing will be retrieved from routine services at a later date, some of it after school entry.

Each child was seen between the age of 4½ and 5 years by one of three developmental pediatricians (J. D., A. J, or L. M.), who did not know the original cord blood findings. Twenty percent were seen in-hospital, and the remainder were seen at home. The examiners monitored each other's performance during a short pilot study carried out to assess the feasibility and acceptability of the protocol and improve inter-observer agreement.

Statistical methods

Sample size. The number of children in groups A, B, C, and E was limited by the sample size of the original study. It was anticipated that the rate of poor outcomes in group D would be 10%.[18] Power calculations showed that a sample of 80 children from group D would be sufficient to detect a threefold increase in the most acidotic group (group A), with 85% power and at a significance level of 0.05.

Tenth percentile cutoff points. These were determined for each test from the distribution of scores for the 192 children in the study and were used for internal comparison between groups. They were not intended as population standards.

Table III. Griffiths subscale scores in each group

	A (*n* = 63)	*B* (*n* = 18)	*C* (*n* = 37)	*Ds* (*n* = 64)	*E* (*n* = 10)
Locomotor*					
Mean	109.4	107.8	110.3	112.0	103.2†
SD	11.6	10.8	13.5	9.1	11.7
Personal/social*					
Mean	107.2	107.6	106.7	108.3	103.9
SD	10.1	11.1	10.9	10.8	7.3
Hearing/speech*					
Mean	118.3	117.6	118.7	118.3	107.1‡
SD	14.7	13.3	18.3	15.9	13.7
Eye/hand coordination					
Mean	113.0	108.9	109.5	111.4	106.3
SD	17.4	19.3	17.0	17.8	24.0
Performance					
Mean	111.9	107.4	110.0	111.2	110.5
SD	19.2	15.1	15.3	19.8	23.0

*Locomotor: one child from groups A, B, and C not tested; personal/social: one child from group C not tested; and hearing/speech: one child from group A not tested.

†Significant difference between group Ds and group E: $p < 0.01$.

‡Significant difference between group Ds and group E: $p < 0.05$.

Comparisons between groups. Comparisons were made initially between groups A, B, C, and Ds and then between groups E and Ds.

In comparisons between groups A, B, C, and Ds, average values were compared by means of a one-way analysis of variance; the Kruskal-Wallis version of this test[19] was used for data that were not normally distributed. Comparisons between proportions were made with the χ^2 test.

In comparisons between groups Ds and E, average values were compared with the *t* test or for data that were not normally distributed the Mann-Whitney U test. Proportions were compared with the χ^2 test.

Muliple regression analysis. Each variable was entered stepwise into the regression equation and remained in the equation only as long as its regression coefficient was statistically significant at the 0.05 level. Categoric variables, such as social class, were treated as a number of binary variables.[20]

Significance levels. This study was not designed to test a single hypothesis but rather to explore whether the groups differed in any particular areas of performance. Following convention, we have reported results as statistically significant whenever p was <0.05. However, because about 50 different significance tests were carried out, there is a very high probability that at least one spuriously "significant" result will have occurred, and a "significant" result even at the 0.01 level should be treated with caution.

Results

Some social and other factors thought likely to affect test scores are shown in Table II. There were no statistically significant differences in the distribution of these characteristics between groups.

Griffiths subscale scores. Mean scores on the five Griffiths subscales were similar across groups A, B, C, and Ds (Table III). Group E had significantly lower mean scores than group Ds on the locomotor subscale and hearing/speech subscale ($p < 0.01$ and <0.05, respectively).

Multiple regression analyses were used to identify factors showing a significant association with the Griffiths scores. Neither pH ≤ 7.10, base deficit >12 mmol/L, nor 1-minute Apgar score ≤3 were significant factors. Higher social class, playgroup, nursery, or school attendance for five or more sessions per week, female sex, and lower age at testing were statistically significantly associated with higher scores on three subscales: personal/social, hearing/speech, and eye/hand coordination. Social class alone was associated with the performance subscale score, but there were no statistically significant associations with the locomotor score. The child's place in the family, current outpatient attendance or therapy, poor hearing reported by the parent, and the place of assessment (home or hospital) were not significant explanatory factors for any of the subscale scores.

The poorest performers on all subscales (those scoring below the tenth percentile) were in general evenly distributed throughout groups A to D (Table IV). However, on the locomotor scale, only one child from group Ds (1.6%) scored below the tenth percentile compared with nine (14.5%) of the children in group A. The difference between the four groups just failed to reach statistical significance at the 0.05 level, although a direct comparison between group A and group Ds was significant ($p < 0.05$). There was no difference between the groups in the proportion of children scoring below the tenth percentile on two or more of the Griffiths

Table IV. Percentage in each group with Griffiths subscale score <tenth percentile

	A (*n* = 63)	*B* (*n* = 18)	*C* (*n* = 37)	*Ds* (*n* = 64)	*E* (*n* = 10)
Locomotor*	14.5	11.8	13.9	1.6†	20.0
Personal/social*	11.1	5.6	11.1	9.4	20.0
Hearing/speech*	9.7	5.6	8.1	9.4	20.0
Eye/hand coordination	9.5	5.6	8.1	10.9	30.0
Performance	12.7	11.1	8.1	7.8	10.0
≥3 scores <10th percentile	9.7	5.9	8.6	6.3	10.0
1 or 2 scores <10th percentile	16.1	17.6	20.0	15.6	60.0
No scores <10th percentile	74.2	76.5	71.4	78.1	30.0

Significant difference between A and D_S, $p < 0.05$.

*Locomotor: one child from groups A, B, and C not tested; personal/social: one child from group C not tested; and hearing/speech: one child from group A not tested.

subscales. Although the number of children in group E is small, seven of 10 scored below the tenth percentile in one or more of the five subscales.

Keyhole pegboard test. The overall mean time for completion of the test was 60.7 seconds (SD 33.4) for the preferred hand and 69.4 seconds (SD 32.8) for the nonpreferred hand. There was no difference between groups in the mean time taken or in the number of poor performers (<tenth percentile).

Using the preferred hand, two children failed to complete the test within 3 minutes, both of whom were from group A. Using the nonpreferred hand, six children were considered failures: four of 63 from group A, one of 37 from group C, and one of 64 from group Ds.

For each child, the difference between completion times with the preferred and nonpreferred hands was calculated. There was no difference in the mean value of this measure between the five groups.

Tramline square. There was no difference in scores between groups A, B, C, and Ds. Group E scored higher (i.e., made more errors) than group Ds on both wide and narrow tramline squares and with both preferred and nonpreferred hands. The difference was statistically significant only for the narrow square, which was drawn with the nonpreferred hand ($p < 0.05$).

Rutter score. Twenty-two of the 192 children (11.5%) scored 12 or more. This is consistent with the findings of Watt et al.[12] for this modified version of the scale. There was no statistically significant difference in total Rutter scores between groups A, B, C, and Ds. However, children in group E scored significantly higher (i.e., were more behavior disordered) than those in group Ds ($p < 0.05$). The behaviors reported in this questionnaire fall into two groups that reflect either conduct-type problems (e.g., fighting, aggression, or tempers) or neurotic-type problems (e.g., shyness, fearfulness, etc.). An excess of conduct-type problems differentiated group E from group Ds.

Test for the Reception of Grammar (TROG). There was no difference between the groups in the numbers of children scoring below the tenth percentile on the Test for the Reception of Grammar.

Table V. Percentage in each group with different levels of overall impairment

*Grades**	*A* (*n* = 63)	*B* (*n* = 18)	*C* (*n* = 37)	*Ds* (*n* = 64)	*E* (*n* = 10)
1	39.7	22.2	40.5	39.1	10.0
2	28.6	22.2	24.3	29.7	30.0
3	22.2	50.0	24.3	20.3	30.0
4	9.5	5.6	10.8	10.9	30.0
TOTAL	100.0	100.0	100.0	100.0	100.0

*1, No impairment; 2, nonspecific poor performance on single items but overall appeared normal; 3, definite identified area(s) of impairment—not necessarily requiring referral; overall less severe level of impairment than grade 4; 4, definite impairment—usually requiring referral including global delay; lateralizing signs, and severe deficit in specific area.

Auditory discrimination and neurologic screening. The picture-pointing test results for auditory discrimination were invalidated by the presence of acute catarrhal upper respiratory tract conditions in many of the children at the time of testing. Despite a short pilot study, interrater reliability on the neurologic examination was not sufficiently established to allow us to present the findings with confidence.

Overall performance. Impairment codes were ascribed by consensus on review of total outcome information available for each child. Four levels of impairment were defined (Table V). The distribution of impairment codes showed no differences between groups. Of the 192 children, 21 (10.9%) were coded as definitely and significantly impaired (code 4); an additional 48 (25.0%) were considered definitely but less severely impaired (code 3).

Primary analysis: Summary. There was no statistically significant difference between groups A, B, C, and Ds, but a greater proportion of children in group A than group Ds scored below the tenth percentile on the Griffiths locomotor subscale (Table IV).

The mean score for group E was significantly lower

Table VI. Griffiths subscale scores: Groups Ds and E and acidotic low Apgar score group

	Group Ds (n = 64)	*Group E (nonacidotic; low Apgar) (n = 10)*	*Acidotic; low Apgar (n = 8)*
Locomotor			
Mean	112.0	103.2	111.9
SD	9.1	11.7	14.7
Personal/social			
Mean	108.3	103.9	107.9
SD	10.8	7.3	10.7
Hearing/speech			
Mean	118.3	107.1	117.0
SD	15.9	13.7	21.4
Eye/hand coordination			
Mean	111.4	106.3	103.5
SD	17.8	24.0	20.8
Performance			
Mean	111.2	110.5	104.5
SD	19.8	23.0	24.0

than that for group Ds on the Griffiths locomotor subscale ($p < 0.01$) and the hearing/speech subscale ($p < 0.05$) (Table III). Group E also did statistically significantly worse than group Ds in the narrow tramline test performed with the nonpreferred hand ($p < 0.05$). Children in group E had a significantly higher Rutter score (i.e., were more behavior disordered) than those in group Ds ($p < 0.05$).

Further analyses

Griffiths "profile." Like other investigators, we found that the population means for all five Griffiths subscales were greater than 100.[21] In addition, they differed considerably from one another (Table III). This meant that a given score on one subscale did not denote the same level of achievement, relative to other children, as the same score on another subscale. Therefore to compare relative achievement across all five subscales, the scores were standardized by being expressed as a number of standard deviations from the mean for that subscale.

Therefore consistency of performance across the Griffiths subscales could be measured for each child in two ways: by the standard deviation of the five standardized Griffiths scores and by the range. Thus children who performed consistently well (or consistently badly) on all five subscales had a small range of scores. Multiple regression analysis was used to determine factors significantly associated with consistency of performance across the five tests. All the explanatory variables previously tested were again examined. The only statistically significant factor was a 1-minute Apgar score of ≤3, which decreased the consistency between scores.

Further analysis suggested that this decrease in consistency arose because the minimum standardized Griffiths score for children with low Apgar scores was relatively depressed. The mean minimum score for the 18 children with low 1-minute Apgar score was −1.29 (SD 0.76). In other words, in the Griffiths subscale on which they performed worst, these children scored an average 1.29 standard deviations below the mean. This was significantly lower ($p < 0.02$) than the mean minimum score for the children in group Ds (−0.79: SD 0.77). There was no difference in the maximum standardized scores for the two groups: 0.94 (SD 0.86) for children with low Apgar scores compared with 0.88 (SD 0.90) for group Ds. The minimum scores for children with low Apgar scores were not concentrated in any particular area of performance.

Very acidotic children (n = 27). Since it was possible that a failure to demonstrate differences between groups A, B, C, and Ds was related to a relatively high cutoff point for pH (1 SD below the mean), those children from groups A and B with an umbilical artery pH of ≤7.04 (2 SD below the mean) were compared with group Ds (normal acid-base status).

The very acidotic children did not perform worse than those in group Ds on any of the tests. Indeed, on overall performance 52% were graded as totally unimpaired (grade 1) compared with 39% in group Ds and 28% of the less acidotic children (pH 7.05 to 7.10). Neurotic-type behavior problems were also underrepresented within this very acidotic group.

All children with Apgar scores of 3 or below. Because our results have suggested some deficit in performance among children in group E (i.e., low Apgar score at 1 minute without acidosis), the results of all infants in the cohort with Apgar scores of ≤3 (at 1 minute) (both nonacidotic and acidotic) were further analyzed. Therefore the seven infants from group A and one infant from group B with Apgar score of ≤ 3 were considered, as well as those from group E. As Table VI shows, the eight acidotic children with low Apgar scores had higher mean scores than those in group E on three subscales: locomotor, personal/social and hearing/speech. On these subscales, acidotic children

with low Apgar scores had mean scores very close to those of the control group Ds. On the two other subscales, the acidotic low Apgar score group performed somewhat worse than group E. None of the differences between the acidotic children with low Apgar scores and group E was statistically significant.

Comment

Sykes et al.[6] demonstrated that during the intrapartum period there is a poor correlation between clinical evidence of depression as measured by Apgar score and umbilical artery pH and base deficit except at the extremes of acidosis. This confirmed the original findings of James et al.,[3] which have been since corroborated by Lumley and Wood[22] and Dijxhoorn et al.[23]

The relationship between neonatal neurologic state and acid-base status at birth is equally uncertain. Dijxhoorn et al.[24] showed that umbilical artery pH had a low predictive value in detecting term infants with neurologic abnormality, although Huisjes et al.[25] pointed out that in already-compromised infants (i.e., preterm or growth-retarded infants), neonatal neurologic signs showed an association with preceding acidosis, and this may well be an important distinction.

Sheridan-Pereira et al.[26] described a syndrome of distal hypertonia, abnormal movements, and altered neonatal neurobehavior in infants with an umbilical venous pH of <7.20. In that study both Apgar score and cord venous pH were significantly, although not strongly, predictive of abnormal neonatal neurology.

This uncertainty of the significance of acid-base status in predicting neurologic status either at birth or in the neonatal period is extended to observation at subsequent follow-up. It has been assumed on the basis of limited animal experimental evidence that a severe acidotic response is associated with neurologic deficit.[27] Low et al.[28] followed up at age 5 years, 37 infants with abnormal acid-base status in umbilical artery blood at birth (buffer base <34 mEq/L). They found no cases of cerebral palsy or other major or minor neurologic handicap and no significant differences in development from that found in a control group.

In a subsequent study, at 1 year, Low et al.[29] demonstrated a gradient of major deficit among high-risk infants of varying gestational ages as metabolic acidosis in umbilical artery blood increased. A recent study by Ruth and Raivio[30] found that umbilical arterial pH <7.16 was not a good predictor of developmental abnormality at 1 year of age. Except for these studies, there is a remarkable lack of information on the late outcome of infants who were "acidotic" at birth. Touwen and Huisjes[31] found that acidemia was associated with abnormal neonatal neurologic findings, particularly in growth-retarded fetuses, but not with abnormal neurologic findings at 4 to 6 years old.

In our study 11% of the children examined had a definite impairment. This proportion is similar to that described by Brimblecombe et al.[18] As expected, we demonstrated that higher social class, female sex, and nursery school attendance were associated with significantly better performance on some Griffiths subscales.

However, our results show no relationship between umbilical artery acid-base status in term infants and poor neurodevelopmental outcome at age 4½ years. Theoretically this might have been because infants with severe acidosis at birth died and those who survived survived intact—the "all or none effect."[32] However, only three deaths occurred among the term infants in the original cohort, and these were attributable to congenital malformations. None had intrapartum acidosis.

It is interesting to compare our study with that of Ruth and Raivio.[30] The birth cohorts studied are numerically similar, but unlike ours, the Finnish study included preterm infants. Notably, the degree of acidosis at birth was minimal compared with that in the cohort on which our study was based.[6] The value of pH 2 SD below the mean was 7.16 for vaginal deliveries and 7.25 for cesarean deliveries compared with 7.04 in our hospital. A further difference was that our follow-up measures were sensitive to lesser degrees of handicap, and the children were 3 years older. This is reflected by the fact that the Finnish study, which was initially based on well-baby clinic screening, found only 5% with adverse outcome, both definite and "slight." By contrast, using our criteria 11% of the children in our group with normal acid-base status at birth (Ds) had definite impairment. An additional 20% had mild impairments that would not have been identified by less sophisticated outcome measures. Yet even with these more sensitive outcome measures, we were unable to show a relationship between acidosis at birth and later outcome.

Progressive acidosis is a physiologic response to labor. We suggest that the capacity to respond to intrapartum stress by a further acidotic shift may also be a physiologic adaptive adjustment that bodes well for future neurologic integrity. Indeed, in our study unimpaired children (coded 1) predominate among those who were severely acidotic. A similar finding was made by Ruth and Raivio.[30] Of the six children in their study who were severely acidotic at birth, only one was "slightly abnormal" at age 1 year. Therefore we speculate that the absence of an acidotic shift may be a sign of a compromised fetus. This may explain our observation that of the children who had an Apgar score of <4, those who were not acidotic appeared to fare worse in some areas than those who were acidotic. Our numbers were small, but the findings concur with those of Dijxhoorn et al.[23] These findings need to be explored further in a larger study.

Our results, if confirmed, would carry clinical and medical-legal implications. Until the relationships be-

tween the measurements that have been assumed to be indirect markers of fetal asphyxia and the ultimate neurodevelopmental outcome of the infant have been further clarified, the greatest caution must be exercised before assignment of the cause of later impairment to asphyxia as defined by these markers alone.

We are grateful to Ms. Jenny Daniels, Mrs. Jane Mitchell, and Mrs. Joan Kidd for invaluable help in tracing the children and arranging the assessments, and to Mrs. Bridget Bryant for computer programming. Dr. Adrian Grant, National Perinatal Epidemiology Unit, and Professor A. Turnbull, Nuffield Department of Obstetrics and Gynaecology, Oxford, provided helpful advice and comment. We also thank Miss Philippa Claiden, Miss Sarah Ayers, and Mrs. Olive Goddard for preparation of the manuscript.

REFERENCES

1. Pape KE, Wigglesworth JS. Hemorrhage, ischaemia and the perinatal brain. In: Clinics in developmental medicine. Nos. 69-70. London: William Heinemann, 1979.
2. Dennis J. The long term effects of intrapartum cerebral damage. In: Crawford JW, ed. Risks of labour. Bristol: John Wiley and Son, 1985:157-88.
3. James LS, Weisbrot IM, Prince CE, Holaday DA, Apgar V. The acid-base status of human infants in relation to birth asphyxia and the onset of respiration. J Pediatr 1958;52:379-94.
4. Freeman JM. Prenatal and perinatal factors associated with brain disorders. U.S. Department of Health and Human Services, 1985; NIH Publication no. 85-1149:359-439.
5. Van den Berg P, Schmidt S, Gesche J, Saling E. Fetal distress and the condition of the newborn using cardiotocography and fetal blood analysis during labour. Br J Obstet Gynaecol 1987;94:74-5.
6. Sykes GS, Molloy PM, Johnson P, et al. Do Apgar scores indicate asphyxia? Lancet 1982;1:494-6.
7. Ounsted MK, Osborn ML, Sleigh G, Good FJ. A method of development assessment at four years and some associated findings. Early Hum Dev 1979;3:1-19.
8. Office of Population Censuses and Surveys. Classification of occupations, 1980. London: Her Majesty's Stationery Office, 1980.
9. Griffiths R. The abilities of young children. Bucks, United Kingdom: Association for Research in Infant and Child Development, 1970. Available from: The Test Agency, Cournswood House, North Dean, High Wycombe, Bucks, United Kingdom.
10. Bishop DVM. Test for the reception of grammar (TROG), Department of Psychology, University of Manchester, 1983.
11. Rutter M, Graham P, Yule W. A neuropsychiatric study in childhood. In: Clinics in developmental medicine. Nos. 35-6. London: William Heinemann, 1970.
12. Watt H, Bax M, Whitmore K. Child psychology services in the Inner City infant school. Monograph. London: Thomas Coram Research Unit, 1980.
13. Renfrew CE, Geary L. Prediction of persisting speech defects. Br J Disord Commun 1973;9:37-41.
14. Matthews C, Cleveland CS, Hopper CL. Neurological patterns in multiple sclerosis. Dis Nerv System 1970;31:161-70.
15. Frostig M, Welty-Lefever D, Whittlesey JRB. A developmental test of visual perception for evaluating normal and neurologically handicapped children. Percept Mot Skills 1961;12:383-94.
16. Tanner JM, Whitehouse RH, Takaishi M. Standards from birth to maturity for height, weight, height velocity and weight velocity: British children 1965. Arch Dis Child 1966;41:454-71.
17. Ounsted MK, Moar VA, Scott A. Head circumference charts updated. Arch Dis Child 1985;60:936-9.
18. Brimblecombe RSW, Rubissow J, Edelsten AD, Jones J. A review of infant handicap and neonatal mortality in relation to the use of a special care unit. In: Brimblecombe FSW, Richards MPM, Robertson NRC, eds. Separation and special care baby units. In: Clinics in developmental medicine. No. 68. London: William Heinemann, 1978.
19. Siegel S. Nonparametric statistics for the behavioural sciences. Kogakusha, Tokyo; McGraw-Hill, 1956:184-93.
20. Armitage P. Statistical methods in medical research. New York: John Wiley & Sons, 1971:320-4.
21. Hanson R, Aldridge Smith J, Hume W. Achievements of infants on items of the Griffiths scales: 1980 compared with 1950. Child Care Health Dev 1985;11:91-104.
22. Lumley J, Wood C. Fetal acidosis. Aust N Z J Obstet Gynaecol 1969;9:144-52.
23. Dijxhoorn MJ, Visser GHA, Fidler VJ, Touwen BCL, Huisjes HJ. Apgar score, meconium and acidaemia at birth in relation to neonatal neurological morbidity in term infants. Br J Obstet Gynaecol 1986;93:217-22.
24. Dijxhoorn MJ, Visser GHA, Huisjes HJ, Fidler VJ, Touwen BCL. The relation between umbilical pH values and neonatal neurological morbidity in full term AFD infants. Early Hum Dev 1985;11:33-42.
25. Huisjes HJ, Touwen BCL, Hoekstra J, et al. Obstetrical-neonatal neurological relationships. A replication study. Eur J Obstet Gynaecol Reprod Biol 1980;10:247-56.
26. Sheridan-Pereira M, Grant A, Chalmers I, McDonald D. A pattern of neonatal behaviour associated with low umbilical cord venous pH [Abstract]. European Congress of Perinatal Medicine, Dublin, Ireland, 1984.
27. Myers RE. Experimental models of perinatal brain damage: relevance to human pathology. In: Gluck L, ed. Intrauterine asphyxia and the developing fetal brain. Chicago: Year Book Medical Publishers, 1977:37-97.
28. Low JA, Galbraith RS, Muir DW, Pater EA, Karchmar EV. Intrapartum fetal hypoxia: a study of long-term morbidity. AM J OBSTET GYNECOL 1983;145:129-34.
29. Low JA, Galbraith RS, Muir DW, Killen HL, Pater EA, Karchmar EJ. Factors associated with motor and cognitive deficits in children after intrapartum fetal hypoxia. AM J OBSTET GYNECOL 1984;148:533-9.
30. Ruth VJ, Raivio KO. Perinatal brain damage: predictive value of metabolic acidosis and the Apgar score. Br Med J 1988;297:24-7.
31. Touwen BCL, Huisjes HJ. Acidaemia and its neurological effects. In: Clinch J, Matthews T, eds. Proceedings of IX European Congress of Perinatal Medicine. Dublin, Ireland: MTP Press, 1984:99-102.
32. Niswander KR, Gordon M, Drage JS. The effect of intrauterine hypoxia on the child surviving to 4 years. AM J OBSTET GYNECOL 1975;121:892-9.

The effect of elevated blood glucose on the electroencephalogram and cerebral metabolism during short-term brain ischemia in fetal sheep

Conrad R. Chao, MD, A. Roger Hohimer, PhD, and John M. Bissonnette, MD
Portland, Oregon

The effect of cerebral ischemia on cerebral metabolism and the electroencephalogram was studied with and without prior glucose infusion in near-term normoxic fetal sheep. At normal blood glucose levels, the electroencephalogram decreased in amplitude during ischemia. At elevated blood glucose levels the electroencephalographic amplitude was much less attenuated by ischemia although Fast Fourier Transforms revealed a shift toward slower frequencies. Under either normal or elevated blood glucose conditions, ischemia caused cerebral oxygen consumption to decrease, glucose uptake to increase, and a net efflux of lactate to occur. Elevated blood glucose appears to help maintain electroencephalographic activity during ischemia, perhaps by fueling additional anaerobic energy production. The relationship between the electroencephalogram, brain metabolism, and brain damage remains to be defined. (Am J Obstet Gynecol 1989:161:221-8.)

Key words: Fetal brain, metabolism, electroencephalogram, glucose, ischemia

Previous studies of the relationship between cerebral metabolism and the electroencephalogram (EEG) during intrauterine hypoxic-ischemic conditions have focused on the response to arterial hypoxic hypoxia, a state in which both arterial blood pressure and cerebral blood flow are elevated.[1, 2] However, Wagner et al.[3] and Ting et al.[4] have emphasized the importance of hypotension and the resultant cerebral ischemia in the genesis of the metabolic and histopathologic changes of fetal brain injury. We recently demonstrated that reducing cerebral perfusion pressure by brachiocephalic artery occlusion, while maintaining flow to most of the body, generates a state of cerebral ischemic hypoxia. The associated metabolic derangements were consistent with tissue hypoxia (low venous oxygen tension), anaerobiosis (lactate efflux), and perhaps brain glucose depletion.[5]

The studies by Myers et al.[6] in adult and juvenile animals have emphasized the adverse effect of hyperglycemia in the genesis of hypoxic-ischemic brain injury. These studies emphasized the importance of glycogen stores and tissue lactate accumulation in regions of the brain that undergo injury. Fetal and neonatal studies of hyperglycemia have not yielded consistent results. Blomstrand et al.[1] reported that hyperglycemia was associated with a decrease in cerebral oxygen consumption and a trend toward deterioration in the somatosensory evoked potential in hypoxic fetal sheep. Conversely, Mann et al.[2] reported that glucose loading had no effect on the umbilical venous P_{O_2} required to induce isoelectricity. All of these studies were carried out in fetal sheep; however, the discrepancy may be due in part to the three different end points selected: neurohistology,[6] somatosensory evoked potential,[1] and an isoelectric EEG.[2] Vannucci et al.[7] and Voorhies et al.[8] found that glucose given before hypoxia in newborn rats did not increase histologic brain injury and was associated with increased duration of survival during hypoxia. The differences between these findings and those of Myers et al.[6] may be explained in part by the different species or differences in central nervous system development at the time of study.

We have sought to test the hypothesis that in near-term fetal sheep elevations of blood glucose immediately before short-term, incomplete, global cerebral ischemia would partially prevent the attenuation of the EEG. Additionally we have attempted to determine if blood glucose elevations before ischemia would alter the cerebral uptake of glucose and oxygen or the efflux of lactate. In a previous study[5] we noted that when measurements are begun 2 minutes after fetal cerebral ischemia is instituted, oxygen consumption was de-

From the Department of Obstetrics and Gynecology, Oregon Health Sciences University.
Supported by National Institutes of Health grants HD 10034-12 and HL 7596-02 and by the American Heart Association, Oregon Affiliate.
Received for publication November 3, 1988; revised February 3, 1989; accepted March 8, 1989.
Reprint requests: Conrad R. Chao, MD, Department of Obstetrics and Gynecology, Columbia University, 630 W. 168th St., New York, NY 10032.

Table I. Blood oxygen, glucose, and lactate concentrations

		Ischemia	
	Control	*Normal glucose*	*Elevated glucose*
Arterial P_{O_2} (mm Hg)	23.4 ± 0.7	23.4 ± 0.9	23.3 ± 0.8
Venous P_{O_2}* (mm Hg)	18.3 ± 0.8	7.6 ± 0.4	8.5 ± 0.6
Arterial P_{CO_2} (mm Hg)	50.7 ± 1.4	48.7 ± 0.6	50.1 ± 0.9
Arterial oxygen content (mmol/L)	3.9 ± 0.2	3.8 ± 0.2	3.9 ± 0.1
Venous oxygen content* (mmol/L)	2.8 ± 0.1	0.6 ± 0.1	0.9 ± 0.1
Arterial glucose† (mmol/L)	1.32 ± 0.09	1.28 ± 0.09	2.52 ± 0.13
Venous glucose‡ (mmol/L)	1.15 ± 0.12	0.46 ± 0.07	1.69 ± 0.09
Arterial lactate (mmol/L)	1.35 ± 0.09	1.67 ± 0.11	1.50 ± 0.10
Venous lactate* (mmol/L)	1.36 ± 0.10	1.81 ± 0.10	1.73 ± 0.07

*Both ischemic values different from control ($p < 0.01$).

†Elevated glucose values different from control and normal glucose ischemia ($p < 0.01$).

‡All groups different from each other ($p < 0.01$).

pressed. In this study we wished to evaluate the role of glucose availability during this period of depressed cerebral metabolism that occurs shortly after ischemia is initiated.

Material and methods

Surgical procedures. At 120 to 129 days of gestation, eight ewes of mixed Western breed were anesthetized with 7 to 10 mg/kg intravenous thiamyl sodium followed by positive-pressure ventilation with 1% halothane in 50% nitrous oxide and 50% oxygen. Hysterotomy was performed and polyvinyl catheters inserted into both fetal brachial arteries and advanced until the tips approached the brachiocephalic artery. A hind limb vein catheter was placed and advanced into the inferior vena cava. A trephine was used to remove a 1 cm circular portion of the skull in the midline to expose the sagittal sinus midway between the coronal and lambdoidal sutures. The sinus was then punctured with a needle and a polyvinyl catheter inserted caudally to the vicinity of the confluens (about 1 cm). Stainless steel electrodes (Cooner Wire, Chatsworth, Calif.) were placed supradurally 1 cm lateral to the midline at the junction of the lambdoidal and sagittal sutures through small burr holes. A left lateral thoracotomy was then performed and an inflatable occluder (In Vivo Metrics, Healdsberg, Calif.) placed around the common brachiocephalic artery, which supplies both forelimbs and carotid arteries in this species. All fetal incisions were closed and a polyvinyl catheter was sewn to the fetal skin for amniotic fluid pressure measurement.

The maternal abdomen was closed and one million units of penicillin G was deposited in the amniotic fluid. After operation animals were allowed at least 4 days to recover before any studies. Animal housing, care, and use were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Experimental procedures

Physiologic measurements. Ewes were allowed food and water as desired throughout the study period. The catheters were connected to strain gauges (Statham, Oxnard, Calif), which had been calibrated with mercury manometers. Mean arterial pressures were derived electronically (0.3 Hz low-pass filter, Beckman type 9853 coupler, Beckman Instruments, Fullerton, Calif.). The pressures from the brachial artery, which was distal to the occluder, and sagittal sinus were digitized; the difference (the cerebral perfusion pressure) was calculated by a computer, and the perfusion pressure was automatically regulated by changing the amount of saline solution in the balloon occluder. In this manner perfusion pressure could be prospectively specified and controlled.

Cerebral blood flow measurements. Regional cerebral blood was measured with radionuclide-labeled microspheres and the reference sample technique.[9, 10] For each blood flow determination a well-dispersed suspension of approximately 2×10^6 15 μm microspheres in 6% dextran was injected over 15 seconds into the hind limb vein followed by a flush of 3 ml saline. Reference sample blood was withdrawn from a brachial artery at a rate of 2.14 ml/min for a period beginning just before the injection and continuing for 3 minutes after injection. The isotopes utilized were cerium 141, chromium 51, strontium 85, niobium 95, and scandium 46. After completion of the experiments the animal was killed with intravenous barbiturate and phenytoin. A postmortem examination was performed to verify catheter placement and to remove the brain. The brain tissues were counted with the reference samples in a

Table II. Hemodynamic and metabolic measurements during ischemia

		Ischemia	
	Control	*Normal glucose*	*Elevated glucose*
Perfusion pressure* (mm Hg)	42 ± 1	12 ± 1	10 ± 1
Cerebral blood flow* (ml/min/100 gm)	121 ± 9	33 ± 3	31 ± 3
Oxygen consumption† (μmol/min/100 gm)	128 ± 3	103 ± 6	94 ± 9
Glucose consumption (μmol/min/100 gm)	21 ± 4	26 ± 2	25 ± 3
Lactate flux* (μmol/min/100 gm)	−2.0 ± 2.2	−4.2 ± 0.8	−7.1 ± 1.5
Glucose/oxygen quotient*	0.95 ± 0.15	1.53 ± 0.10	1.62 ± 0.16
Glucose/oxygen/lactate†	0.91 ± 0.16	1.40 ± 0.10	1.39 ± 0.16

*Both ischemic values different from control ($p < 0.01$).
†Both ischemic values different from control ($p < 0.02$).

multichannel analyzer (Model 601, Packard/Nuclear Data, Schaumburg, Ill.). Isotopic separation and blood flow calculation were performed by methods that have been previously described.[9-11]

Blood sampling and analysis. Arterial and sagittal venous blood samples (3 ml) were anaerobically obtained. Sagittal sinus blood has been validated as a means of sampling exclusively cerebral venous drainage, because extracerebral contamination is negligible.[12] Blood gas and pH determinations were made at 39° C with a model 3BGS blood gas analyzer (Radiometer, Copenhagen). Oxygen contents were measured with a Lex-O_2-Con (Lexington Instruments, Waltham, Mass.). Glucose and lactate were measured in duplicate in frozen-thawed whole blood with glucose oxidase–hydrogen peroxide and lactate oxidase–hydrogen peroxide methods (YSI 23A glucose analyzer, Yellow Springs Instruments, Yellow Springs, Ohio). The instrument was calibrated daily with standard solutions. Intrareplicate reproducibility was within 4%.

Electroencephalographic recordings. Fetal EEG signals were amplified and displayed on a strip chart recorder (model R612, 9853 coupler, Beckman) and digitized at 95 Hz with an analog-to-digital converter (Data Translation DT2801, Marlborough, Mass.) and the Labtech Notebook software (Laboratory Technologies Corporation, Wilmington, Mass.). An independent 100 μV voltage source was used for calibration. Signals were electronically filtered by a 30 Hz low-pass filter.

Fast Fourier Transforms and power spectral analyses were performed over 25-second intervals by the Fast Fourier Transform subroutine in the Labtech Notebook package. The EEG during the 25-second normotensive period immediately before the initiation of ischemia was recorded as the control recording for that experiment; the 25 seconds beginning at the time of blood sampling, approximately 2 minutes after the onset of ischemia, was recorded as the experimental EEG. Total EEG power for a given ischemic experiment was determined by summing the power spectra in 1 Hz brackets over the range 0 to 30 Hz. This was then expressed as a percent change from the total EEG power of the control recording for that experiment. Power frequency distributions were calculated by dividing the power spectra into δ-range (0 to 3 Hz), θ-range (4 to 7 Hz), α-range (8 to 11 Hz), σ-range (12 to 14 Hz), and β-range (15 to 30 Hz) and dividing the power contained in each band by the total power in that recording.

Protocol. We have previously shown that 2 to 5 minutes of ischemia produces a decrease in cerebral oxygen consumption and an efflux of lactate from the fetal brain[5]; therefore a similar short-term protocol was used. After control measurements of cerebral blood flow, blood gases, and arterial and sagittal venous glucose and lactate, animals were randomized to receive either a 10 ml infusion of 7.5% glucose in saline solution in the hind limb vein or saline solution only. The EEG recording was then begun and the infusion given intravenously over 1 minute. After 1 minute the balloon occluder was inflated to reduce cerebral perfusion pressure to approximately 10 mm Hg. After 2 minutes of ischemia, arterial and venous samples were taken, the EEG recorded, and another microsphere measurement was made. The balloon occluder was then released. After at least a 2-hour interval, the experiment was repeated with the alternative treatment. In a number of the animals the ischemic experiments were repeated in the reverse order.

Calculations and data analysis. Metabolic fluxes were calculated as the product of arteriovenous difference times the cerebral blood flow. The glucose/oxygen molar quotient was calculated as six times the glucose uptake divided by the oxygen uptake. The glu-

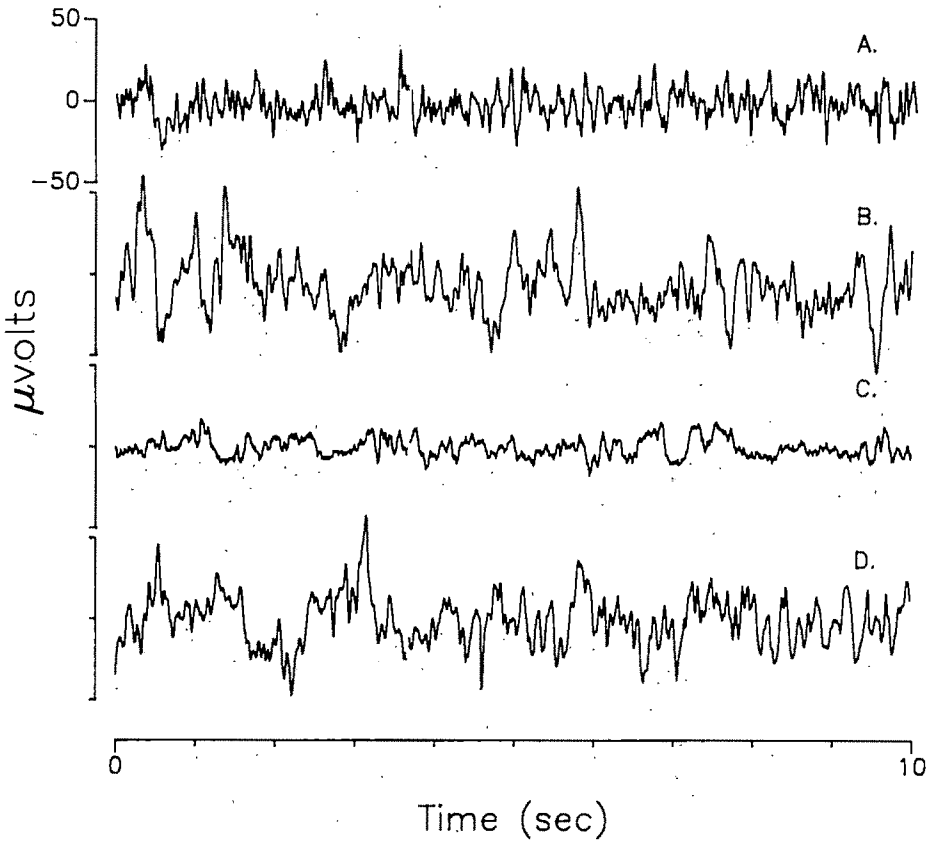


Fig. 1. EEG recordings from fetus during control conditions in low-voltage *(A)* and high-voltage *(B)* states. EEG amplitude is greatly attenuated after 2 minutes of cerebral ischemia at normal blood glucose levels *(C)* but is much less attenuated if blood glucose levels were elevated before ischemia *(D)*.

cose/lactate/oxygen molar quotient was calculated as the quantity six times the glucose uptake minus two times the lactate efflux divided by the oxygen uptake. Grouped means and SEMs between control (no occlusion), ischemic, and elevated blood glucose ischemic conditions were calculated for the eight control measurements and for 13 normoglycemic ischemic and 10 elevated glucose ischemic experiments. Statistical significance of continuous data was inferred by analysis of variance followed, when indicated, by post hoc least significant difference tests. Significance for data that were not normally distributed was determined by Kruskal-Wallis analysis of variance by ranks and χ^2. Spearman rank order correlation was performed to correlate EEG changes with the metabolic and blood chemical measurements.

Results

Metabolic measurements. Blood gas tensions, blood glucose concentrations, and blood lactate concentrations are presented in Table I. The arterial blood gas tensions and lactate concentrations were comparable and not different from control under both ischemic conditions. As shown in Table II, perfusion pressure and blood flow were significantly reduced by the balloon occlusion to approximately 25% of control in both normal and high blood glucose ischemic conditions. During ischemia, the widened arteriovenous difference for oxygen content resulted in significant decreases in venous oxygen content and Po_2, reflecting the ischemic hypoxia with glucose and nonglucose experiments being similarly affected. The arteriovenous difference for glucose widened during ischemia to approximately 0.8 mmol/L in both cases. Whereas venous glucose fell to 0.46 mmol/L in normoglycemic ischemia, when arterial glucose was high, the sagittal venous (outflow) blood from the brain maintained a value (1.69 mmol/L) in excess of the control arterial glucose concentration (Table I).

Oxygen consumption (Table II) decreased significantly between control and both ischemic groups. The ischemic groups were not different from each other. There was an increase in glucose uptake that did not reach significance; glucose-infused and saline solution–infused means were essentially the same. Mean lactate efflux was significantly increased above control in both

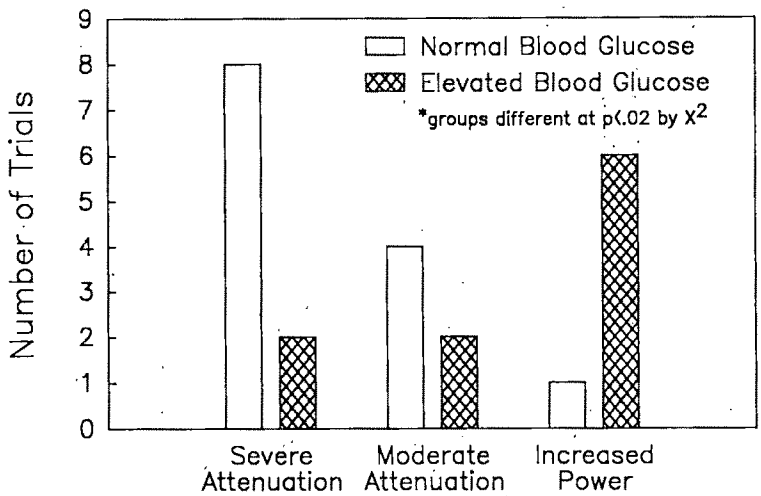


Fig. 2. Elevation of blood glucose before ischemia increases total EEG power during ischemia relative to normoglycemic ischemia.

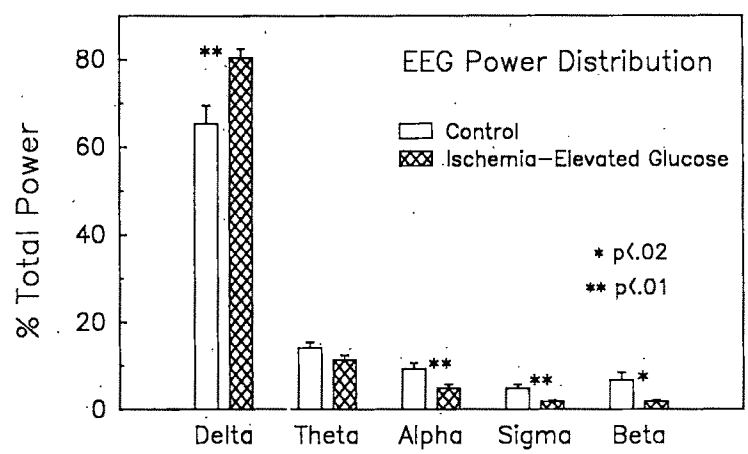


Fig. 3. Cerebral ischemia after elevation of blood glucose concentrations was associated with a shift of power toward slower frequencies.

ischemia groups, and they were not significantly different from each other. The glucose/oxygen quotient was significantly increased above control in both ischemia groups and they likewise did not differ from each other. Similarly, the glucose/lactate/oxygen quotient was significantly increased above control in both ischemia groups but was not different between the hyperglycemic and normoglycemic ischemic groups.

Electroencephalographic findings. A series of digitized recordings from a typical animal are presented in Fig. 1. The high- and low-voltage electrocortical states[13, 14] can be easily distinguished. Both ischemic periods were initiated from the low-voltage state. To the naked eye, the amplitude of the EEG of the elevated glucose ischemic experiment clearly exceeds that of the normal blood glucose ischemia; summation of frequency bands for total power determination demonstrates that the elevated blood glucose tracing contains 371% of control power, whereas the normoglycemic recording exhibits only 33% of control power. Thus in this example the administration of glucose appears to preserve electrocortical function during cerebral ischemia. It is difficult to distinguish the elevated glucose ischemia from the high-voltage control with respect to amplitude or apparent frequency distribution.[14]

Because the total power percent changes from control were not normally distributed, they were analyzed in a nonparametric manner by dividing them into three groups: a >50% increase from control power (severe attenuation), a <50% decrease from control (moderate attenuation), or an increase relative to control (increased power). Fig. 2 depicts the changes in power expressed in terms of glucose status; the tendency for prior glucose infusion to maintain or augment EEG amplitude and power during ischemia was highly significant.

Table III. Cerebral metabolism with alterations in EEG during ischemia

	Increased power	*Moderate attenuation*	*Severe attenuation*
Cerebral blood flow* (mg/min/100 gm)	37 ± 3	38 ± 3	28 ± 4
Oxygen consumption† (μmol/min/100 gm)	109 ± 6	109 ± 9	87 ± 9
Glucose consumption (μmol/min/100 gm)	26 ± 2	30 ± 3	23 ±3
Lactate flux (μmol/min/100 gm)	−7.5 ± 2.1	−4.9 ± 1.9	−4.6 ± 0.8
Arterial oxygen content (mmol/L)	3.8 ± 0.2	3.6 ± 0.3	4.0 ± 0.2
Venous oxygen content (mmol/L)	0.8 ± 0.1	0.7 ± 0.1	0.7 ± 0.1
Arterial glucose (mmol/L)	2.1 ± 0.2	1.6 ± 0.4	1.7 ± 0.2
Venous glucose† (mmol/L)	1.4 ± 0.2	0.8 ± 0.3	0.8 ± 0.2

*$p < 0.03$, Spearman rank order correlation.
†$p < 0.07$, Spearman rank order correlation.

During ischemia there was a significant decrease in power in higher-frequency bands and an increase in the lower-frequency bands relative to control in the glucose-infused group (Fig. 3); this effect was not statistically demonstrable in the normoglycemic ischemia group.

The EEG and metabolism. Table III presents metabolic fluxes for the EEG subclasses. Change in total EEG power was significantly correlated with cerebral blood flow, oxygen consumption, and venous glucose concentration, although the correlations with oxygen consumption and venous glucose were of borderline significance. There was, however, no correlation between EEG functional class and venous oxygen content, glucose uptake, or lactate efflux.

Comment

We have demonstrated that elevations in blood glucose before cerebral ischemia maintain EEG amplitude and power when compared with normoglycemic ischemia. Additionally, when glucose was elevated, the proportion of the power in slower frequency bands increased during ischemia, while that in faster frequency bands decreased.

During ischemia, oxygen consumption fell while glucose uptake increased, and a net lactate efflux was observed. Because arteriovenous differences for glucose and oxygen increase during ischemia, the venous concentrations fall. Thus venous blood and perhaps brain tissue were both hypoxic and had low concentrations of glucose during ischemia in the absence of a glucose infusion. These findings confirm our previous observations of severe ischemia.[5] In this current study there were no significant differences between prior glucose-infused and saline solution–infused ischemic trials with regard to these metabolic parameters, although there was a trend toward greater lactate efflux with elevated glucose. The ischemic glucose/oxygen quotient was greater than unity in either glucose condition, indicating that more glucose was taken up than could be oxidized by the measured consumption of oxygen. Thiringer et al.[15] noted a similar relationship between glucose/oxygen quotient and deterioration in somatosensory evoked potential during hypoxemia; a mild impairment was associated with quotients of 1.42 to 1.43, whereas severe impairment was associated with a quotient of 2.1.

Even when the measured lactate efflux is accounted for with the glucose/lactate/oxygen quotient, a portion of glucose uptake is stoichiometrically unaccountable, suggesting that lactate production may exceed the measured efflux because of membrane transport limitations, as we have previously speculated.[5] It should be noted, however, that a recent study has shown that brain lactate levels measured by in vivo nuclear magnetic resonance spectroscopy were not elevated in newborn lambs given a glucose infusion after 20 minutes of brain ischemia.[16] Glucose infusion failed to elevate brain lactate above that seen in noninfused ischemic lambs despite an increase in glucose arteriovenous differences.

Taken together, these observations suggest that during cerebral ischemia, oxygen consumption and glucose oxidation are inadequate to meet the needs of the cells; glucose uptake increases to fuel anaerobic glucose metabolism, resulting in production and net efflux of lactate. Energy production from this excess glucose is limited to anaerobic glycolysis. In the normoglycemic conditions, EEG power could not be maintained despite these adaptations.

Glucose infusion before ischemia did not enhance glucose uptake or lactate efflux during ischemia, al-

though it may have increased lactate production and improved electroencephalographic function. It is important to note that the Fick technique measures net fluxes of substrates rather than metabolic rates. There will be a close relationship between net fluxes and metabolic rates for substrates that cannot be stored in tissue, such as oxygen, whereas metabolic rates for substrates such as carbohydrates will be the sum of net flux and net change in the tissue pool. Although we did not measure tissue concentrations, it is likely that the tissue concentration of glucose before the initiation of ischemia was increased by the glucose load. Thus endogenous stores of glucose were probably greater after glucose loading.

The amount of endogenous stores becomes important because venous concentrations of glucose in these experiments become very low in normoglycemic ischemia because of the combined effects of the increase in net influx and the decrease in blood flow. Previous studies of metabolite concentrations in the fetal sheep cerebral cortex during combined hypoxia-hypotension demonstrated a whole tissue extract glucose concentration of 0.44 mmol/kg, which is slightly less than our measured venous concentration.[3] It is interesting to note that venous oxygen contents were the same in all three classes of EEG impairment, whereas venous glucose concentration was significantly higher when function was preserved than when experiments were done in which the EEG was attenuated (Table III). This suggests but does not establish that the low glucose concentration, rather than the low oxygen concentration, limited neural function. Furthermore, the low glucose concentration may have limited tissue lactate production during normoglycemic ischemia.

Elevations in blood glucose may then help maintain electrical activity during ischemia because of anaerobic glycolysis of the increased stores. This would also explain the trend toward increased efflux of lactate. Therefore direct measurement of the tissue concentrations would be necessary to confirm these speculations.

Our data conflict with those of Blomstrand et al.[1] and Mann et al.,[2] who reached other conclusions regarding the effect of glucose loading on the EEG during intrauterine hypoxia-ischemic conditions. Blomstrand et al. studied anesthetized fetal sheep during maternal hypoxia. On a scale of 0 to 5, with 5 representing the most severe deterioration of somatosensory evoked potential, arterially hypoglycemic fetal sheep (0.5 mmol/L) demonstrated a mean score of 1.22, whereas in hyperglycemic fetuses (2.25 mmol/L) the mean value increased nonsignificantly to 1.88. Oxygen consumption in this experiment fell to 48 μmol/min/100 gm, suggesting a more severe derangement of metabolism. Spontaneous EEG results were not reported. Mann et al. studied anesthetized fetal sheep during maternal hypoxia and found that the arterial Po_2 at the onset of isoelectricity was not different between elevated blood glucose and euglycemic fetuses. However, because cerebral blood flow and metabolism and cerebral venous oxygen tensions were not studied, it is not possible to compare the severity of the hypoxic insult with that of our experiment. The fact that all fetuses became isoelectric in that experiment suggests that the insult was more severe.

Hypoxic slowing of the EEG has been described in human adults[17] and in fetal sheep.[18] We were able to statistically demonstrate this effect in fetuses with elevated blood glucose levels. As noted in the Results section, a similar trend was observed during normoglycemic ischemia. It is also difficult to precisely characterize the frequency composition of a near-isoelectric waveform with Fast Fourier Transform.

Interestingly, Ishikawa and McDowell[19] found that electrical activity in lightly anesthetized, normoxic, hypotensive cats approached isoelectricity at cerebral blood flows of about 20 to 25 ml/min/100 gm, close to the severely affected EEG class in this experiment. Other investigators[20] have found the critical flow threshold to be closer to 15 to 20 ml/min/100 gm in adults. It is possible that in fetal animals the lower arterial oxygen concentration causes loss of function at higher blood flows during ischemia.

Although we could not differentiate the "increased power" recordings from those of a typical control high-voltage state, visually or with Fast Fourier Transform analysis, the metabolic measurements differed from that of typical high voltage. Oxygen consumption was reduced to a mean of 109 μmol/min/100 gm, and a lactate efflux of 7.5 μmol/min/100 gm was detected; the stoichiometry of the measured fluxes implies that anaerobic consumption of glucose occurs. This suggests that the high-voltage state previously described during hypoxia[21] may be metabolically distinct from the high-voltage state observed during normoxia.

It is not clear whether it is beneficial to maintain EEG amplitude and power in the face of tissue hypoxia-ischemia. Duffy et al.[22] have suggested that the depression of function during severe hypoxia may represent a protective mechanism to conserve energy, perhaps because of a decrease in oxygen consumption. It is also possible that low tissue concentrations of glucose may also act as a protective signal to prevent either further energy expenditure, lactate production, or both. The studies of Myers et al.[6] suggest that, at least in adult and juvenile animals, glucose administration is associated with histologic evidence of brain damage after hypoxia. For these reasons we would strongly recommend that no clinical inference be made from this study until more is known about the relationship between

glucose concentrations and brain damage. Clearly, there is a need for further study of the complex interaction of brain function, metabolic substrates, oxygen, and blood flow in the genesis of brain injury during development.

In conclusion, we have demonstrated that elevated blood glucose before noncomplete fetal brain ischemia maintains EEG power whereas power is lost during normoglycemic ischemia. This suggests that substrate availability may limit energy metabolism during severe cerebral ischemia in the sheep fetus. Anaerobic metabolism of increased endogenous tissue stores of substrate may support the persistent electrical function observed during ischemia after prior glucose infusion. Further study with measurement of tissue concentrations of carbohydrate substrates will be necessary for confirmation.

The assistance of Sharon Knopp, Neil Notaroberto, Keri Stepper, and Gail Willeke is greatly appreciated.

REFERENCES

1. Blomstrand S, Hrbek A, Karlsson K, Kjellmer I, Lindecrantz K, Olsson T. Does glucose administration affect the cerebral response to fetal asphyxia? Acta Obstet Gynecol Scand 1984;63:345-53.
2. Mann LI, Prichard JW, Symmes D. The effect of glucose loading on the fetal response to hypoxia. AM J OBSTET GYNECOL 1970;107:610-8.
3. Wagner K, Ting P, Westfall M, Yamaguchi S, Bacher J, Myers R. Brain metabolic correlates of hypoxic-ischemic necrosis in mid-gestational sheep fetuses: significance of hypotension. J Cereb Blood Flow Metab 1986;6:425-34.
4. Ting P, Yamaguchi S, Bacher JD, Killens RH, Myers RE. Hypoxic-ischemic cerebral necrosis in midgestational sheep fetuses: physiopathologic correlations. Exp Neurol 1983;80:227-245.
5. Chao CR, Hohimer AR, Bissonnette JM. Cerebral carbohydrate metabolism during severe ischemia in fetal sheep. J Cereb Blood Flow Metab 1989;9:53-7.
6. Myers RE, deCourten-Myers GM, Wagner KR. Effect of hypoxia on fetal brain. In: Beard RW, Nathanielsz PW, eds. Fetal physiology and medicine. The basis of perinatology. New York: Marcel Dekker, 1984:419-58.
7. Vannucci RC, Vasta F, Vannucci SJ. Cerebral metabolic responses of hyperglycemic immature rats to hypoxia-ischemia. Pediatr Res 1987;21:524-9.
8. Voorhies TM, Rawlinson D, Vannucci RC. Glucose and perinatal hypoxic-ischemic brain damage in the rat. Neurology 1986;36:1115-8.
9. Makowski EL, Schneider JM, Tsoulos NG, Colwill JR, Battaglia FC, Meschia G. Cerebral blood flow, oxygen consumption, and glucose utilization of fetal lambs in utero. AM J OBSTET GYNECOL 1972;114:292-303.
10. Rudolph AM, Heymann MA. The circulation of the fetus in utero: methods for studying distribution of blood flow, cardiac output, and organ blood flow. Circ Res 1967; 21:163-84.
11. Jones MD, Burd LI, Makowski EL, Meschia G, Battaglia FC. Cerebral metabolism in sheep: a comparative study of the adult, the lamb, and the fetus. Am J Physiol 1975;229:235-9.
12. Purves MJ, James IM. Observations on the control of cerebral blood flow in the sheep fetus and newborn lamb. Circ Res 1969;25:651-67.
13. Jost RG, Quilligan EJ, Yeh S, Anderson GG. Intrauterine electroencephalogram of the sheep fetus. AM J OBSTET GYNECOL 1972;114:535-9.
14. Szeto HH, Vo TDH, Dwyer G, Dogramajian ME, Cox M, Senger G. The ontogeny of fetal lamb electrocortical activity: A power spectral analysis. AM J OBSTET GYNECOL 1985;153:462-6.
15. Thiringer K, Blomstrand S, Hrbek A, Karlsson K, Kjellmer I. Cerebral arterio-venous differences for hypoxanthine and lactate during graded hypoxia in the fetal lamb. Brain Res 1982;239:107-17.
16. Hope PL, Cady DT, Ives NK, Gardiner RM, Reynolds EOR. Brain metabolism and intracellular pH during ischemia: effects of systemic glucose and bicarbonate administration studied by ^{31}P and ^{1}H nuclear magnetic resonance spectroscopy in vivo in the lamb. J Neurochem 1988;50:1394-402.
17. Ingvar DH, Sjolund B, Ardo A. Correlation between dominant EEG frequency, cerebral oxygen uptake, and blood flow. Electroencephalogr Clin Neurophysiol 1976;41:268-76.
18. Symmes D, Prichard J, Mann L. Spectral analysis of fetal sheep EEG during hypoxia Electroencephalogr Clin Neurophysiol 1970;29:511-5.
19. Ishikawa T, McDowall DG. Electrical activity of the cerebral cortex during induced hypotension with sodium nitroprusside and trimetaphan in the cat. Br J Anaesthesiol 1980;53:605-11.
20. Symon L. Flow thresholds in brain ischemia and the effects of drugs. Br J Anaesthesiol 1985;57:34-43.
21. Harding R, Poore ER, Cohen GL. The effect of brief episodes of diminished uterine blood flow on breathing movements, sleep states, and heart rate in fetal sheep. J Dev Physiol 1981;3:231-43.
22. Duffy T, Nelson S, Lowry O. Cerebral carbohydrate metabolism during acute hypoxia and recovery. J Neurochem 1972;19:959-77.

Uncoupling of excitation from contraction in uterine smooth muscle in near-term ewes

Deborah L. Reid, PhD, Mark C. Hollister, MD, Susan R. Davidson, MD, Terrance M. Phernetton, BS, and John H. G. Rankin, PhD

Madison, Wisconsin

We infused forskolin in 30% ethanol or 30% ethanol systemically in seven chronically catheterized near-term sheep to determine the response of ovine uterine musculature to cyclic adenosine monophosphate stimulation. Maternal and fetal arterial pressures, fetal venous pressure, intrauterine pressure, and uterine electromyogram activity were monitored continuously. Prostaglandin E_2 was infused at a delivery rate that caused a minimal 5 mm Hg increase in intrauterine pressure with definite contraction-like pressure spikes and associated uterine electromyogram activity. Forskolin (10 mg in 30% ethanol) or ethanol (vehicle) was then infused for 20 minutes. The prostaglandin E_2 challenge was repeated 3 minutes later and again every 30 minutes over a 2½-hour period. Data were analyzed by repeated measures analysis of variance. Infusion of ethanol ($n = 2$) had no apparent effect on either the intrauterine pressure or the uterine electromyogram response to prostaglandin E_2 challenge. Forskolin infusion ($n = 5$) caused an attenuation of the intrauterine pressure response to prostaglandin E_2 for 93 minutes. This effect was greatest 33 minutes after the infusion ended when the integrated pressure signal was 37% of the initial prostaglandin E_2 response. The forskolin infusion had no effect on uterine electromyogram response to prostaglandin E_2. We conclude that forskolin causes an uncoupling of excitation from contraction in the intact near-term ovine uterus. (AM J OBSTET GYNECOL 1989; 161:229-33.)

Key words: Tocolysis, forskolin, cyclic adenosine monophosphate, sheep, pregnancy

Forskolin, the extract of an herb indigenous to India, is a direct stimulator of adenylate cyclase. Recently, we have shown that forskolin is capable of vasodilation of the mature ovine placenta on both the maternal and fetal sides.[1, 2] We also have shown a similar effect on rabbit maternal placental vessels.[3]

Forskolin has been shown to diminish spontaneous contractions in rat myometrial strips.[4] This event has also been demonstrated in the rat and rabbit myometrium in which relaxation was linked to increased cyclic adenosine monophosphate (cAMP) levels.[5, 6] We devised an experiment to determine whether forskolin can diminish the contractile response of ovine uterine smooth muscle to a controlled uterine stimulus. We measured both uterine electromyogram and intrauterine pressure in response to repeated prostaglandin E_2 (PGE_2) challenges to determine which, if either, measurement was affected by a forskolin-induced increase in uterine smooth muscle cAMP.

From the Department of Obstetrics-Gynecology, University of Wisconsin, and Wisconsin Perinatal Center, Southcentral Region, Meriter Hospital/Madison General.

Supported by National Institutes of Health grant HD21082.

Received for publication September 28, 1988; revised February 22, 1989; accepted March 8, 1989.

Reprint requests: Deborah L. Reid, PhD, Meriter Hospital/Madison General, 202 South Park St., Madison, WI 53715.

Methods

Animal preparation. Surgery was performed on seven pregnant ewes at 125 days' mean gestational age. Intramuscular ketamine (10 mg/kg) and atropine (10 μg/kg) were administered to induce anesthesia and reduce tracheal secretion. Anesthesia was maintained throughout surgery with intravenous ketamine infused through a jugular vein catheter. Surgery was performed in accordance with our standard procedure.[7] A fetal hind limb was exposed through a midline laparotomy and hysterotomy. Catheters were inserted into the hind limb artery and vein; the tips were advanced to the abdominal aorta and inferior vena cava, respectively. A catheter with side holes was attached to the fetal skin to monitor intrauterine pressure and the uterine incision was repaired. Three electrode wires were anchored in the myometrium to record the uterine electromyogram. Catheters were placed bilaterally in the maternal femoral arteries and advanced 20 cm. A final catheter was placed in the maternal right carotid artery and advanced to a position inside the left ventricle; placement was confirmed at surgery with a pressure transducer. Antibiotics were administered to the fetus (Chloromycetin, 100 mg/kg), intrauterine space (penicillin, 600,000 units), and ewe (penicillin, 400,000 units, intramuscularly). The catheters and electrode leads were exteriorized through the maternal flank and

Table I. Intrauterine pressure and uterine electromyogram responses to PGE_2 before and after systemic administration of forskolin

	Control	*3 min*	*33 min*	*63 min*	*93 min*	*123 min*	*153 min*
Intrauterine pressure (mm Hg)							
Average	5.4	2.9*	2.4*	3.3*	3.7*	3.9	4.3
SEM	±0.6	±0.6	±0.7	±0.3	±1.0	±0.8	±0.7
Integrated	418.0	181.4*	155.8*	201.0*	241.6*	305.9	315.2
SEM	±77.7	±49.8	±46.5	±37.6	±65.8	±80.3	±64.8
Uterine electromyogram (% control)							
Average	100.0	94.5†	101.6†	96.1	86.0	95.9	90.0
SEM		±6.9	±5.3	±5.8	±8.0	±8.3	±4.2
Integrated	100.0	79.0†	95.9†	82.8	88.4	96.7	85.7
SEM		±6.9	±11.3	±8.8	±15.7	±10.2	±6.1

*$p \leq 0.05$, different from control.
†$n = 4$.

stored in a cloth pouch. The laparotomy was then closed. The maternal jugular vein and left ventricular catheters were secured to the neck with a loosely wrapped elastic bandage. The ewe was placed in a pen with free access to food and water during the 5-day recovery period.

Experimental protocol. The ewe was moved to the laboratory on the day before the experiment. The morning of the experiment a 1 ml arterial sample was taken for analysis of blood gas tension and pH on an Instrumentation Laboratory System 1302 pH/blood gas analyzer (Lexington, Mass.). Measurements were recorded at 37° C and converted by algorithm to equivalent values at 39° C. Fetal arterial and venous pressures, intrauterine pressure, uterine electromyogram, and maternal arterial pressure were recorded with use of pressure transducers (Gould P23XL), Pidas data acquisition system, and Beckman 711 chart recorder. Analog signals were digitized on line. Both the analog and digitized signals were stored on magnetic tape.

PGE_2 (Sigma) and forskolin (Calbiochem) solutions were prepared on the morning of each experiment. Forskolin (10 mg) was dissolved in 7.5 ml of 95% ethanol and mixed with 0.9% saline solution to a final volume of 25 ml. PGE_2 was diluted with saline solution from a stock solution.

PGE_2 (0.012 ± 0.002 mg/min) was infused into the left ventricle for a 3-minute period. An acceptable response to PGE_2 was defined, according to the criteria of Nathanielsz et al.,[8] as an increase in intrauterine pressure ≥5 mm Hg and increased uterine electromyogram activity longer than 4 minutes in duration. If these criteria were not met, the PGE_2 challenge was repeated after a 30-minute period at a higher infusion rate. Generally, a response was seen with the first challenge (0.01 mg/min) but on one occasion the infusion rate had to be increased (0.02 mg/min).

Seven minutes after a successful PGE_2 challenge forskolin (10 mg diluted in 25 ml of 30% ethanol) or vehicle (25 ml of 30% ethanol) was then infused through the maternal left ventricular catheter over a 20-minute period. Three minutes after the forskolin or vehicle infusion was stopped a second PGE_2 challenge was made (T = 3). PGE_2 infusion was repeated every 30 minutes for 2 hours (T = 33, 63, 93, 123, 153).

At the end of the experiment the ewe and fetus were killed with a lethal injection of euthanasia solution (T-61, Hoechst), followed by intracardiac injection of saturated potassium chloride.

Forskolin dosage for this experiment was extrapolated from in utero work on myometrial contractility using 0.2 to 5.0 μmol/L forskolin.[5] The lipid-soluble drug should distribute evenly throughout the maternal and fetal tissues. By use of an estimated body weight of 50 kg, we calculated that 10 mg of forskolin (molecular weight = 410 gm) would result in a final concentration of approximately 0.5 μmol/L forskolin.

Data analysis. The digital tape was read with use of a VAX 8600 mainframe computer. Electrical output signals were converted to appropriate pressure readings with calibration signals recorded on tape. Data files were then imported to local IBM computers and stored on hard disk. Data files were split into separate PGE_2 infusion periods: control, 3 minutes, 33 minutes, 63 minutes, 93 minutes, 123 minutes, and 153 minutes. Polygraph records were examined to determine the time of the start and finish of each period of electromyogram activity and intrauterine pressure response. Electromyogram and intrauterine pressure values were referred to the average value preceding PGE_2 infusion for each period. Average values were computed for each period of activity. Integrated values were calculated by summing each 5-second value during a response. This calculation was made to assess the combined effects of magnitude and duration of each response. Fetal arterial pressure (referred to venous

pressure), maternal arterial pressure, and heart rate were calculated for each episode. The magnitude of the electrical signal from the uterine electromyogram electrodes varied with each sheep, depending on the placement and orientation of the electrodes on the uterus. To correct for interanimal variations, electromyogram signals were normalized so that the magnitude of each control signal was equal. The same correction factor was used for each measurement in an individual animal.

Data were analyzed with repeated-measures analysis of variance at the $p \leq 0.05$ level of significance. Differences between test values and control were accepted as significant ($p \leq 0.05$) when the overall analysis of variance test was significant.

Results

Experiments were performed on seven pregnant ewes on the one hundred thirtieth day of gestation. Arterial oxygen tension (103 ± 4 mm Hg), carbon dioxide tension (35.3 ± 2 mm Hg), pH (7.456 ± 0.02), and hematocrit (28% ± 2.1%) were all within normal range for this laboratory. There were no apparent differences between ewes in the forskolin and vehicle studies and the ewes appeared to be healthy.

Vehicle infusion. In two pregnant ewes, intrauterine pressure and uterine electromyogram responses to PGE_2 challenge were measured before and after an infusion of a 25 ml solution of 30% ethanol. Prostaglandin E_2 challenges were made every 30 minutes for 2½ hours after the infusion. There was no difference in either uterine electromyogram or intrauterine pressure response to PGE_2 at any time during the experiment.

Forskolin infusion. In five pregnant ewes, maternal and fetal arterial pressure, intrauterine pressure and uterine electromyogram responses to PGE_2 were determined before and after an infusion of 400 μg/ml forskolin in 30% ethanol solution. Maternal and fetal arterial pressures were measured continuously throughout the experiment and did not demonstrate a significant change from control values at any time during the study. Mean maternal and fetal arterial pressures measured during the control period were 93 ± 4 and 46 ± 2 mm Hg, respectively.

The intrauterine pressure response to PGE_2 challenge was compared before and at 30-minute intervals after forskolin infusion. Immediately after the forskolin infusion (T = 3 minutes), the magnitude (both integrated signal and average) of the intrauterine pressure increment was sigificantly lower than control (Table I). This effect was maximized 33 minutes after forskolin infusion when the integrated and average intrauterine pressure responses were 37% and 44% of control values, respectively. The response to PGE_2 challenge continued to be significantly lower than control levels through the 63- and 93-minute PGE_2 challenges and returned to control levels after 123 minutes. These data are presented as the percentage of control levels in Fig. 1 for both intrauterine pressure and uterine electromyogram response. The duration of the pressure responses did not change from the control value of 377 ± 51 seconds. Data from one animal are presented in Fig. 2, which illustrates the normal electrical and contractile responses to PGE_2 before forskolin, the abolition of the pressure response after forskolin, and the gradual return toward the control condition.

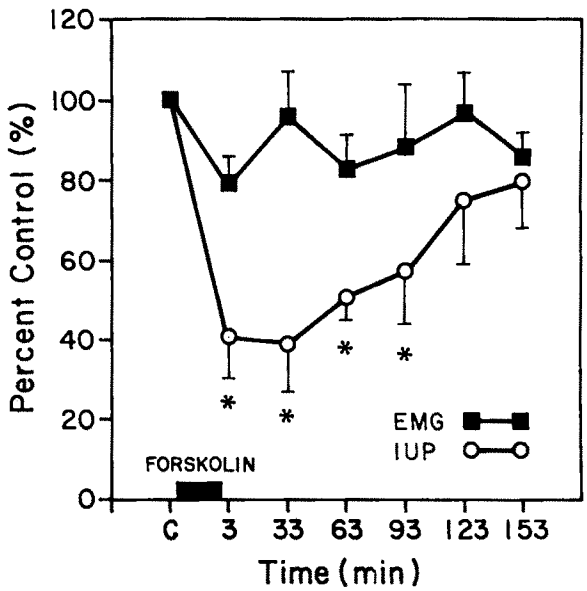


Fig. 1. Effect of forskolin on integrated uterine electromyogram and intrauterine pressure responses to PGE_2 before *(C)* and after forskolin infusion (3 through 153 minutes). Because of electrical failure, $N = 4$ for uterine electromyogam response at 3 and 33 minutes; $N = 5$ for all other data points. *Asterisk* denotes a difference from control values ($p \leq 0.05$).

On two occasions contractile-like intrauterine pressure increments were seen in the absence of electromyogram activity. These were interpreted as electrical failures; the data were not included in the final analysis.

The uterine electromyogram response to PGE_2 challenge showed no changes throughout the experiment. Both the integrated signal and the average voltage remained constant for each animal. The duration of the electromyogram response did not change from its control value of 381 ± 43 seconds.

Comment

Data from seven pregnant ewes were included in this study of the effects of forskolin on the uterine response to PGE_2 challenge. Maternal arterial blood gas tensions and pH, general appetite, and alertness all indicated that the ewes were in good condition. Therefore it is

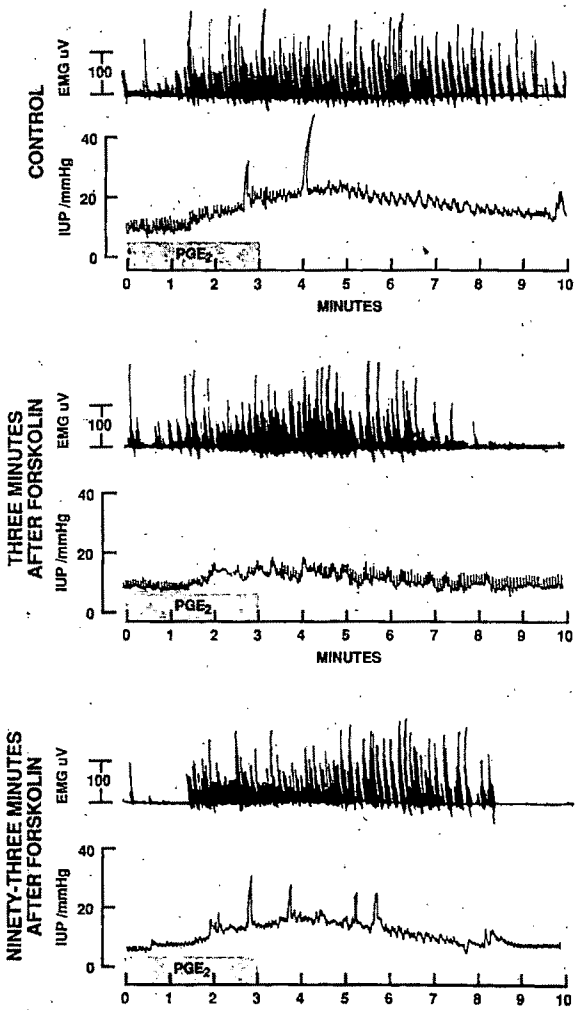


Fig. 2. Effect of forskolin on contractile and electrical responses to PGE_2 challenge in a single animal. Raw data are shown for control condition and for 3 and 93 minutes after forskolin administration. The uterine electrical activity is presented in *upper tracing* and intrauterine pressure *(IUP)* in the *lower panel.*

believed that the data herein constitute a normal physiologic response.

Prostaglandin E_2 was used in this study to stimulate uterine contractions. Prostaglandin E_2 was favored because of its immediate and well-defined effect on the uterus. Both uterine electromyogram and intrauterine pressure responses were apparent within 2 minutes of the onset of PGE_2 infusion. These indicators usually returned to normal within 10 minutes after the PGE_2 infusion was terminated. Intravenous oxytocin infusion is the standard method of labor induction in the clinical realm.[9] This hormone was used in a preliminary study but gave a protracted and irregular response that was deemed unsuitable for quantitative analysis.

This study demonstrates an attenuation of the intrauterine pressure response to PGE_2 challenge in the face of a continued normal uterine electromyographic response after systemic administration of forskolin to the late gestation ewe. Since intrauterine pressure is a physiologic marker of uterine contractility and uterine electromyogram is a marker of uterine electrical activity, this study demonstrates a disruption of the normal excitation-contraction coupling of uterine smooth muscle mechanisms. This dissociation of mechanical contraction from electrical stimulus is a smooth muscle phenomenon that has also been observed in some cases in response to isoproterenol challenge.[10]

The use of intrauterine pressure to interpret uterine contractile activity has been questioned.[8] The amniotic fluid around the late gestation fetus is no longer confluent and an intrauterine pressure catheter does not always accurately reflect an overall change in uterine tone because the pressure from a contraction may be absorbed by the fetus and fail to reveal a pressure increment. There are two reasons this was not considered a major difficulty in this study. First, only those ewes that showed a response to PGE_2 before forskolin infusion were studied. In addition to yielding baseline data, this PGE_2 challenge demonstrated that the intrauterine catheter was functioning well. Second, the consistency of the results suggests that catheter function was not affected during the subsequent 3 hours by random fetal movements.

The possible contributions of the alcohol vehicle to the tocolytic activity demonstrated for forskolin were explored in two near-term ewes. Each ewe received an intravenous infusion of 25 ml 30% ethanol over a 20-minute period. As in the forskolin series, PGE_2 was administered systemically before the infusion and at 30-minute intervals for 2½ hours after the infusion. No effect of the ethanol was apparent on either the intrauterine pressure or uterine electromyogram response. This is most likely attributed to the small quantity of alcohol involved. Doses of ethanol used to effect tocolysis in a clinical setting are more than 20 times greater per kilogram of weight and are administered over much longer periods of time.[11-14]

The molecular basis for the actions of forskolin on smooth muscle remains unclear. Forskolin, a diterpene isolated from the native Indian herb *Coleus forskohlii,* is a direct stimulant of the catalytic subunit of adenylate cyclase.[15] The subsequent rise in cAMP levels has been associated with smooth muscle relaxation in many isolated systems including swine carotid artery, rat aortic rings, and rat myometrial strips.[4, 5, 16-18] Three mechanisms have been proposed for cAMP-mediated smooth muscle relaxation: (1) decreased net calcium ion influx, (2) phosphorylation of calmodulin-dependent myosin light chain kinase, and (3) increased intracellular calcium ion sequestration.[19] Gerthoeffer et al.[16] report that forskolin can relax carotid artery strips with no change

in levels of myosin phosphorylation. This response suggests there is no change in phosphorylation of myosin light chain kinase. However, the role of myosin phosphorylation in smooth muscle relaxation remains controversial. Further reports suggest that forskolin acts by altering intracellular levels of calcium ion, either by internal sequestration or by changes in net calcium ion influx.[17, 20]

The electrical wave reflected in the uterine electromyogram is largely a function of net calcium ion flux across the plasma membrane of uterine smooth muscle cells.[21] The response of the uterine electromyogram to PGE_2 did not change at any time after forskolin infusion. It is therefore unlikely that forskolin causes smooth muscle relaxation by influencing net calcium ion influx during excitation. We surmise that forskolin causes uterine smooth muscle relaxation by causing increased formation of intracellular cAMP, which in turn elicits an increase in intracellular calcium ion sequestration, or by provoking phosphorylation of myosin light chain kinase.

In summary, systemic forskolin infusion caused a decreased contractile response without affecting uterine electrical activity in response to PGE_2 in five near-term pregnant ewes. This study demonstrates that forskolin can influence uterine response to a contractile stimulant such as PGE_2 by an intracellular event independent of cellular excitation.

REFERENCES

1. Rankin JHG, Landauer M, Tian Q, Neaves N, Phernetton TM. Forskolin dilates the maternal ovine placental vascular bed. J Dev Physiol 1988;10:201-8.
2. Rankin JHG, Landauer M, Tian Q, Phernetton TM. Cardiovascular responses to forskolin in the ovine fetus. J Dev Physiol 1988 [In press].
3. Reid DL, Hollister MC, Phernetton TM, Rankin JHG. Effects of forskolin on placental vascular resistance in rabbits. Proc Soc Exp Biol Med 1988;451-4.
4. Smith DD, Marshall JM. Forskolin effects on longitudinal myometrial strips from the pregnant rat: relationship with membrane potential and cyclic AMP. Eur J Pharmacol 1986;122:29-35.
5. Marshall JM, Fain JN. Effects of forskolin and isoproterenol on cyclic AMP and tension in the myometrium. Eur J Pharmacol 1985;107:25-34.
6. Riemer RK, Wu YY, Bottari SP, Jacobs MM, Goldfien A, Roberts JM. Estrogen reduces β-adrenoceptor-mediated cAMP production and the concentration of the guanyl nucleotide-regulatory protein, G_s, in rabbit myometrium. Mol Pharmacol 1988;33:389-95.
7. Landauer M, Phernetton TM, Parisi VM, Clark KE, Rankin JHG. Ovine placental vascular response to the local application of prostacyclin. AM J OBSTET GYNECOL 1985; 151:460-3.
8. Nathanielsz PW, Poore ER, Brodie A, et al. Update on the molecular events of myometrial activity during pregnancy. In: Nathanielsz PW, Parer JT eds. Research in perinatal medicine I. Ithaca, New York: Perinatology Press, 1984:87-111.
9. Pritchard JA, MacDonald PC, Gant NF, eds. Williams' obstetrics. 17th ed. Norwalk, Connecticut: Appleton-Century-Crofts, 1985:367-88.
10. Bulbring E, Ohashi H, Tomita T. Adrenergic mechanisms. In: Bulbring E, Brading A, Jones A, Tomita T, eds. Smooth muscle. London: E. Arnold, 1981:219-48.
11. Lauersen NH, Raghavan KS, Wilson KH, Fuchs F, Niemann WH. Effects of prostaglandin $F_{2\alpha}$, oxytocin and ethanol on the uterus of the pregnant baboon. AM J OBSTET GYNECOL 1973;115:912-8.
12. Lauersen NH, Wilson KH, Fuchs FF. The inhibitory effect of ethanol on oxytocin-induced labor at term. J Reprod Med 1981;26:547-50.
13. Spearing G. Alcohol, indomethacin and salbutamol. Obstet Gynecol 1979;53:171-4.
14. Zlatnik FJ, Fuchs F. A controlled study of ethanol in threatened premature labor. AM J OBSTET GYNECOL 1972;112:610-2.
15. Seamon K, Daly JW. Activation of adenylate cyclase by the diterpene forskolin does not require the guanine nucleotide regulatory protein. J Biol Chem 1981;256:9799-801.
16. Gerthoeffer WT, Trevethick MA, Murphy RA. Myosin phosphorylation and cyclic adenosine 3',5'-monophosphate in relaxation of arterial smooth muscle by vasodilators. Circ Res 1984;54:83-9.
17. Jones AW, Bylund DB, Forte LR. cAMP-dependent reduction in membrane fluxes during relaxation of arterial smooth muscle. Am J Physiol 1984;246:H306-11.
18. Mokhtari A, Dokhac L, Tanfin Z, Harbon S. Forskolin modulates cyclic AMP generation in the rat myometrium: interactions with isoproterenol and prostaglandin E_2 and prostaglandin I_2. J Cyclic Nucleotide Protein Phosphor Res 1985;10:213-27.
19. Schaub MC, Kunz B. Regulation of contraction in cardiac and smooth muscles. J Cardiovasc Pharmacol 1986;8 (suppl 8):S117-23.
20. Hertog AD, Pielkenrood J, Akker JVD. The effect of forskolin on smooth muscle cells of guinea pig taenia caeci. Eur J Pharmacol 1985;106:131-4.
21. Guyton AC. Neuromuscular transmission; function of smooth muscle. In: Guyton AC, ed. Textbook of medical physiology. Philadelphia: WB Saunders, 1981:138-48.

Gestational diabetes in the BHE rat: Influence of dietary fat

Juliana M. Bue, BS, Dorothy B. Hausman, PhD, and Carolyn D. Berdanier, PhD
Athens, Georgia

Two studies were performed to assess the effects of fat feeding on glucose tolerance before, during, and after gestation of BHE rats. In the first study 5% or 22% fat diets were fed from 3 weeks before mating until after gestation. In the second study, these diets were fed from weaning until 400 days of age. Glucose tolerance before and after gestation was not affected by diet in the short-term study. At 19 days' gestation, glucose tolerance had deteriorated in the pregnant rats that were fed with 22% fat diet. In the long-term study, abnormal glucose tolerance was observed before mating in rats that were fed the 22% fat diet. Tolerance improved during gestation and post partum, but still was not normal. Fertility and pup survival were significantly (50%) reduced in rats that were fed the 22% fat diet. These findings suggest that either short-term or long-term feeding of a 22% fat diet to BHE rats results in a model for human gestational diabetes that might be worthy of further study. (AM J OBSTET GYNECOL 1989;161:234-40.)

Key words: BHE rats, glucose tolerance, gestational diabetes, fertility, neonatal survival

Gestational diabetes mellitus, or glucose intolerance with recognition of onset during pregnancy, occurs in approximately 1% to 3% of all pregnancies in human beings.[1-3] It is associated with an increase in fetal and neonatal death as well as with an increase in fetal weight. The risk of maternal death is also increased and it is thought that women at risk for diabetes have more spontaneous abortions and more difficulty with conception. Its pathophysiology is poorly understood, in part, because of the lack of a small inexpensive animal model to use to study the disorder.

The BHE strain of rat has many of the features commonly associated with human adult onset noninsulin dependent diabetes mellitus. It is hyperinsulinemic early in life[4] but this hyperinsulinemia subsides as the pancreas loses its ability to respond to a glucose challenge. Peripheral insulin resistance is also observed in these rats, with compensatory changes in glucose metabolism in the liver.[4-8] These changes include an increase in hepatic glycogenesis,[5] lipogenesis,[4,8] and gluconeogenesis from lactate.[5] As glucose tolerance deteriorates and the hepatic compensations become inadequate, lipemia and glycemia are observed. Lastly, deterioration of renal, vascular, and cardiac tissue occurs and the animal dies of a variety of diseases at middle age. The average life span of the male BHE rat is about 600 days. That of normal Wistar or Sprague-Dawley rats is between 1000 to 1200 days. A diet rich in sucrose can hasten the appearance of lipemia and glucose intolerance and further shorten the life span of these rats. Substitution of a highly saturated fat for an unsaturated fat such as corn oil can likewise alter the time frame for the appearance of these genetic traits and alter gluconeogenic rate,[6] mitochondrial activity,[7] and glucose oxidation.[8] Almost all of the work with BHE rats has been done with male rats to avoid the confounding effects of the estrus cycle on the measurements made. It is quite likely that the female BHE rat may have these same characteristics but little documentation exists that assures us that this is so. Nonetheless, given the genetic tendency of the strain to manifest noninsulin dependent diabetes mellitus, and the report of Arnold and Thye[9] that female BHE rats have an abnormal glucose tolerance, we decided to explore the possibility that female rats might be useful in the study of gestational diabetes. In this article we report results of our work to develop such a model.

Material and methods

Two experiments were conducted. The main difference between the studies was the duration of feeding of the special diets. Experiment 1 consisted of a short-term (3 weeks) feeding period that preceded gestation, whereas experiment 2 consisted of a long-term (25 weeks) feeding period that preceded gestation. The short-term study used 175-day-old rats (12 rats per group), whereas the long-term study rats (20 rats per group) were fed the diets from weaning and ranged from 225 to 250 days of age at mating. The animals were housed in a room controlled for temperature (21 ± 1° C), humidity (45% to 50%), and light (lights on 6 A.M. to 6 P.M.). Food and water were always available. The animals were bred in double-wide hanging wire mesh cages and were removed to single cages after mating. The papers that underlined the cages were

From the Department of Foods and Nutrition, University of Georgia.
Received for publication October 27, 1988; accepted February 21, 1989.
Reprint requests: Dr. Carolyn D. Berdanier, Department of Foods and Nutrition, University of Georgia, Dawson Hall, Athens, GA 30602.

Table I. Diet composition (gm/100 gm)

	5% Fat	*22% Fat*
Corn oil	3	14
Beef tallow	2	8
Lactalbumen	10	6
Casein	10	6
Starch	30	26
Glucose	6	5
Fructose	6	5
Sucrose	23	19
Alphacel	4	5
Vitamin mix*	1.1	1.1
Mineral mix†	4.9	4.9

*Association of Official Agricultural Chemists vitamin mix, U.S. Biochemical Corp., Cleveland, Ohio.

†Bernhardt-Tomerelli salt mix, ICN Corp., Cleveland, Ohio.

examined daily. Mating was determined by the appearanace of a vaginal plug on the papers under the mating cage. Male rats were removed from the cage as soon as the plug appeared. This was considered day 0 of the gestation period. Rapid weight gain in the first week after mating was considered to be evidence of conception. After glucose tolerance tests (GTTs) were conducted at 19 days' gestation, dams were placed in plastic shoe box cages with treated pine shavings as bedding. The dams were monitored until parturition was complete. At parturition, the litter was counted and weighed. The dams were weighed weekly and their food intakes were determined.

Before mating, at 19 days' gestation, and at 10 days post partum, glucose tolerance was determined. Day 19 was selected as representative of late gestation. The GTT consisted of a 6-hour fast that preceded a load dose of glucose (25% glucose solution, 1 ml/100 gm body weight) administered by gavage. Tail blood samples were drawn before glucose administration and at 30, 90, and 150 minutes later. The incremental area or the difference over time between the fasting glucose level and that after the glucose pulse, was calculated and compared with the use of the Student *t* test.[10] Both serum glucose[11] and insulin[12] levels were determined in the short-term study, whereas only the glucose level was determined in the long-term study. Glucose tolerance was determined at 400 days of age in the long-term study. The ratio of the 0 to 150-minute incremental area of glucose to insulin, the insulinogenic index,[13] was calculated and compared. To assess the relationship between insulin concentration and glucose concentration, the insulin/glucose ratio was calculated (μU/ml insulin divided by mg/dl glucose).[13]

The rats were fed either a 5% or 22% fat diet (Tables I and II). The composition of the 22% fat (high-fat) diet was determined on the basis of the composition of the average American diet[14] in terms of sources of calories from the macronutrients. The saturated/unsaturated fat ratio was replicated, as was the ratio of polysaccharides to disaccharides and monosaccharides. Purified ingredients were used to construct the diet. The low-fat or 5% fat diet was created on the basis of the proximate composition of the stock diet usually fed to our rat colony. The same ingredients and ratios within macronutrients were used as for the high-fat diet.

Table II. Diet composition (kcal/100 kcal)

	5% Fat	*22% Fat*
Lipid	11.7	42.5
Protein	20.8	10.3
Carbohydrate	67.5	47.2

In experiment, 1, the progeny were removed from their dam within 2 hours of birth and were killed by decapitation. Blood was collected and was used for the determination of glucose and insulin levels, and the body was used for the determination of its proximate composition. In study 2, litters were size limited to 4 to 6 pups. Male pups were killed at birth, day 3, and day 10 and were used for the histologic examination of the inguinal fat pads. Whole body cross sections were prepared in accordance with the procedures of Hausman and Richardson.[15] Sliced paraffin sections (7 μm) were stained with periodic acid-Schiff reagent to determine the presence of soluble polysaccharides in cell membrane and were counterstained with Harris hematoxylin to distinguish cellular nuclei. Inguinal adipocytes in periodic acid-Schiff–stained histologic sections were identified and diameters were determined on 50 randomly selected inguinal adipocytes per animal.

Results

Experiment 1. This experiment was conducted to examine the effect of short-term, high-fat feeding on glucose tolerance during gestation and gestation outcome. Several differences in food intake were obesrved: rats fed the high-fat diet ate significantly more than rats fed the low-fat diet (29.3 ± 1.7 versus 24.9 ± 1.0 kcal/100 gm body weight per day) during the initial week on the experimental diets and gradually diminished their intake during the next 2 weeks so that food consumption was similar (25.7 ± 1.5 versus 25.1 ± 1.4 kcal/100 gm body weight per day) in both groups at mating. Food consumption was increased during each week of pregnancy in the low-fat rats and only during the second trimester in the high-fat rats. Significant differences in food intake between the low-fat and high-fat rats were observed during the first and third trimesters ($p < 0.05$). Despite the observed differences in food intake, the body weights of the two dietary groups were not significantly different, nor was body weight gain during gestation affected by diet. Rats fed

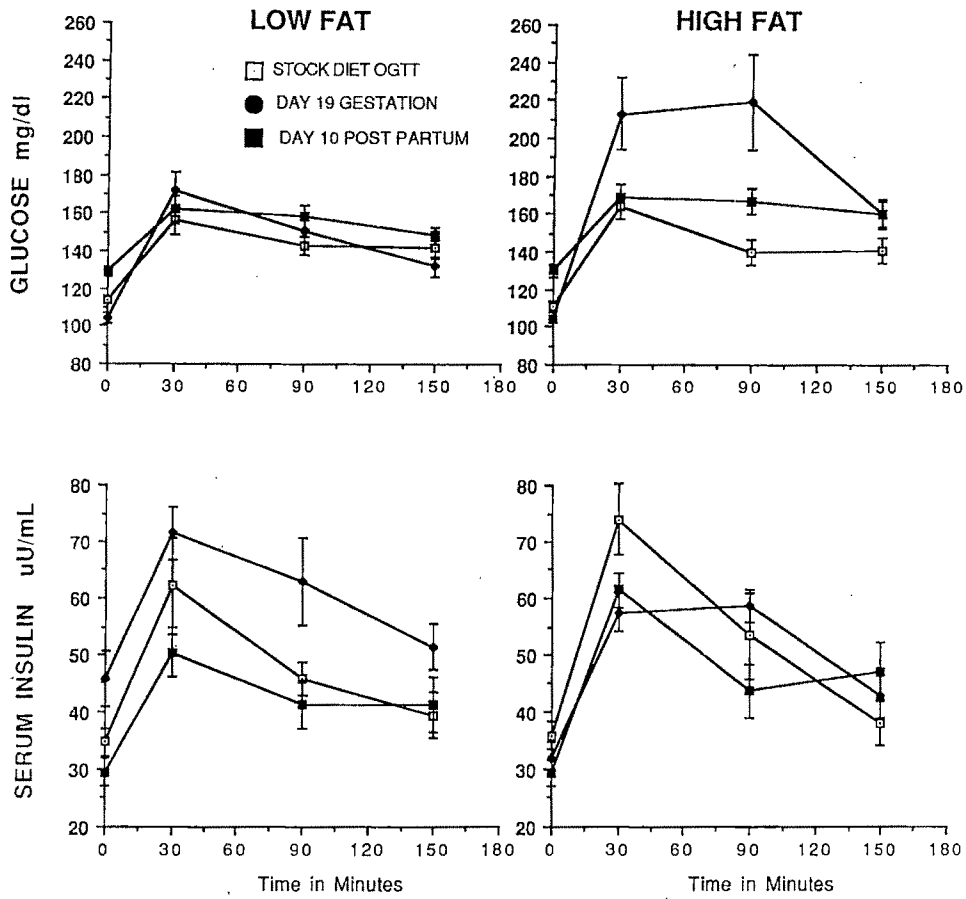


Fig. 1. Effects of short-term low-fat or high-fat feeding on glucose and insulin response to oral glucose load before and after gestation (experiment 1).

the low-fat diet gained 94 gm ± 8.8 gm, whereas rats fed the high-fat diet gained 86.6 gm ± 8 gm. Low-fat dams had 11.4 ± 0.9 pups per litter and high-fat dams had 9.3 ± 0.5 pups per litter.

Basal levels of serum glucose, measured after a 6-hour fast, increased when animals were changed from the stock diet to either of the purified diets (112 mg/dl ± 1.7 mg/dl on stock versus 131.3 mg/dl ± 4.5 mg/dl and 129.6 mg/dl ± 2.9 mg/dl on low-fat and high-fat diets, respectively). Pregnancy in low-fat rats led to a decrease in basal serum glucose levels and an increase in basal serum insulin levels (Fig. 1). This is consistent with the study of Martin et al.[16] that used rats of a normal strain. In the high-fat rats, pregnancy was associated with a decrease in basal serum glucose levels and no change in basal serum insulin levels. Basal serum glucose and insulin levels returned to initial values by day 10 post partum in both low-fat and high-fat rats.

Glucose tolerance was similar in both dietary groups in the nonpregnant state (before breeding and on day 10 post partum) but was increased by pregnancy to a greater extent in the high-fat rats than in the low-fat rats. The 0 to 150-minute mean incremental glucose area (Fig. 2) was increased in the pregnant low-fat rats compared with their nonpregnant counterparts. It is likely that this was a result of the pregnancy-induced reduction in basal serum glucose levels. The mean incremental glucose area was further increased in the pregnant high-fat rats. A significant difference in the 0 to 150-minute mean incremental glucose area was observed between the two dietary groups during pregnancy. This difference indicated a greater degree of glucose intolerance in the high-fat rats. This impairment was only temporary; by day 10 post partum, glucose tolerance in the high-fat rats had improved. The 0 to 150-minute mean incremental glucose area of the postpartum high-fat rats was comparable to that of the postpartum low-fat rats. The insulin response of the pregnant rats to the oral GTT was similar to that in the nonpregnant state regardless of diet (Figs. 1 and 2). The insulinogenic index was similar in the low-fat and high-fat rats before breeding. During pregnancy, the insulinogenic index decreased in the high-fat rats and increased slightly in the low-fat rats. A significant difference in insulinogenic index was observed during

pregnancy between the two dietary groups ($p < 0.05$). During the postpartum period, the insulinogenic index increased in the high-fat rats to a value that was similar to that of the postpartum low-fat rats.

The effects of maternal diet on selected parameters in newborn BHE rat pups is not presented because, despite the observed differences in maternal glucose tolerance, there were no differences in average pup weight, serum glucose and insulin levels, pancreatic soluble protein, pancreatic insulin content, and body composition.

Experiment 2. This experiment was conducted to examine the effect of long-term high or low fat feeding on glucose tolerance during gestation and gestation outcome. Food intakes (approximately 25 kcal/100 gm body weight per day) by both diet groups were similar throughout the experiment.

Rats fed the two diets differed in their body weight gain. Those fed the high-fat diet gained more weight than those fed the low-fat diet. However, most of the difference in weight gain was attributed to four of the 20 rats fed the high-fat diet. These four rats became obese. When this small subset was removed and was treated as a separate group, the diet difference in weight gain was not significant. Weight gain during gestation was not affected by diet. Low-fat dams gained 84.1 gm ± 8.3 gm and high-fat dams gained 97.9 gm ± 9.6 gm during pregnancy. At 400 days of age, rats fed the high-fat diet weighed 400 gm ± 10 gm and those fed the low-fat diet weighed 360 gm ± 10 gm.

At 200 days of age, glucose tolerance was significantly depressed in high-fat rats, as compared with low-fat rats (Fig. 3). Basal levels of serum glucose measured after a 6-hour fast decreased when the rats were pregnant. This is consistent with the reports of an improvement in glucose tolerance with pregnancy in streptozocin diabetic rats.[16] Glucose tolerance was improved in high-fat rats during gestation and at 10 days post partum as compared with tolerance before breeding. At each of the testing times the high-fat rats' glucose tolerance was less than that of the low-fat rats. Their 30-minute post dose blood glucose values were higher and the rate of decline in blood glucose from 30 to 150 minutes was less. These results are in contrast with those of experiment 1 in which glucose tolerance was depressed in rats that were fed the high-fat diet only during gestation.

Compared with the short-term study in which diet had no effect on fertility or pregnancy outcome, the long-term feeding of the high-fat diet significantly affected fertility as well as production (Table III). Fertility (conception and successful completion of pregnancy) was decreased fifty percent in dams that were fed the high-fat diet. Of the 20 rats that were fed the high-fat diet, only half conceived. Of those that conceived, 30%

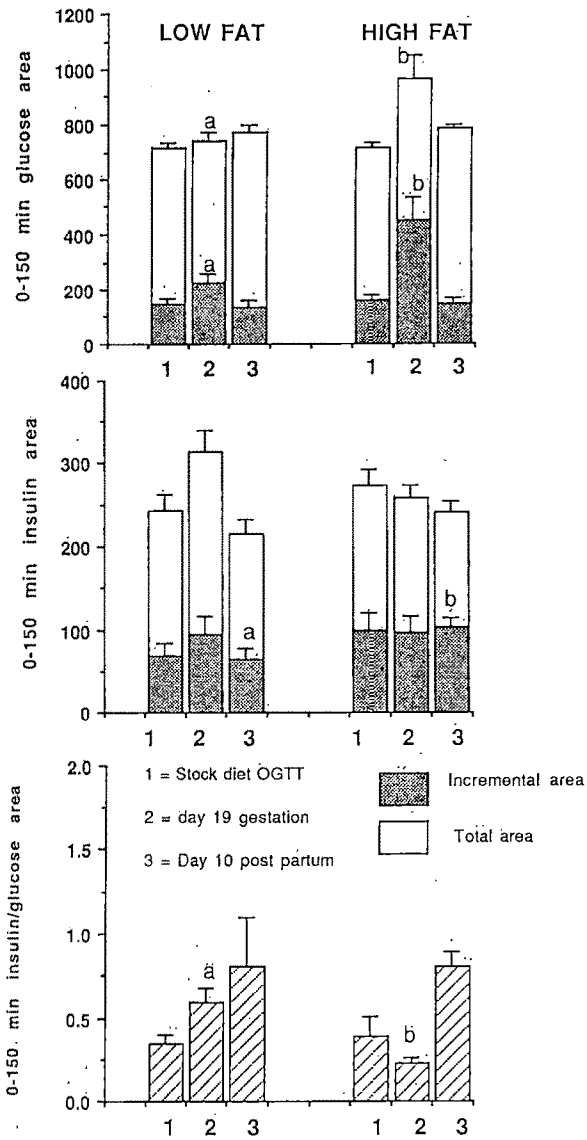


Fig. 2. Effects of short-term low-fat or high-fat feeding on total and mean incremental areas under glucose tolerance curves obtained before, during, and after gestation. Superscripts *(a, b)* indicate significant differences because of diet.

did not rear young. The pups of these dams either died at birth or shortly thereafter. Of the rats that were fed the low-fat diet, 35% did not conceive and 15% of these did not rear young. Diet had no effect on litter size, litter weight, or average pup weight. As previously mentioned, four of the rats that were fed the high-fat diet became obese. Only one of these conceived. However, all of her pups died at birth. Of the pups born to the dams fed the high-fat diet, 25% died at birth. Of the pups born to the low-fat dams, 17% died at birth. The usual mortality rate in our stock diet–fed rat colony is <10%.

Diet also affected gestation time, which in turn probably affected neonatal mortality. The average gestation

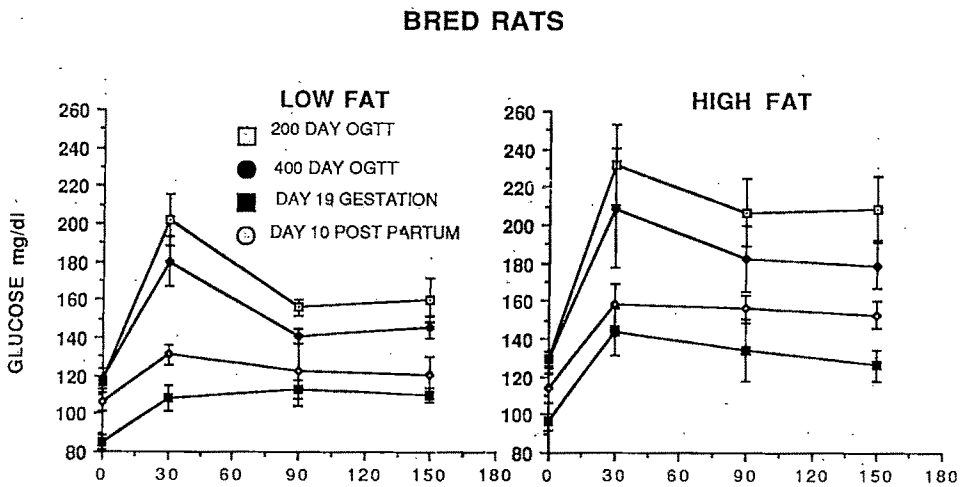


Fig. 3. Effects of long-term high-fat feeding and gestation on glucose tolerance of female BHE rats.

Table III. Effect of maternal diet on fertility, litter size, litter weight, average body weight, and lactation (experiment 2)

	Diet	
	5% Fat	*22% Fat*
Number of dams initially	20	20
Number of dams that conceived	13	10
Number of dams that bore young	13	10
Number of dams that reared pups	11	7
Percentage of dams that gave birth to live young	55% (11:20)	35% (3:20)
Percentage of dams that did not lactate	15% (2:13)	30% (3:10)
Average length of gestation (days)	22.7	22.9
Average litter size	6.2 ± 1.1	7.0 ± 1.1
Average litter weight (gm)	37.8 ± 6.3	41.2 ± 5.7
Average pup weight (gm)	6.3 ± 0.2	6.0 ± 0.2
Total number pups	80	65
Numbers of pups that died during neonatal period	14	16
Percentage of pups that died	17% (14:80)	25% (16 65)

time was 22.7 days for the low-fat dams 22.9 days for the high-fat dams. Five of the low-fat group had a 23-day gestation and one had a 25-day gestation. Seven of the high-fat group had a 23-day gestation and one had a 24-day gestation. The normal gestation period of stock-fed dams in our BHE colony is 21 days. All of the pups of the dams with 24- and 25-day gestation periods died. One of the pups of a high-fat dam with a 23-day gestation was malformed and died. The high-fat dam with the 24-day gestation had only two pups, which weighed 8 gm and 7.9 gm, respectively. Both pups died at birth. Both low-fat and high-fat dams had small litters. The average litter size for the low-fat dams was six, whereas the average litter size for the high-fat dams was seven. The usual litter size of our colony BHE rats is 12 with pup weights ranging from 5 gm to 6 gm. Because we wanted to follow up these dams to 400 days of age we did not look for evidence of resorptions. Hence, we can only assume that fetal death and resorption had occurred, which could account, in part, for the small litter sizes. As in the short-term study, the surviving pups were normal in all respects. We did not observe macrosomia in the surviving young. Birth weight and size of inguinal pad adipocytes of offspring were not affected by dam's diet; however, there were differences in growth. By 10 days of age, low-fat pups weighed significantly more (27.4 gm ± 0.79 gm versus 21.1 gm ± 1.7 gm) than the high-fat pups. This increase in body weight was not accompanied by an increase in fat cell size.

Comment

The purpose of this work was to develop a rat model that might be useful for the study of gestational diabetes as it occurs in human beings. It has long been recognized that noninsulin dependent diabetes mellitus has a strong genetic component and so we used a rat strain with a genetically determined predisposition for noninsulin dependent diabetes mellitus. In human beings, it is also recognized that women who show evidence of gestational glucose intolerance may later have noninsulin dependent diabetes mellitus. Gestational diabetes has been considered an early warning signal of the latent disease. Before our use of the special diets we conducted several preliminary studies* of the pregnant

*PhD dissertation. Dorothy B. Hausman. Studies on the development of a rat model for human gestational diabetes. Athens, Georgia: University of Georgia, 1985 (C. D. Berdanier, dissertation director).

BHE and normal or streptozocin-treated Sprague-Dawley rats. These studies revealed that the BHE rats that were fed a stock diet that consisted of a variety of natural ingredients (mostly cereals and grains) did not show evidence of impaired glucose tolerance during gestation. Neither age nor parity influenced this finding. Streptozocin treatment did elicit impaired glucose tolerance but this impairment was not solely associated with gestation. Therefore we concluded that streptozocin diabetic rats were not good models for gestational diabetes nor were the BHE rats that were fed the stock diet.

With the realization that male BHE rats require special purified diets for the expression of their noninsulin dependent diabetes mellitus phenotype, we then decided to study the BHE female rat within this context. Inasmuch as the stock diet is not a replicate of the human diet, we decided to compose a diet that resembled (in terms of its proximate composition) a diet consumed by the average American.

This was a serendipitous choice because female BHE rats manifested many of the characteristics of the human woman who has glucose intolerance during pregnancy. In the first study we showed that the BHE rats that were fed the high-fat diet did indeed show evidence of glucose intolerance during pregnancy but not before or after pregnancy. We did not observe the macrosomia or other perinatal problems in the pups that we expected if our rats truly modeled human beings. Perhaps the species differences in the level of maturity of the progeny at birth might be an important consideration. Rat pups, compared with human infants, are relatively immature. They are blind, hairless, and have very primative stubs for limbs. The human infant, in contrast, usually has all of its faculties and has relatively well developed arms, hands, legs, and feet.

In the second study, the dams used were reared on the low- or high-fat diets and then were bred. It is of interest that we observed several additional breeding problems that might have been related to the phenotypic expression of the noninsulin dependent diabetes mellitus genotype and that might be analogous to the human condition. These problems consisted of decreased fertility, presumed increased fetal death, increased neonatal death, decreased ability to lactate with the consequence of pup death because we elected not to hand feed these pups, and prolonged gestation times. Those dams that had prolonged gestation times gave birth to fewer live pups and these pups were larger and more mature in terms of their development than normal pups. Whether this prolonged gestation time is analogous to the human woman with gestational diabetes is unknown because human beings with this condition usually are closely supervised. Their glucose intolerance is treated and the pregnancy is brought to term as soon as is deemed medically desirable to achieve a successful pregnancy outcome. Failure to bring the pregnancy to term could result in sudden fetal death or abnormalities, primarily dystocia, related to the abnormal size of the infant. In this study we did not treat the glucose intolerance nor did we terminate the pregnancies at the end of the normal 21-day gestation period. Whether the large size of the pups we observed was a result of the prolonged gestation or gestational diabetes per se is not known.

Several investigators have studied the rat as a possible animal model for gestational diabetes.[16-18] Some of the features of diabetes have been elicited but none, so far, have developed a model that is normoglycemic before and after gestation but hyperglycemic during gestation. For example, Neufeld and Corbo[17] used the streptozocin-treated rat and found that maternal and fetal glucose levels were significantly elevated as was in vitro hepatic insulin receptor binding. They also found that insulin degradation was faster in the progeny of the diabetic dam than in the progeny of the control dam. They did not report on the glucose-insulin relationship during either the pre- or postgestation period but one can assume that the streptozocin treatment elicited a long-term glucose intolerance and not just one that occurred during pregnancy. Thus the streptozocin-treated rat is a good model for insulin-dependent pregnant human beings but not for the gestationally diabetic woman. Catlin et al.[18] induced fetal hyperinsulinemia by the injection of fetuses with 2 units of insulin 2 days before birth. They produced pups that were 12% heavier at birth and that showed evidence of a greater growth velocity than their saline solution–treated litter mates. Although the liver and muscle of the insulin-treated pups contained more lipid than the tissues of the saline solution–treated pups, rates of fatty acid synthesis were not different. They produced large pups but did not measure cell size so we do not know if these pups were macrosomic. Perhaps the next iteration of the present work might focus on the development of a procedure that will elicit not only the glucose intolerance during late pregnancy but also macrosomia in living pups. Inasmuch as high-sucrose feeding has been shown to increase insulin production and release, perhaps raising the sucrose level in the high-fat diet would have this effect. Further work is needed to more fully develop the BHE rat fed a 22% fat diet as a model for human gestational diabetes.

Addendum

Since this manuscript was submitted, we have learned that peripheral fat cell glucose transport, insulin binding, and receptor affinity are normal in these rats and the genetic error resulting in noninsulin dependent diabetes mellitus is only in the liver.

REFERENCES

1. Freinkel N. Of pregnancy and progeny. Diabetes 1980; 29:1023-35.
2. Pederson J. Aspects of carbohydrate metabolism in normal pregnancy and gestational diabetes. In: The pregnant diabetic and her newborn. 2nd ed. Balitmore: Williams and Wilkins, 1977:22-45.
3. Metzger BE, Freinkel N. Inquiries into the pathogenesis of gestational diabetes. In: Camerini-Davolos RA, Hanover B, eds. Treatment of early diabetes. New York: Plenum Press, 1979:201-8.
4. Berdanier CD. Hepatic metabolism in glycemic/hyperlipidemic BHE rats: evidence for a membrane error in maturity-onset diabetes. In: Shafrir E, Renold AE, Lessons from animal diabetes. London: John Libby, 1984: 210-21.
5. Berdanier CD. Rat strain differences in gluconeogenesis by isolated hepatocytes. Proc Soc Exp Biol Med 1982; 169:74-9.
6. Wander RC, Berdanier CD. Effects of type of dietary fat and carbohydrate on gluconeogenesis in isolated hepatocytes from BHE rats. J Nutr 1986;116:1156-64.
7. Deaver OE, Wander RC, McCusker RH, Berdanier CD. Diet effects on membrane phospholipid fatty acids and mitochondrial function in BHE rats. J Nutr 1986;116: 1148-55.
8. Berdanier CD, Baltzell JK. Comparative studies of the responses of two strains of rats to an essential fatty acid deficient diet. Comp Biochem Biophys 1986;85A:725-7.
9. Arnold TS, Thye FW. Effects of an oral contraceptive on plasma growth hormone and glucose tolerance in two strains of rats. Am J Clin Nutr 1987;30;381-7.
10. SAS Institute, SAS User's Guide. Helwig JT, Coucil KA, eds. Raleigh, North Carolina SAS Institute Inc., 1979.
11. Sigma technical bulletin 510. St. Louis: Sigma Chemical, 1982.
12. Hales CN, Randle PJ. Immunoassay of insulin with insulin antibody precipitate. Biochem J 1963;88:137-46.
13. Yen SSC, Tsai CC, Vela P. Gestational diabetogenesis: quantitative analysis of glucose-insulin interrelationship between normal pregnancy and pregnancy with gestational diabetes. AM J OBSTET GYNECOL 1971;111:792-800.
14. Dietary goals for the United States. 2nd ed. Washington, D.C.: Senate Select Committee on Nutrition and Human Needs, 1977.
15. Hausman GJ, Richardson RL. Cellular and vascular development in immature rat adipose tissue. J Lipid Res 1983;24:522-32.
16. Martin A, Zorzano A, Caruncho I, Herrera E. Glucose tolerance tests and in vivo response to intravenous insulin in the unanesthetized late pregnant rat and their consequences to the fetus. Diabetes Metab 1986;12:302-7.
17. Neufeld ND, Corbo LM. Insulin-receptor development in normal and diabetic pregnancies. Role of membrane fluidity. Diabetes 1986;35:1020-6.
18. Catlin EA, Cha C-J, Oh W. Postnatal growth and fatty acid synthesis in overgrown rat pups induced by fetal hyperinsulinemia. Metab 1985;34:1110-4.

Make researching easier with the Ten-Year Cumulative Index (1978-1987) to the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY

The Ten-Year Cumulative Index is a complete guide to more than 30,000 pages of original articles, case reports, letters, and editorials published in the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY from 1978 through 1987 (volumes 130 to 157). Numbering more than 800 pages, the Cumulative Index includes both a subject and an author index. This hard-cover volume is $64.50 ($69.00 international). Prices include shipping. *Payment must accompany all orders and must be in U.S. funds, drawn on a U.S. bank.*

Contact The C.V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146 USA; phone (314) 872-8370, ext. 394. In the U.S., call toll-free: 1-800-325-4177, ext. 394. Please allow six weeks for delivery.

Effect of inhibiting prostaglandin synthesis in pregnant sheep with 4-aminoantipyrine under normothermic and hyperthermic conditions

Peter Andrianakis, BSc(Hons.), David W. Walker, PhD, Meredith M. Ralph, PhD, and Geoffrey D. Thorburn, MD

Clayton, Victoria, Australia

Infusion of the prostaglandin synthetase inhibitor 4-aminoantipyrine at a rate of 20 mg/min via the fetal tarsal vein during normothermic conditions (ambient temperatures = 24° C) significantly decreased the concentrations of prostaglandins E_2 and $F_{2\alpha}$ in maternal and fetal arterial plasma and in uterine and umbilical vein plasma. The infusion was associated with a small but significant rise in fetal temperature and a fall in fetal arterial pH. Respiratory alkalosis developed in fetuses during hyperthermia by raising the ambient temperature to 43° C for 8 hours. In contrast, infusion of 4-aminoantipyrine during hyperthermia produced fetal metabolic acidosis as indicated by a fall in fetal pH and an increase in $Paco_2$, even though the ewe remained hypocapnic. Four of the 10 fetuses died during or shortly after the 4-aminoantipyrine infusion during hyperthermia. These results indicate that uteroplacental prostaglandin synthesis is essential to allow the fetus to adapt to an increase in body temperature and suggests that prostaglandin synthetase inhibitors should be used with caution during pregnancy. (Am J Obstet Gynecol 1989;161:241-7.)

Key words: Prostaglandin, 4-aminoantipyrine, hyperthermia, fetal death

Prostaglandins (PGs) are present in both the maternal and fetal circulations from early pregnancy in sheep and increase during gestation, particularly before and during labor.[1] Much of this PG is probably produced by the placenta, since the concentrations of PGE_2 and $PGF_{2\alpha}$ are higher in uterine and umbilical vein plasma than in maternal and fetal arterial plasma, respectively.[2]

The role of PGs for fetal growth and development is not understood, but PGE_2 has both cardiovascular and endocrine effects in the fetus,[3, 4] and both PGE_2 and $PGF_{2\alpha}$ are responsible in part for the nonlabor uterine contractions present throughout much of pregnancy in sheep.[5] PG concentrations are increased after surgery in growth-retarded fetal lambs[6] and during hypoglycemia in fetal lambs and foals.[7-9] It has been suggested that PGE_2 may act as a circulating hormone in the fetal lamb, and because of its actions on the adrenal cortex and lung, it may act to accelerate organ maturation and fetal viability.[6, 8] PGs may also regulate uteroplacental and umbilical blood flows,[10] although this has recently been questioned.[11]

We have shown that there is an increase in the concentrations of PGE_2 and $PGF_{2\alpha}$ in uterine vein and umbilical vein plasma when pregnant sheep are subjected to hyperthermia.[12] Prolonged hyperthermia during pregnancy may result in fetoplacental growth retardation,[13] but it is unclear if the increased synthesis and release of PGs contribute to this growth deficit or are part of the compensatory changes by which the fetus adapts to the increased tissue temperatures. In this study we show that inhibition of PG synthesis during hyperthermia leads to severe fetal acidosis, sometimes resulting in fetal death, which suggests that the increase in PG synthesis is important for fetal adaptation to increased temperatures that may occur naturally from time to time in pregnancy.

Material and methods

Surgical procedures. Ten pregnant Border Leicester/Merino ewes were used. Surgery was carried out at 118 to 122 days' gestation, and at least 7 days elapsed before the fetuses were studied.

Anesthesia was induced by intravenous administration of 5% sodium thiopentone and maintained, after intubation, with 1% to 2% halothane in oxygen and nitrous oxide (50:50 v/v). With the use of sterile procedures, a paramedial incision was made in the maternal abdominal wall, thereby exposing the pregnant uterus. A polyvinyl catheter was placed in the uterine vein (inner diameter 0.86 mm; outer diameter 1.50

*PhD dissertation. Dorothy B. Hausman. Studies on the development of a rat model for human gestational diabetes. Athens, Georgia: University of Georgia, 1985 (C. D. Berdanier, dissertation director).

From the Department of Physiology, Monash University.
Supported by a grant from the National Health and Medical Research Council of Australia to G. D. T.
Received for publication May 13, 1988; revised January 4, 1988; accepted February 16, 1989.
Reprint requests: David W. Walker, PhD, Department of Physiology, Monash University, Clayton, Victoria 3168, Australia.

Table I. Fetal body temperatures, blood gases and pH, and plasma glucose concentrations before and at the end of a 150-minute infusion of 4-aminoantipyrine (20 mg/min) into fetal tarsal vein at an environmental temperature of 24° C and 43° C; $N = 10$ except where shown otherwise in parentheses

	Before 4AAP	*During 4AAP*
T_a = 24° C		
Body temperature (° C)	39.1 ± 0.06	39.4 ± 0.09†
pH_a	7.342 ± 0.01	7.310 ± 0.01†
$PaCO_2$ (mm Hg)	48.5 ± 1.30	55.3 ± 1.30†
PaO_2 (mm Hg)	17.8 ± 1.60	18.5 ± 0.80
O_2 saturation (%)	49.3 ± 2.6	52.0 ± 2.7
Glucose (mmol/L)	0.72 ± 0.06(9)	0.78 ± 0.11(9)
T_a = 43° C		
Body temperature (° C)	41.3 ± 0.23*	41.6 ± 0.23*
pH_a	7.414 ± 0.02*	7.177 ± 0.08‡
$PaCO_2$ (mm Hg)	35.8 ± 2.20*	39.8 ± 2.97*
PaO_2 (mm Hg)	15.60 ± 1.10	13.2 ± 1.74‡
O_2 saturation (%)	46.0 ± 5.6(7)	35.5 ± 7.5(8)
Glucose (mmol/L)	0.86 ± 0.14(9)	1.15 ± 0.17(9)‡

4AAP, 4-Aminoantipyrine; T_a, ambient temperature.
*Significant change caused by heat.
†Significant change caused by 4-aminoantipyrine infusion.
‡Significant change caused by heat and 4-aminoantipyrine infusion.

mm). Through a small hysterotomy, a fetal hindlimb was exposed, and similar polyvinyl catheters were inserted 5 to 6 cm into the tarsal vein and femoral artery. A polyvinyl catheter was also inserted in the umbilical vein via a cotyledonary tributary vein. A temperature probe (two temperature-sensitive diodes in series encased in silicone and calibrated in a water bath for temperatures between 35° and 45° C) was placed deep in the groin of the fetus and secured between the muscles close to the femoral artery. A temperature probe was also attached to the greater omentum in the abdomen of the ewe. A maternal arterial catheter was inserted in the carotid artery via an incision in the neck of the ewe.

In three sheep nonocclusive electromagnetic flow probes (Micron Instruments Ltd., California) were placed around the main uterine artery.

Experimental protocol. On the day of an experiment, each fetus received two infusions of 4-aminoantipyrine (10 gm/dl, 0.2 ml/min) via the tarsal vein, beginning at approximately 8:00 AM and 4:00 PM; each infusion lasted for 2½ hours. Blood samples (3 ml) were taken from the fetal femoral artery, maternal carotid artery, and uterine and umbilical veins at 60 and 5 minutes before and at 90, 110, 130, and 150 minutes after the start of each infusion and 1 and 2 hours after completion of the second infusion. Samples were collected from the four vessels within 1 minute of each other and kept on ice until they were divided into aliquots.

At the completion of the first 4-aminoantipyrine infusion, the room temperature was raised to 43° ± 1° C from approximately 10:00 AM until 6:00 PM. The second 4-aminoantipyrine infusion started when the ewe had been subjected to 5½ hours of hyperthermia. Fetal and maternal temperatures were recorded at regular intervals through this time.

From each sample of blood, 0.5 ml was used to measure pH, blood gases, and oxygen saturation by means of Radiometer ABL30 and OSM3 equipment; packed cell volume was also determined from this aliquot. The remaining blood was divided into (1) a 1.5 ml sample placed in a polypropylene tube containing lithium heparin and (2) a 1 ml sample placed in a polypropylene tube containing aspirin (0.05 mg) and ethylenediaminetetraacetic acid (0.7 mg). After centrifugation at 2500 rpm for 10 minutes at 4° C, the plasma from the lithium heparin tubes was stored at −20° C until used for determination of 4-aminoantipyrine, glucose, and $PGF_{2\alpha}$. The plasma from the aspirin/ethylenediamine tetraacetic acid samples was mixed with an equal volume of a solution containing methoxyamine hydrochloride (final concentration, 0.12 mol/L in 1.0 mol/L sodium acetate bufer, pH 5.6) and left overnight at room temperature before storage at −20° C. This plasma was used for assay of PGE_2.

Assays and recordings. 4-Aminoantipyrine was assayed in 0.1 ml of plasma by means of the colorimetric assay of Mendelsohn and Levin[14] as described by Huckabee.[15] Glucose was measured enzymatically by the method of Trinder.[16] $PGF_{2\alpha}$ was measured by direct radioimmunoassay with antiserum raised in rabbits (kindly given by Dr. M. Mitchell). The sensitivity of the assay, which was defined as the concentration of $PGF_{2\alpha}$ corresponding to the bound count that was significantly ($p < 0.05$) less than that of the zero concentration, was 0.18 nmol/L. The intraassay coefficient of variation was <10%, and interassay coefficients of variation were

Table II. Maternal body temperatures, blood gases and pH, and plasma glucose concentrations before and at the end of a 150-minute infusion of 4-aminoantipyrine (20 mg/min) into fetal tarsal vein at an environmental temperature of 24° C and 43° C; $N = 10$ except where shown otherwise in parentheses

	Before 4AAP	*During 4AAP*
T_a = 24° C		
Body temperature (° C)	38.0 ± 0.06	38.2 ± 0.18
pH_a	7.460 ± 0.01	7.444 ± 0.01
$Paco_2$ (mm Hg)	34.92 ± 1.25	37.42 ± 1.87
PaO_2 (mm Hg)	108.56 ± 2.37	110.30 ± 3.37
O_2 saturation (%)	96.51 ± 1.12	95.27 ± 0.75
Glucose (mmol/L)	2.55 ± 0.21(9)	2.62 ± 0.19(9)
T_a = 43° C		
Body temperature (° C)	40.2 ± 0.28*	40.6 ± 0.29*
pH_a	7.583 ± 0.03*	7.600 ± 0.03*
$Paco_2$ (mm Hg)	23.55 ± 2.28*	19.94 ± 1.16*
PaO_2 (mm Hg)	130.95 ± 5.73*	132.51 ± 7.86*
O_2 saturation (%)	98.19 ± 1.03	97.42 ± 1.16
Glucose (mmol/L)	2.88 ± 0.21(9)	2.8 ± 0.18(9)

*Significant change caused by heat.

11.6% at 2.66 nmol/L and 15.6% at 1.65 nmol/L. Cross-reactivities with other PGs and metabolites were <0.4% except for thromboxane B_2 (6.21%), 6-keto-$PGF_{1\alpha}$ (5.4%), 13,14-dihydro $PGF_{2\alpha}$ (4.2%), and PGD_2 (3%).

Plasma PGE_2 was measured as PGE_2-methyloxime by means of an antiserum raised in goats against methyloximated PGE_2.[8, 17] Sensitivity of the assay was 0.05 nmol/L. The intraassay coefficient of variation was <10%, and interassay coefficients of variation were 10% at 14.58 nmol/L and 15.6% at 43.24 nmol/L.

Fetal and maternal temperatures were recorded when blood samples were collected. Phasic uterine artery blood flow was recorded from the electromagnetic probe; calibrations were made in vitro as described by the manufacturers (Micron Instruments Ltd.).

Calculations. Blood gases were corrected to the prevailing temperature with the use of the equations published by Lotgering et al.[18] Uterine and umbilical blood flows were calculated from the formulas described by Meschia et al.[19] The average concentration difference of 4-aminoantipyrine between uterine vein and maternal artery and umbilical vein and fetal femoral artery determined from the four pairs of samples was used to derive an average value for uterine and umbilical blood flows, respectively. 4-Aminoantipyrine was assumed to distribute between plasma and red blood cells as for antipyrine, and blood concentrations were calculated from plasma water concentrations as described by Meschia et al.[19] Loss of 4-aminoantipyrine from the fetus other than by placental transfer was assumed to be negligible.

Statistical analyses. Data are presented as mean ± standard error of the mean. Comparison between groups and within treatments have been made by means of analysis of variance. Analysis of uteroplacental blood flows was carried out by unpaired t tests.

Results

Temperature, blood gases, and plasma glucose. During the infusion of 4-aminoantipyrine into the fetus under normothermic (ambient temperature = 24° C) conditions, a small but significant rise in fetal body temperature was noted, (+0.3° C, $p < 0.05$; Table I). This increase was associated with a significant decrease in fetal arterial pH (−0.032, $p < 0.05$) and an increase in arterial P_{CO_2} (+6.8 mm Hg, $p < 0.001$), indicating that 4-aminoantipyrine infusion resulted in fetal acidemia with a respiratory component (Table I). There was no significant change in maternal body temperatures or blood gases during 4-aminoantipyrine infusion under normothermic conditions.

After 5½ hours of hyperthermia (ambient temperature = 43° C), there was a significant rise in maternal body temperature (+2.2° C, $p < 0.001$; Table II). Fetal body temperature was also significantly elevated (+2.2, $p < 0.001$) so that the fetomaternal temperature gradient was maintained. The rise in temperature resulted in maternal and fetal respiratory alkalosis (Tables II and I, respectively). There was no significant change in arterial P_{O_2} of the fetus during hyperthermia.

The infusion of 4-aminoantipyrine into the fetus during hyperthermia did not further change maternal or fetal body temperature or maternal arterial pH, P_{CO_2}, P_{O_2}, or oxygen saturation (Table II). However, infusion of 4-aminoantipyrine during hyperthermia significantly reduced fetal arterial pH and P_{O_2} (Table I). The acidemia was followed by fetal death either during, or within 5 hours of the end of the hyperthermia in four of the 11 trials. No fetal death occurred after two prolonged (3-hour) 4-aminoantipyrine infusions in the sheep kept at normal ambient temperatures (data not shown).

Plasma glucose concentrations in both maternal and fetal arterial circulations were unchanged by the in-

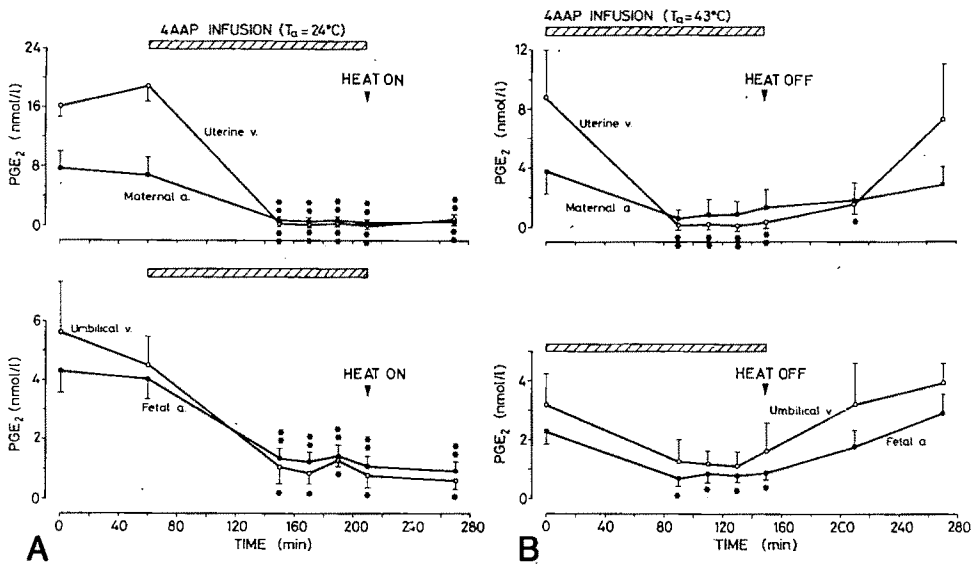


Fig. 1. Concentrations of PGE_2 in fetal and maternal circulations before and during infusion of 4-aminoantipyrine at 20 mg/min into fetal tarsal vein at ambient temperatures (T_a) of **(A)** 24° C and **(B)** 43° C. Second infusion of 4-aminoantipyrine was started 5½ hours after onset of heating.

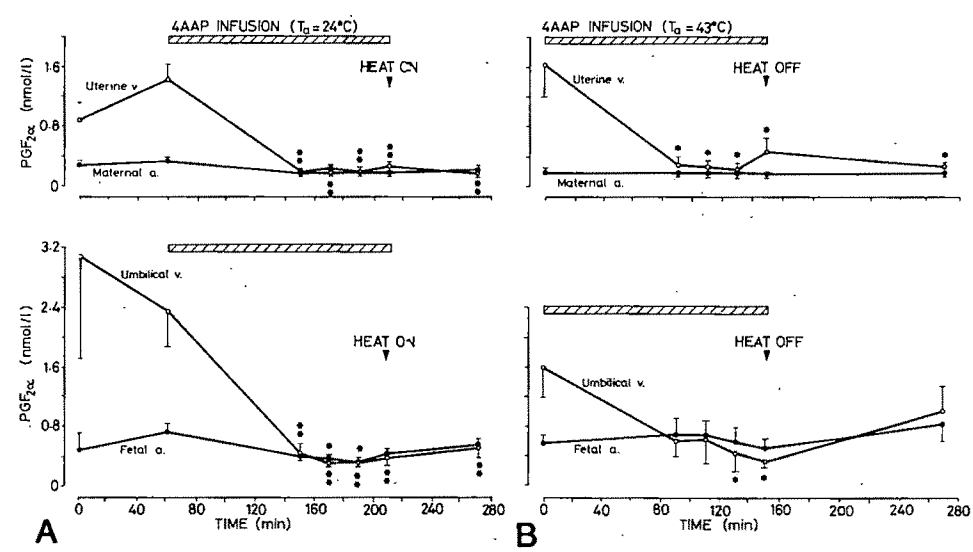


Fig. 2. Concentrations of $PGF_{2\alpha}$ in fetal and maternal circulations before and during infusion of 4-aminoantipyrine at 20 mg/min into fetal tarsal vein at ambient temperatures (T_a) of **(A)** 24° C and **(B)** 43° C. Second infusion of 4-aminoantipyrine was started 5½ hours after onset of heating.

fusion of 4-aminoantipyrine at normothermic temperatures (Tables I and II). During hyperthermia, the infusion of 4-aminoantipyrine into the fetus resulted in a significant increase in fetal plasma glucose concentrations (+0.29 mmol/L, $p < 0.05$) with no change in maternal plasma glucose concentrations.

PGE_2 and $PGF_{2\alpha}$ concentrations. After 90 minutes of 4-aminoantipyrine infusion into the fetus under normothermic or hyperthermic conditions, there was a significant reduction in the plasma concentrations of PGE_2 and $PGF_{2\alpha}$ in both the maternal and fetal circulations (Figs. 1 and 2). The inhibition of PG synthesis persisted for as long as 2 hours after the infusion. Under normothermic conditions, infusion of 4-aminoantipyrine caused a significant reduction in PGE_2 in all four vessels and in $PGF_{2\alpha}$ in the uterine and umbilical veins and fetal artery (Fig. 1). After 5½ hours of hyperthermia and immediately before the start of the second 4-aminoantipyrine infusion, plasma PGE_2 and $PGF_{2\alpha}$ concentrations had recovered to control levels. During

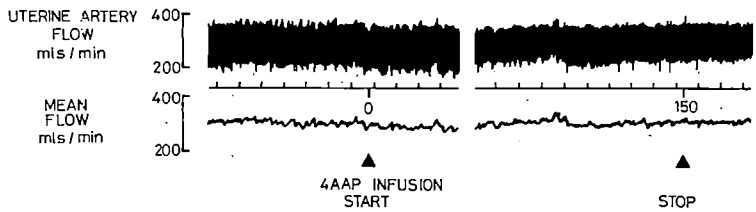


Fig. 3. Blood flow measurement in one uterine artery by electromagnetic flow probe before and 150 minutes after start of infusion of 4-aminoantipyrine at 20 mg/min into fetal tarsal vein.

Table III. Uterine and umbilical blood flows (milliliters per minute) in pregnancy sheep (125 to 140 days' gestation) before and after 8 hours of hyperthermia

Uterine blood flow			*Umbilical blood flow*		
Experiment	T_a = 24° C	T_a = 43° C	*Experiment*	T_a = 24° C	T_a = 43° C
1	1676 ± 200	1757 ± 325*	1	709 ± 20	*
2	1103 ± 100	1484 ± 251	2	1542 ± 127	629 ± 30†
3	1293 ± 204	2204 ± 581	3	585 ± 83	517 ± 60
4	1142 ± 32		4	639 ± 7	
5	1214 ± 55	1471 ± 80‡	5	727 ± 72	814 ± 143
6	1522 ± 377	1394 ± 356	6	843 ± 101	473 ± 17†
7	923 ± 46	2134 ± 653	7	709 ± 97	830 ± 84
8	1409 ± 350	2523 ± 134‡			
9	1996 ± 575	1593 ± 192			
Mean uterine blood flow (ml/min)	1364 ± 110	1820 ± 147‡	Mean umbilical blood flow (ml/min)	822 ± 124	653 ± 74

*Fetal metabolic acidemia; fetus died.
†$p < 0.001$.
‡$p < 0.05$.

hyperthermia, infusion of 4-aminoantipyrine again caused a significant decrease in the concentrations of PGE_2 and $PGF_{2\alpha}$ in uterine vein and fetal femoral artery plasma and of $PGF_{2\alpha}$ concentrations in the umbilical vein plasma (Figs. 1 and 2). PGE_2 concentrations in maternal artery and umbilical vein plasma did not decrease during this infusion in hyperthermia (in contrast to the significant decrease observed under normothermic conditions), but it should be noted that plasma concentrations in these vessels remained lower than the initial control values obtained at the beginning of the experiment. This suggests that the first infusion of 4-aminoantipyrine may have had a prolonged inhibitory effect on PG synthesis, which was still present after 5½ hours of hyperthermia.

Uterine and umbilical blood flows. The infusion of 4-aminoantipyrine into the fetal tarsal vein at normothermic temperatures (n = 3) did not change the blood flow measured in one main uterine artery by means of nonocclusive electromagnetic flow meters (Fig. 3).

The mean uterine blood flow, which was calculated from the steady-state concentration difference of 4-aminoantipyrine between uterine vein and maternal arterial plasma, increased from 1364 ± 110 ml/min before hyperthermia to 1820 ± 147 ml/min after 8 hours of hyperthermia (n = 8; $p < 0.05$, Table III). Mean umbilical blood flow, which was calculated from the steady-state concentrations of 4-aminoantipyrine in umbilical vein and fetal arterial plasma, was 822 ± 124 ml/min during normothermia (Table III). In experiments in which the fetus survived, mean umbilical blood flow was reduced, but this was not significant. In two of these experiments, umbilical blood flow was significantly lower than the control value during hyperthermia, and fetal pH fell significantly during hyperthermia (−0.168 and −0.315 pH units).

Comment

These results show that inhibition of PG synthesis with 4-aminoantipyrine during hyperthermia leads to a compromise of fetal condition, as shown by the development of fetal acidemia and fetal death. The acidemia is particularly significant when it is considered that, under this level of heat stress, fetal lambs usually develop a respiratory alkalosis as a result of maternal hyperventilation.[20-22] It is unclear if the fetal hyperglycemia observed during hyperthermia was caused by catecholamine release because of the acidemia[23] or may have been the result of diminished insulin responsive-

ness caused by the reduction in plasma PGs.[24] The reduction in umbilical blood flow in some experiments seems to be related to the metabolic changes, since reduction of PG synthesis alone does not alter placental blood flow.[11] Concurrent fetal acidemia and decreased umbilical blood flow have been noted in other experimental situations (e.g., during maternal hyperglycemia[24, 25]), but the mechanisms contributing to these changes have not been delineated. However, it is clear that the presence of 4-aminoantipyrine and the reduction of plasma PGs under hyperthermic conditions lead to a disturbance of fetal metabolism, metabolic acidemia, and decreased umbilical blood.

The fact that such extreme acidosis occurred during hyperthermia when PG synthesis was suppressed and that four of the 10 fetuses died indicates that the increase in plasma PG concentrations that normally occurs during hyperthermia[12] has an important role in enabling the fetus to adjust to the stress of hyperthermia. The cardiovascular and endocrine effects of PGE_2 in fetal sheep include the maintenance of a dilated ductus arteriosus,[26] release of cortisol,[4] and augmentation of insulin secretion during hyperglycemia.[24] Although the impact of hyperthermia on fetal metabolism is not fully understood, plasma glucose and cortisol concentrations were not consistently increased when fetal and maternal body temperatures were raised by 1° to 2° C.[12, 20] Also, the increase in fetal arterial pH appears to be almost entirely caused by the decrease in arterial P_{CO_2} and does not obscure a change in fetal metabolism that otherwise tends to decrease arterial pH. This study suggests that PGs play an important role in allowing fetal and maternal body temperatures to rise without causing a pathologic disturbance of fetal metabolism.

Morishima et al.[27] noted that in anesthetized pregnant baboons hyperthermia resulted in increased uterine activity. We have observed that this also occurs in unanesthetized pregnant sheep (unpublished observations), and this may be caused by the increase in both PGE_2 and $PGF_{2\alpha}$ concentrations during hyperthermia. The complete cessation of uterine activity found by El Badry et al.[28] and us[29] to occur during 4-aminoantipyrine infusion confirms that PGs are important for nonlabor uterine activity. It is unclear if this preterm activity has important effects on fetal homeostasis or development. Harding and Poore[30] have shown that nonlabor contractions are associated with a decrease in uterine blood flow. During hyperthermia when there would be a tendency for vascular dilatation, inhibition of PG synthesis may allow greater dilatation to occur within the myometrium, perhaps resulting in a relative shift in uterine blood flow away from the cotyledons. We have shown that hyperthermia is normally associated with the maintenance or increase in total uterine blood flow (this study and that of Andrianakis et al.[22]), but the partitioning of the flow between myometrium and placenta is not known.

Uteroplacental blood flow, as measured in this study by the steady-state method with 4-aminoantipyrine infusion, was significantly increased during hyperthermia. Although this could be considered a result of the inhibition of PG synthesis, we have also observed that uterine blood flow is increased during hyperthermia when there is an increase in maternal and fetal plasma PG concentrations.[22] These observations, together with those obtained with electromagnetic flow meters (this study and Cashner et al.[11]), question the importance of PGs in the regulation of uterine blood flow in vivo as reported by others.[10]

There was no significant change in mean umbilical blood flow during hyperthermia as measured by the steady-state technique in this study. However, there was a clear decrease in umbilical blood flow in the two experiments in which the arterial pH decreased. In four other trials the fetuses became severely acidotic and died soon after the start of the 4-aminoantipyrine infusion during heat. That these changes are the result of the interaction of 4-aminoantipyrine infusion is shown by the observation that heat alone results in fetal alkalosis without fetal loss.[20-22] Further work is required to determine whether these effects of 4-aminoantipyrine are caused by the inhibition of PG synthesis, resultant fetal metabolic acidemia and hyperglycemia, or other pharmacologic effects of 4-aminoantipyrine.

These observations suggest PG synthetase inhibitors should be used with caution during pregnancy in humans. Although it is apparent that short-term inhibition of PG synthesis does not normally affect fetal condition adversely (although a small fall in fetal arterial pH and rise in temperature were noted), the fetus is no longer able to adapt if a rise in body temperature is imposed at this time. If a pregnant woman uses aspirin or other PG synthetase inhibitors and then experience a rise in body temperature because of one of a number of factors (e.g., exercise, hot environment, or fever), the ability of the fetus to cope with this challenge might be compromised.

We thank Julie Norman for help with the surgery and Lynne Hepburn for typing the manuscript.

REFERENCES

1. Challis JRG, Dilley SR, Robinson JS, Thorburn GD. Prostaglandins in the circulation of fetal lamb. Prostaglandins 1976;11:1040.
2. Mitchell MD, Brunt J, Clover L, Walker DW. Prostaglandins in the umbilical and uterine circulations during late pregnancy in the ewe. J Reprod Fertil 1980;58:283.
3. Novy MJ, Piasecki G, Jackson BT. Effect of prostaglandins E_2 and $F_{2\alpha}$ on umbilical blood flow and fetal hemodynamics. Prostaglandins 1974;5:543.
4. Louis TM, Challis JRG, Robinson JS, Thorburn GD.

Rapid increase of fetal corticosteroids after prostaglandin E_2. Nature 1976;264:797.
5. Olson DM, Lye SJ, Skinner K, Challis JRG. Prostanoid concentrations in maternal and fetal plasma and amniotic fluid and intrauterine tissue prostanoid output in relation to myometrial contractility during the onset of adrenocorticotrophin induced preterm labor in sheep. Endocrinology 1985;116:389.
6. Challis JRG, Hart I, Louis TM, Mitchell MD, Jenkin G. Prostaglandins in the sheep fetus: Implications for fetal function. In: Coceani F, Olley PM, eds. Advances in prostaglandin and thromboxane research. Vol 4. New York: Raven Press, 1978:115-32.
7. Fowden AL, Silver M. The effect of the nutritional state on uterine prostaglandin F metabolite concentrations in the pregnant ewe during late gestation. Q J Exp Physiol 1983;68:337.
8. Fowden AL, Harding R, Ralph MM, Thorburn GD. The nutritional regulation of prostaglandin E in concentrations in the fetus and pregnant ewe during late gestation. J Physiol 1987;394:1.
9. Silver M, Fowden AL. Uterine prostaglandin F metabolite production in relation to glucose availability in late pregnancy: the possible influence of diet and time of delivery in the mare. J Reprod Fertil 1982;32(suppl):511.
10. Rankin JHG. Role for prostaglandins in the regulation of the placental blood flows. Prostaglandins 1976;11:343.
11. Cashner KA, Skillman CA, Brockman D, Mack C, Clark KE. Effect of antipyrine on prostaglandin levels and uterine and umbilical blood flow. AM J OBSTET GYNECOL 1987;155:1305.
12. Andrianakis P, Walker DW, Ralph MM, Thorburn GD. Effect of hyperthermia on prostaglandin concentrations in the umbilical and uterine circulations. In: 14th Proceedings, Society for Study of Fetal Physiology. Groningen, The Netherlands, 1987:C19.
13. Alexander G. Birth weight of lambs: influences and consequences. Size at birth. Ciba Found Symp 1974:215-46.
14. Mendelsohn D, Levin NW. A colorimetric method for the estimation of antipyrine in plasma or serum. S Afr J Med Sci 1960;25:13.
15. Huckabee WE. Uterine blood flow and oxygen consumption in the unrestrained pregnant ewe. Q J Exp Physiol 1972;57:12.
16. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann Clin Biochem 1969;6:24.
17. Kelly RW, Deam S, Cameron MJ, Seamark RF. Measurement by radioimmunoassay of prostaglandins as their methyl oximes. Prostaglandins Leukot Essent Fatty Acids 1986;24:1.
18. Lotgering FK, Gilbert RD, Longo LD. Exercise responses in pregnant sheep: oxygen consumption uterine blood flow and blood volume. J Appl Physiol 1983;55:834.
19. Meschia G, Cotter JR, Makowski EL, Barron DH. Simultaneous measurement of uterine and umbilical blood flows and oxygen uptakes. Q J Exp Physiol 1968;52:1.
20. Davies AN, Walker DW, McMillen IC, Thorburn GD. The acute effects of maternal hyperthermia on the foetal lamb. In: Lindsay DR, Pearce DT, eds. Reproduction in sheep. Canberra, Australia: Australian Academy of Science and Australian Wool Corporation, 1984:153-7.
21. Oakes GF, Walker AM, Ehrenkranz RA, Cefalo RC, Chez RA. Uteroplacental blood flow during hyperthermia with and without respiratory alkalosis. J Appl Physiol 1976; 41:197.
22. Andrianakis P, Walker DW, Thorburn GD. Effects of hyperthermia on uterine and umbilical blood flows in sheep [Abstract]. Aust Perinatol Soc 1987:26.
23. Jones CT, Robinson RO. Plasma catecholamines in fetal and adult sheep. J Physiol 1975;248:15.
24. Philipps AF, Matty PJ, Porte PJ, Raye JR. Inhibition of glucose-induced insulin secretion by indomethacin and sodium salicylate in the fetal lamb. AM J OBSTET GYNECOL 1984;148:481-7.
25. Crandell SS, Fisher DJ, Morriss FH. Effects of ovine maternal hyperglycemia on fetal regional blood flows and metabolism. Am J Physiol 1985;249:E454.
26. Coceani F, Olley PM, Bodach E. Lamb ductus arteriosus: effect of prostaglandin synthesis inhibitors on the muscle tone and the response to prostaglandin E_2. Prostaglandins 1975;9:299.
27. Morishima HO, Glaser B, Niemann WH, James LS. Increased uterine activity and fetal deterioration during maternal hyperthermia. AM J OBSTET GYNECOL 1975;121: 531.
28. El Badry A, Figueroa JP, Poore ER, et al. Effect of fetal intravascular 4-aminoantipyrine infusions on myometrial activity (contractures) at 125 to 143 days' gestation in the pregnant sheep. AM J OBSTET GYNECOL 1984;150:474-9.
29. Andrianakis P, Walker DW, Ralph MM, Thorburn GD. Effect of 4-aminoantipyrine on prostaglandin concentrations in uterine and umbilical circulations of pregnant sheep [Abstract]. Endocrinol Soc Aust 1987:115.
30. Harding R, Poore ER. The effects of myometrial activity on fetal thoracic dimensions and uterine blood flow during late gestation. Biol Neonate 1984;45:244.

Progesterone antagonist lilopristone: A potent abortifacient in the common marmoset

Chander P. Puri, PhD, Rohini K. Patil, BSc, Sanjiv D. Kholkute, PhD, Walter A. G. Elger, MD, and Xavier R. Swamy, BA

Bombay, India, and Berlin, Federal Republic of Germany

The effects of a progesterone antagonist ZK 98.734 (lilopristone) on implantation, early pregnancy, and midpregnancy were studied in the common marmoset, *Callithrix jacchus jacchus*. Treatment (5 mg/day intramuscularly for 3 consecutive days) on day 10 (n = 8) after the midcycle peak in estradiol levels in mated animals induced a premature drop in plasma progesterone levels and shortened the ovarian cycle length. Treatment on day 20 (n = 5) or day 40 (n = 5) induced a drop in progesterone levels and decidual collapse. In three animals treated on day 40, vaginal bleeding was observed within 46 hours of the initiation of treatment. Treatment on day 80 resulted in expulsion of the fetuses with a mean induction abortion interval of 39 hours (range, 20 to 48 hours). The progesterone antagonistic effects of ZK 98.734 could be a result of the decrease in progesterone synthesis by the corpus luteum and/or placenta in addition to the interference with the progesterone binding to its cellular receptors in the target organ. Our study suggests that ZK 98.734 has potential for fertility regulation. Clinical trials for postcoital contraception, induction of menstruation, and early abortifacient effects are warranted. (AM J OBSTET GYNECOL 1989;161:248-53.)

Key words: Progesterone antagonist, lilopristone, abortifacient, progesterone, common marmosets

Progesterone is indispensable for the nidation of fertilized ovum and maintenance of pregnancy. The blocking of progesterone biosynthesis[1-3] or inhibiting its action at the target organ[4, 5] interferes with these physiologic events. Recently three progesterone antagonists (RU 486, mifepristone; ZK 98.299, onapristone, and ZK 98.734, lilopristone) have been identified that compete for hormone binding at the target site and thus prohibit progesterone from eliciting hormonal effects. These compounds are being evaluated as postcoital contraceptive, implantation inhibitor, menstrual inducer, or early abortifacient.

RU 486 is a progesterone antagonist that blocks progesterone synthesis[6, 7] and intercepts progesterone action at the cellular level.[4] In nonpregnant monkeys[8-10] and women[6, 11] it is an effective menstrual inducer. Treatment with RU 486 induces vaginal bleeding in pregnant women in the majority of cases; however, the expulsion of conceptus is often incomplete.[12, 13] Treatment within 10 days of missed periods induces complete abortion in about 80% of the women and the efficacy is further reduced (average, 63%) when the treatment is delayed.[14] In some cases the incidence of vaginal bleeding is heavy and requires emergency curettage.[15] This clinical efficacy is lower than that achieved by prostaglandin analogs.[16] However, when RU 486 is used in combination with prostaglandin analogs, the abortifacient efficacy is increased to 95%.[17, 18]

ZK 98.299 also has high affinity for progesterone receptors in the endometrium of bonnet monkeys[5] and humans.[19] Its administration during the midluteal phase decreases serum progesterone levels and induces menstruation in the bonnet monkeys.[20] Its administration during early pregnancy induces vaginal bleeding in all the animals but the conceptus is expelled only in about 60% of the animals (unpublished observations). In guinea pigs, priming with ZK 98.299 markedly enhances uterine prostaglandin sensitivity.[21] ZK 98.299 has not been clinically evaluated; however, it appears that it may also require a supplementary oxytocic agent for complete abortion, as does RU 486.

Structurally ZK 98.734 more closely resembles RU 486 than ZK 98.299.[22] Its antiglucocorticoid activity is less than the other two compounds.[22] It is a more potent abortifacient than ZK 98.299 or RU 486 in guinea pigs.[21] In the nonpregnant common marmoset, administration of ZK 98.734 during the midluteal phase induces a drop in plasma progesterone levels and shortens the ovarian cycle length.[23] Similarly, its administration during early pregnancy induces resorption. ZK 98.734 also has a high affinity for progesterone receptors.[23] Therefore it is possible that ZK 98.734 would be

From the Institute for Research in Reproduction (ICMR), Parel, Bombay and the Department of Experimental Gynecology and Fertility Control, Schering Ag, Berlin.

Received for publication October 17, 1988; revised February 3, 1989; accepted February 23, 1989.

Reprint requests: Chander P. Puri, PhD, Institute for Research in Reproduction (ICMR), Parel, Bombay-400012, India.

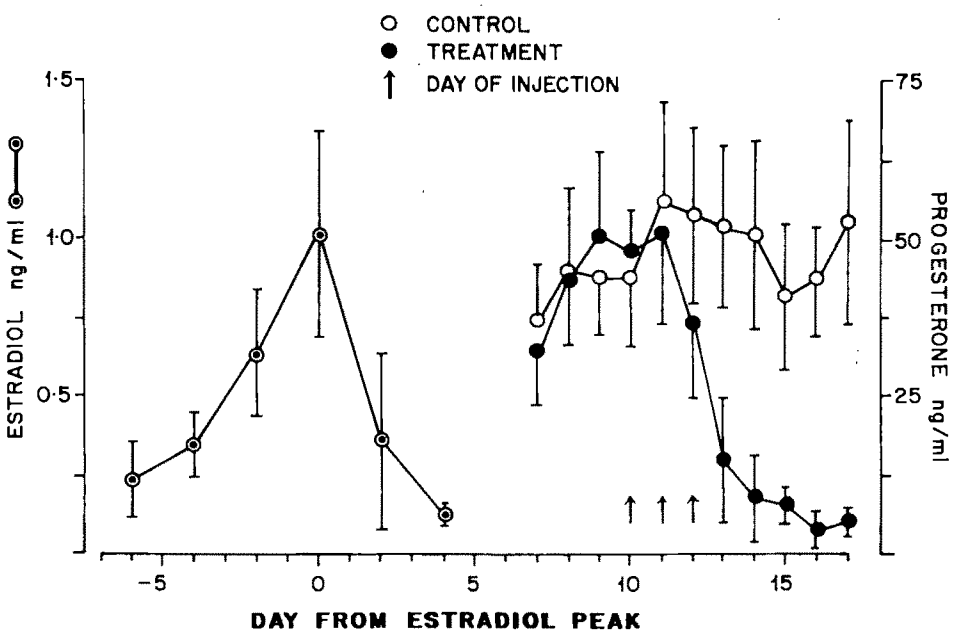


Fig. 1. Effect of ZK 98.734 on luteal phase progesterone levels. Occurrence of midcycle peak in estradiol levels followed by rise in progesterone levels indicates that these animals had ovulated. Progesterone levels (mean ± SD) of five animals in group 1 (control) that became pregnant and eight animals treated with ZK 98.734 (group 2) are shown. Progesterone levels on day 13 were significantly less in group 2 than in group 1 animals.

a more potent abortifacient than the other two compounds.

This study was undertaken to evaluate the efficacy of ZK 98.734 in pregnancy termination in the common marmoset, *Callithrix jacchus jacchus*. ZK 98.734 was administered at four stages of gestation: (1) around the time of implantation, (2) immediately after the ovarian cycle length is extended, which corresponds to missed periods in menstruating nonhuman primate species, (3) on day 40 and, (4) on day 80 after the midcycle peak in estradiol levels (i.e., before and after the luteoplacental shift in progesterone synthesis). Plasma levels of progesterone were estimated to determine the effects of ZK 98.734 on corpus luteum function and on placental progesterone production. The reproductive endocrine profile of gonadal hormones has been extensively studied in this species of nonhuman primates.[24, 25] Like other species of nonhuman primates, ovulation occurs in the common marmoset about 24 hours after the luteinizing hormone surge or 36 hours after the midcycle peak in estradiol levels.[26] Implantation occurs on approximately day 9 to 10 after ovulation.[27] A corpus luteum is necessary for pregnancy sustenance for nearly 48 days after ovulation.[28]

Material and methods

Animals. Common marmosets weighing between 350 and 450 gm were used. The animals were bred at the Primate Facility of the Institute and were housed in rooms maintained at 26° C and controlled photoperiod of 14 hours of light and 10 hours of darkness. They were fed a diet of bread, fresh fruits, vegetables, milk, and water. The details of the management and husbandry have been previously published.[29]

Antiprogestin. ZK 98.734 (11β-(4-dimethylaminophenyl)-17β-hydroxy-17α-(3-hydroxy-prop-1(Z)-enyl-4,9(10)-estradien-3-one) was dissolved in benzyl benzoate at 90° C and was further diluted with castor oil (1:4). A dose of 5 mg in 0.2 ml of the vehicle was injected intramuscularly. Only vehicle was injected in the control group.

Administration of ZK 98.734 or vehicle. ZK 98.734 or vehicle was injected into five groups of animals. The treatment was initiated with respect to the day of midcycle peak in estradiol levels (day 0) as follows: group 1 (n = 8), vehicle from day 10 to day 12; group 2 (n = 8) ZK 98.734 from day 10 to day 12; group 3 (n = 5) ZK 98.734 from day 20 to day 22; group 4 (n = 5) ZK 98.734 from day 40 to day 42, and group 5 (n = 7) ZK 98.734 from day 80 to day 82, unless the animal aborted before the third injection.

Selection of pregnant animals. Common marmosets show postpartum ovulation and in our colony approximately 80% of the animals become pregnant in the first ovarian cycle after delivery.[29] Therefore, marmosets were selected immediately after delivery. The day of parturition was considered day 1. The females were allowed to continue in family groups, with an adult male and their young ones. On postpartum day 15, the males were removed from the cages. Blood samples were col-

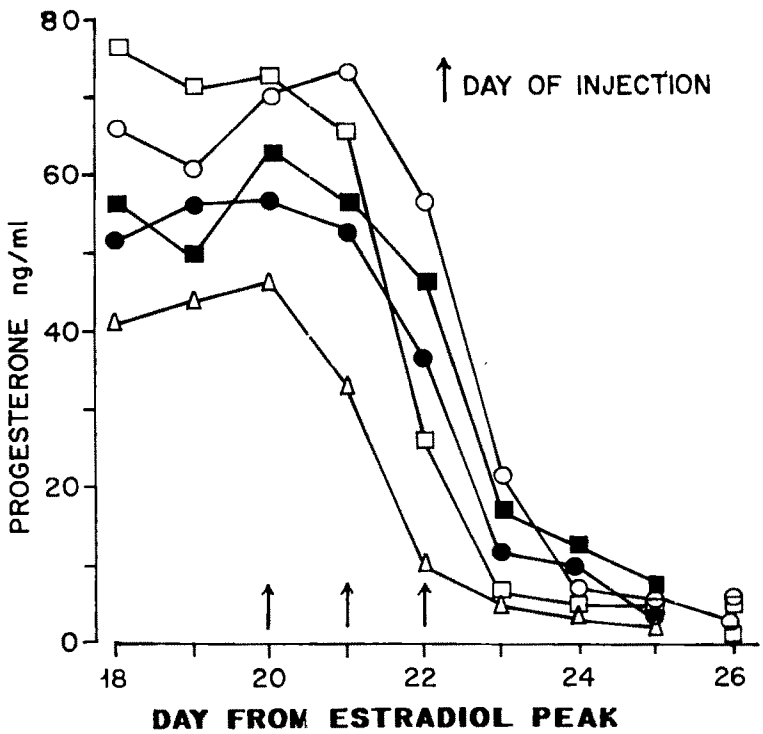


Fig. 2. Effect of ZK 98.734 on pregnancy. Progesterone levels >40 ng/ml on day 20 are indicative of fertile mating. Administration of ZK 98.734 on day 20 to day 22 caused significant decrease in plasma progesterone levels. Pregnancy was terminated in all animals.

lected on alternate days until day 15. Estradiol levels were analyzed immediately to determine the day of midcycle peak in estradiol levels. In groups 1 and 2, in which the treatment was initiated on day 10 after estradiol peak, pregnancy was not confirmed at the time of treatment. Results were analyzed with regard to the basis of hormone levels and the outcome of mating between the control and treatment groups. In the other groups, blood sampling was again started on day 15 and samples collected once daily until day 20. Plasma progesterone levels were immediately estimated and the animals in which progesterone levels on day 20 were >40 ng/ml were considered pregnant.[29] In our colony, marmosets have an ovarian cycle length of 26.6 ± 2.4 days (mean ± SD, n = 14), the midcycle peak in estradiol levels occurs on day 9.2 ± 0.7, and the luteal phase is of 17.4 ± 1.3 days. The pregnant animals were distributed in three groups (groups 3 to 5).

Collection of blood samples. Blood samples (0.3 ml) were collected from the femoral vein once daily for 2 days before and 7 days after the treatment was initiated. Plasma was separated and kept frozen below −20° C until further processed.

Estimation of hormone levels. Plasma levels of estradiol and progesterone were analyzed by specific radioimmunoassay methods. The reagents were provided by the World Health Organization under the Programme of Matched Assay Reagents for the Radioimmunoassay of Hormones in Reproductive Physiology.[30] Estradiol levels were measured with an antiserum raised against estradiol-3CMO-bovine serum albumin (1:210,000) and 2,4,6,7-^{3}H-estradiol (99 Ci/mmol). Mouse monoclonal antibody raised against progesterone-3-CMO-BSA (1:210,000) and 1,2,6,7-^{3}H-progesterone (89 Ci/mmol) were used for progesterone assay. Serum aliquots of 50 μl and 10 μl in duplicate were used for the estimation of estradiol and progesterone levels, respectively. The sensitivities of the assay methods were 5 pg/tube (100 pg/ml) for estradiol and 15 pg/tube (1.5 ng/ml) for progesterone. The coefficients of intraassay and interassay variation (n = 13) were 9.5% and 13.4%, respectively, for estradiol and 8.4% and 12.6%, respectively, for progesterone.

Statistical analysis. Radioimmunoassay data were analyzed with a logit-log transformation of the data on an Apple IIe computer. The differences in progesterone levels between control and treated animals were evaluated by Student's t test.

Results

Effect on pregnancy.

Groups 1 and 2. In group 1 (control, n = 8) five animals became pregnant, thereby indicating a pregnancy rate of 62.5%. The pregnancy was confirmed by the rising levels of progesterone beyond day 17 and subsequent increase in uterine size. Administration of vehicle on day 10 to day 12 had no effect on the luteal function and the pregnancy.

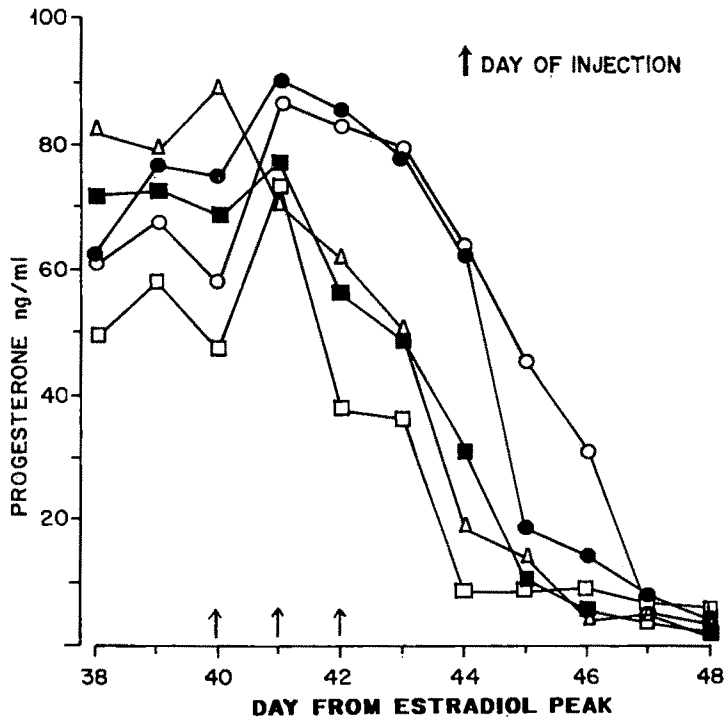


Fig. 3. Administration of ZK 98.734 on day 40 to day 42 caused decrease in plasma progesterone levels. Abdominal palpation was suggestive of resorption of conceptus.

In ZK 98.734-treated animals (group 2), the occurrence of a midcycle peak in estradiol levels followed by a rise in plasma progesterone levels indicated that these animals had ovulated. Administration of ZK 98.734 caused a premature decrease in plasma progesterone levels (Fig. 1). Progesterone levels on day 13 were significantly less ($p < 0.001$) in treated animals compared with the control group. The luteal phase was curtailed and none of these animals continued with pregnancy.

Groups 3 and 4. The administration of ZK 98.734 on day 20 (group 3) or day 40 (group 4) caused a significant decrease in plasma progesterone levels (Figs. 2 and 3). Vaginal bleeding was observed between 32 and 46 hours after the initiation of treatment in three animals treated on day 40. The conceptus was not expelled. Abdominal palpation of the animals in group 4 was suggestive of resorption of conceptus. Pregnancy was terminated in all animals in both the groups.

Group 5. Administration of ZK 98.734 on day 80 induced abortion in all the animals (Table I). Fetuses were expelled between 20 and 48 hours of the initiation of treatment. The mean plasma progesterone levels immediately before the initiation of treatment were 64 ± 11 ng/ml; 72 hours later the levels were 7 ± 3 ng/ml.

Comment

Our study demonstrates that ZK 98.734 is a potent abortifacient in the common marmoset. Its administration at approximately the time of implantation in mated animals inhibited nidation or disrupted the implanted ovum. Because blood samples were collected on alternate days, the day of estradiol peak could be ± 1 day. Therefore it is possible that treatment was initiated just before implantation in some animals. A significant decrease in peripheral plasma progesterone levels and ovarian cycle length were suggestive of pregnancy termination. The posttreatment cycles were ovulatory and of normal duration. Similarly, when the treatment was initiated on day 20 or day 40, a decrease in progesterone levels was observed and the conceptus was resorbed. Conversely, when the treatment was initiated after the luteoplacental shift in progesterone synthesis and when embryonic development was near completion,[31] the fetuses were expelled. Therefore this study suggests that ZK 98.734 has the potential for use as a postcoital method of contraception, as a menstrual inducer, and in the termination of pregnancy.

The abortifacient effects of ZK 98.734 in the common marmoset appear to be similar to that in guinea pigs.[21] In guinea pigs, treatment on postconception day 4 prevents nidation, treatment on day 8 induces decidual collapse and bleeding, and treatment on day 43 induces expulsion of the conceptus. Whereas ZK 98.734 and RU 486 have comparable antinidatory potency, ZK 98.734 induces expulsion more rapidly and at higher rate compared with RU 486.[21]

ZK 98.734 and progesterone are mutually competi-

Table I. Effect of ZK 98.734 on pregnancy in common marmoset

Animal No.	*Total dose (mg)*	*Outcome*	*Induction-abortion time (hr)*	*No. of foetuses*	*Mean weight per foetus (gm)*
147	5	Aborted	20	2	1.7
149	10	Aborted	45	1	1.5
151	10	Aborted	48	1	4.0
107	10	Aborted	45	1	2.1
131	10	Aborted	27	1	1.4
159	10	Aborted	47	1	2.2
111	10	Aborted	42	3	1.5

Treatment (5 mg/day) was initiated on day 80.

tive for binding to progesterone receptors in the uterine cytosol of common marmosets[23] and bonnet monkeys.[5] Moreover, ZK 98.734 also exchanges the progesterone-labeled receptor sites in the myometrium of bonnet monkeys.[5] It is therefore possible that the progesterone antagonistic effects of ZK 98.734 could be mediated by interfering with the binding of progesterone to its cellular receptors. However, a significant decrease in plasma progesterone levels observed in this study suggests that the antinidatory or abortifacient effects could also be a result of the reduced progesterone production by the corpora lutea and the placenta. Whether the decrease in progesterone production is the result of direct action of ZK 98.734 on the ovary or is the result of withdrawal of gonadotropin support is not known. In the rat, administration of ZK 98.734 on postcoital day 5 induces structural changes in the corpora lutea that are characteristic of luteolysis; these effects are similar to those observed with RU 486.[32] RU 486 decreases the level of luteal 3β-hydroxysteroid dehydrogenase, which is followed by a decrease in serum progesterone levels in the rat.[33] Similarly, under in vitro conditions RU 486 decreases the activity of 3β-hydroxysteroid dehydrogenase and progesterone production by the human granulosa cells.[7] In the rat ovary, RU 486 binds to progesterone and glucocorticoid receptors,[34] suggesting the possible involvement of progesterone antagonists in progesterone receptor-mediated effects on the ovary. Similarly, the abortifacient effects of ZK 98.734 or structurally related compounds, when administered after the luteoplacental shift in progesterone synthesis, could be a result of a decrease in progestesrone synthesis by the placenta. RU 486 impairs the production of progesterone, human chorionic gonadotropin, and human placental lactogen by the syncytiotrophoblasts in culture.[35] The effects of RU 486 on human chorionic gonadotropin and human placental lactogen are reversed by exogenous progesterone and not with cortisol. RU 486 is rapidly absorbed from the maternal circulation to the fetus through the placenta of rhesus monkeys.[36] Therefore these observations support the possibility of direct action of RU 486 and structurally related compounds on the inhibition of progesterone action in the trophoblast. However, further studies are needed to elucidate the mechanism of action of progesterone antagonists.

Epostane, an inhibitor of progesterone synthesis, is also a potent abortifacient in rhesus monkeys[37] and in humans.[2, 3] Its abortifacient efficacy is quite comparable to that of RU 486.[38] However, the termination of pregnancy with epostane requires repeated administration of the compound for several days and the incidence of side effects such as nausea and vomiting is very common.[2] Moreover, it has not yet been conclusively resolved in primates whether epostane is a selective inhibitor of ovarian and placental progesterone biosynthesis or whether it also affects progesterone production by the adrenal glands, an effect that would not be desirable in view of the role of progesterone as an intermediary in glucocorticoid biosynthesis. Conversely, the use of progesterone receptor-blocking agents would be preferable because of their selective and specific mode of action. Moreover, progesterone receptors are almost exclusively found in reproductive organs and are sensitive to the hormone only at appropriate times.[39] Since ZK 98.734 is a very potent abortifacient in the common marmosets, it can be clinically evaluated as a postcoital method of contraception, "once-a-month pill," or for the termination of early pregnancy.

We thank the World Health Organization for providing reagents for the estimation of steroid hormones. We also thank S. Kadam for assistance with the art work.

REFERENCES

1. Webster MA, Phipps SL, Gillmer MDG. Interruption of first trimester human pregnancy following epostane therapy. Effect of prostaglandin E pessaries. Br J Obstet Gynaecol 1985;92:963-8.
2. Birgerson L, Olund A, Odlind V, Somell C. Termination of early human pregnancy with epostane. Contraception 1987;35:111-20.
3. Selinger M, MacKenzie IZ, Gillmer MD, Phipps SL, Ferguson J. The effect of epostane, a 3β-hydroxy steroid dehydrogenase inhibitor, on maternal and fetal steroid

levels during mid-gestation. Br J Obstet Gynaecol 1988; 95:596-601.
4. Gravanis A, Schaison G, George M, et al. Endometrial and pituitary responses to the steroidal antiprogestin RU 486 in post-menopausal women. J Clin Endocrinol Metab 1985;60:156-63.
5. Pongubala JMR, Elger WAG, Puri CP. Relative binding affinity of antiprogestins ZK 98.299 and ZK 98.734 for progesterone receptors in the endometrium and myometrium of bonnet monkeys. J Recept Res 1987;7:903-20.
6. Schaison G, George M, Le Strat N, Reiberg A, Baulieu EE. Effects of the antiprogesterone steroid RU 486 during mid luteal phase in normal women. J Clin Endocrinol Metab 1985;61:484-9.
7. Dimattina M, Albertson B, Seyler DE, Lorianx DL, Falk RJ. Effect of the antiprogestin RU 486 on progesterone production by cultured human granulosa cells: inhibition of the ovarian 3β-hydroxy steroid dehydrogenase. Contraception 1986;34:199-205.
8. Healy DL, Baulieu EE, Hodgen GD. Induction of menstruation by an antiprogesterone steroid (RU 486) in primates: site of action, dose-response relationships and hormonal effects. Fertil Steril 1983;40:253-7.
9. Asch RH, Rojas FJ. The effects of RU 486 on the luteal phase of the rhesus monkey. J Steroid Biochem 1985; 22:227-30.
10. Shortle B, Dyrenfurth I, Ferin M. Effects of an antiprogesterone agent (RU 486) on the menstrual cycle of the rhesus monkey. J Clin Endocrinol Metab 1985;60:731-5.
11. Herrmann W, Wyss R, Riondel A, et al. The effects of an antiprogesterone steroid on women: interception of the menstrual cycle and of early pregnancy. CR Acad Sci [D] Paris 1982;294:933-8.
12. Kovacs L, Sas M, Resch BA, et al. Termination of very early pregnancy by RU 486—an antiprogestational compound. Contraception 1984;29:399-410.
13. Cameron IT, Michie AF, Baird DT. Therapeutic abortion in early pregnancy with antiprogestogen RU 486 alone or in combination with prostaglandin analogue (Gemeprost). Contraception 1986;34:459-68.
14. Van Look PFA. Post-ovulatory method of fertility regulation. In: Deczfalusy E, Griffin PD, Khanna J, eds. Research in human reproduction. Geneva: World Health Organization, 1987:153-76.
15. Shoupe D, Mishell DR Jr, Brenner PF, Spitz IM. Pregnancy termination with a high and medium dosage regimen of RU 486. Contraception 1986;33:455-61.
16. WHO Task Force on Post-ovulatory Methods for Fertility Regulation. Menstrual regulation by intramuscular injections of 16-phenoxy tetranor PGE_2methyl sulfonylamide or vacuum aspiration. A randomized multicentre study. Br J Obstet Gynaecol 1987;94:949-56.
17. Bygdeman M, Swahn ML. Progesterone receptor blockage. Effect on uterine contractility and early pregnancy. Contraception 1985;32:45-51.
18. Rodger MW, Baird DT. Induction of therapeutic abortion in early pregnancy with mifepristone in combination with prostaglandin pessary. Lancet 1987;2:1415-8.
19. Puri CP, Pongubala JMR, Jayaraman S, Hinduja IN, Elger WG. Antifertility action of antiprogestins. Gottingen, FDR Germany: XI Congress of the International Primatological Society, 1986:191.
20. Puri CP, Elger WG, Pongubala JMR. Induction of menstruation by antiprogesterone ZK 98.299 in cycling bonnet monkeys. Contraception 1987;35:409-21.
21. Elger W, Fahnrich M, Beier S, Qing SS, Chwalisz K. Endometrial and myometrial effects of progesterone antagonists in pregnant guinea pigs. Am J Obstet Gynecol 1987;157:1065-74.
22. Neef G, Beier S, Elger W, Henderson D, Wiechert R. New steroids with antiprogestational and antiglucocorticoid activities. Steroids 1984;44:349-72.
23. Puri CP, Kholkute SD, Pongubala JMR, Patil RK, Elger WAG, Jayaraman S. Effect of antiprogestin ZK 98.734 on the ovarian cycle, early pregnancy, and on its binding to progesterone receptors in the myometrium of marmoset, *Callithrix jacchus*. Biol Reprod 1988;38:528-35.
24. Harding RD, Hulme MJ, Lunn SF, Henderson C, Aitken RJ. Plasma progesterone levels throughout the ovarian cycle of the common marmoset *(Callithrix jacchus)*. J Med Primatol 1982;11:43-9.
25. Kholkute SD, Puri CP. Reproductive endocrine profile of the common marmoset, *Callithrix jacchus*. In: Puri CP, and Anand Kumar TC, eds. Proceedings of the seventeenth annual conference of the Endocrine Society of India. Bombay: 1987,66-76.
26. Heger HW, Neubert D. Timing of ovulation and implantation in the common marmoset, *Callithrix jacchus*, by monitoring of estrogens and 6-hydroxypregnenolone in urine. Arch Toxicol 1983;54:41-52.
27. Hearn JP. Endocrinology and timing of implantation in the marmoset monkey *(Callithrix jacchus)*. Prog Reprod Biol 1980;7:262-72.
28. Hearn JP. The endocrinology of reproduction in the common marmoset, *Callithrix jacchus*. In: Kleiman DG, ed. The biology and conservation of marmosets. Washington, DC: Smithsonian Institute, 1978:163-71.
29. Kholkute SD. Plasma estradiol and progesterone levels during the post-partum ovulation and early pregnancy in the common marmoset *(Callithrix jacchus)*. Primates 1984; 25:538-44.
30. Sufe SB, Donaldson A, Jeffcoate SL. WHO method manual in programme for the provision of matched assay reagents for the radioimmunoassay of hormones in reproductive physiology. Geneva: World Health Organization, 1987.
31. Phillips IR. The embryology of the common marmoset *Callithrix jacchus*. Adv Anat Embryol Cell Biol 1976;52:1-47.
32. Singh G, Singh MM, Maitra SC, et al. Luteolytic action of two antiprogestational agents (RU 38486 and ZK 98734) in the rat. J Reprod Fertil 1988;83:78-83.
33. Kawano T, Okamura H, Tajima C, Fukuma K, Katabuchi H. Effect of RU 486 on luteal function in the early pregnant rat. J Reprod Fertil 1988;83:279-85.
34. Schreiber JR, Hsueh AJW, Baulieu EE. Binding of the antiprogestin RU 486 to rat ovary steroid receptors. Contraception 1983;28:77-85.
35. Das C, Catt KJ. Antifertility actions of the progesterone antagonist RU 486 include direct inhibition of placental hormone secretion. Lancet 1987;2:599-601.
36. Wolf JP, Chillik CF, Itskovitz J, et al. Transplancental passage of a progesterone antagonist in monkeys. Am J Obstet Gynecol 1988;159:238-42.
37. Creange JE, Anzalone AJ, Potts GO, Schane HP. WIN 32, 729, a new, potent interceptive agent in rats and rhesus monkeys. Contraception 1981;24:289-99.
38. Birgerson L, Odlind V. Early pregnancy termination with antiprogestins: a comparative clinical study of RU 486 given in two dose regimens and epostane. Fertil Steril 1987;48:565-70.
39. Baulieu EE. RU 486: an antiprogestin steroid with contragestive activity in women. In: Baulieu EE, Segal SJ, eds. The antiprogestin steroid RU 486 and human fertility control. London: Plenum Press, 1985:1-25.

Fetal death: Diagnosis and management

To the Editors:

I read the article (Fetal death: Diagnosis and management. AM J OBSTET GYNECOL 1987;157:583-9) by Roy M. Pitkin with great interest and admiration. The purpose of the article was to review the causes and diagnoses of fetal death and to discuss several laboratory tests that offer a much higher yield in the identification of the causes of unexplained stillbirths Our protocol[1] for evaluation of pre- and perinatal death is similar to that proposed by J. C. Carey[2] and others. We use a mnemonic to remember possible causes of unexplained fetal death: MIMAMET (which in Hebrew literally means "from what died?") M = malformations; I = infections; M = muscular; A = autoimmune; M = metabolic; E = endocrine; and T = transfusion.

Despite their rarity, endocrine causes of fetal death (hyperthyroidism and hyperparathyroidism) should be taken in account. The perinatal death rate was 50% in cases of thyrotoxicosis diagnosed in utero[3] and fetal loss has been reported to occur most often late in the pregnancies of untreated cases. Also, overlooked and untreated primary hyperparathyroidism has deleterious effects on both mother and fetus.[4] Many (20%) of the patients with primary hyperparathyroidism were completely without evident symptoms during their pregnancy.[4] For these reasons it appears that calcium, thyroxine, and thyroid-stimulating hormone tests should be routinely performed for every unexplained fetal death.

We also believe that the search for infectious causes of fetal death, such as listeriosis, should be extended to *Ureaplasma, Mycoplasma,* anaerobes, and especially to viral infections (herpes, cytomegalovirus). Benirschke and Robb[5] have demonstrated a high frequency of latent endometrial herpes simplex virus type 2 infection, which produces a true intrauterine herpes simplex virus infection in the embryo or fetus and was an important cause of fetal death.

We continue to take into account muscular causes of fetal death, although reports suggest that myasthenia gravis probably does not increase a pregnant patient's risk of intrauterine death.[6]

Despite these additions, Pitkin's review is very interesting and provides useful and most valuable information for current clinical practice.

Paul Merlob, MD

Neonatal Department
Beilinson Medical Center and
Sackler School of Medicine
University of Tel Aviv
49 100 Petah Tikva, Israel

REFERENCES

1. Lerman-Sagie T, Merlob P. Evaluation of perinatal death—suggestion for a protocol and review of the literature. Harefuah 1987;113:246.
2. Carey JC. Diagnostic evaluation of the stillborn infant. Clin Obstet Gynecol 1987;30:342.
3. Pekonen F, Teramo K, Makinen T, et al. Prenatal diagnosis and treatment of fetal thyrotoxicosis. AM J OBSTET GYNECOL 1984;150:893.
4. Kristoffersson A, Dahlgren S, Lithner F, et al. Primary hyperparathyroidism in pregnancy. Surgery 1985;97:326.
5. Benirschke K, Robb YA. Infectious causes of fetal death. Clin Obstet Gynecol 1987;30:284.
6. Genkins G. Myasthenia gravis and pregnancy. In: Cherry SH, Berkowitz RL, Kase NG, eds. Medical, surgical and gynecological complications of pregnancy. 3rd ed. Baltimore: Williams and Wilkins, 1985:447.

Response declined

Data sought to link adverse pregnancy outcome with maternal serum α-fetoprotein levels in black gravid women

To the Editors:

Macri et al. (Maternal serum α-fetoprotein screening. AM J OBSTET GYNECOL 1987;157:820-2.) document that "the mean of the distribution of maternal serum α-fetoprotein levels in black gravid women is significantly higher than that in other gravid women." He then goes on to state that these women will be subjected to unnecessary anxiety, expense, inconvenience, and potential maternal or fetal harm." In addition, the incidence of open neural tube defects is lower for black women than that for others. Whereas his data should obviously be used for the prediction of open neural tube defects in blacks, it should not be forgotten that elevated maternal serum α-fetoprotein values also predict other pregnancy complications. In the United States, compared with white mothers, black mothers have an increased incidence of pregnancy complications and low birth weight infants. Perhaps this shift in maternal serum α-fetoprotein values for blacks is not normal, but in reality it is related to this increased incidence of adverse pregnancy outcome. Thus it would be helpful if Macri et al. compared their data in black women with regard to birthweight, stillbirth, and spontaneous abortion.

Roger R. Lenke, MD

Maternal-Fetal Medicine
Center for Women and Children
3000 Arlington Ave.
Toledo, OH 43699

Reply

To the Editors:

As evidenced by our publication in 1978,[1] we have long shared Lenke's interest in the association between elevated maternal serum α-fetoprotein and the higher incidence of complications and low birth weight in pregnancies of black women. This laboratory has developed a large data base on black gravid women. Com-

pletion of the onerous task of securing and analyzing data with regard to perinatal complications including low birth weight will enable us to make known our findings on this association.

James N. Macri, PhD
Ramana V. Kasturi, BE, MS
Mannshya G. Hu, PhD
David A. Krantz, BS
Timothy J. Douros
Paul Sajda
Edward J. Cook, PhD

Research Division
NTD Laboratories, Inc.
383 Old Country Road
Carle Place, NY 11514

REFERENCE

1. Macri JN, Weiss RR, Libster B, Cagan M. Maternal serum α-fetoprotein and low birth-weight. Lancet 1978;1:660.

Frequency and timing of intravascular intrauterine transfusions reconsidered

To the Editors:

In our country the incidence of severe fetoneonatal hemolytic disease is still rather elevated because of the lack of effective prophylactic planning. In the past radiologically[1,2] and then ultrasonographically[3] guided intrauterine intraperitoneal transfusions helped us to improve neonatal survival in such cases. At present we perform intravascular intrauterine transfusions because the good results of this procedure reported in the literature[4-7] are encouraging. Furthermore, the intravascular route allows us to start treatment before 25 weeks' gestation, to obtain immediate hematologic data, and to determine the amount of blood volume needed to increase fetal hematocrit.

We were surprised to read the extremely good perinatal results reported by Dr. Michael L. Socol et al. (Percutaneous umbilical transfusion in severe rhesus isoimmunization. AM J OBSTET GYNECOL 1987;157: 1369-75). In their study, all hydropic changes in the treated fetuses resolved completely after the transfusions. In our experience, however, the rate of hydropic resolution is much lower, as it is in the other studies published in the literature.[8]

In the past 18 months we have performed a total of 90 intravascular transfusions on 33 patients with severe fetal Rh hemolytic disease. Before the initial transfusion, 16 (48%) of the fetuses had hydrops with ascites, edema, and pericardial effusion. Only four of these (25%) showed hydropic remission after transfusional therapy. A total of 12 (36%) fetuses died in utero. A total of 21 (64%) treated fetuses were born alive; of these, 11 died in the early neonatal period (4 of hydrops fetalis, 1 of cardiac arrest during the exchange transfusion, 2 of cardiac failure, 3 of hyaline membrane disease, and 1 of extreme immaturity).

Anti-D titers have been shown to increase (in some cases from 7.31 μg to 35.2 μg) after intravascular intrauterine transfusions. This antibody rise might result from fetomaternal hemorrhage probably in response to vascular disruption caused by the use of transfusion needles. This forces us to reconsider the frequency of intravascular intrauterine transfusions and the most appropriate time to initiate treatment.

Our results differ from those stated by Dr. Socol et al. Furthermore, we believe that Dr. Socol's findings do not reflect the real danger and irreversibility of most hydropic cases despite intravascular intrauterine transfusions.

Liliana S. Voto, MD
Miguel Margulies, MD

Section of Maternal-Fetal Medicine
Hospital Juan A. Fernández
University of Buenos Aires School of Medicine
Av. Pueyrredón 1746 2A
1119 Buenos Aires, Argentina

REFERENCES

1. Margulies M, Margulies M, Voto LS. Diagnóstico, pronóstico y tratamiento de las pacientes Rh negativas isoinmunizadas, Parte I. OGLA 1982;40:279.
2. Margulies M, Margulies M, Voto LS. Diagnóstico, pronóstico y tratamiento de las pacientes Rh negativas isoinmunizadas. Parte II. OGLA 1982;40:363.
3. Margulies M, Voto LS, Zapaterio J, Margulies ND, Cremona JC. Transfusión fetal intrauterina con la ayuda de la ultrasonografía de tiempo real. Rev Soc Obst Gin (Buenos Aires) 1983;LXII:308.
4. Rodeck CH, Holman CA, Karnicki J, Kemp JR, Whitmore DN, Austin MA. Direct intravascular fetal transfusion by fetoscopy in severe rhesus isoimmunization. Lancet 1981; 1:625.
5. Rodeck CH, Nicolaides KH, Warsof SL, Fysh WJ, Gamsu HR, Kemp JR. The management of severe rhesus isoimmunization by fetoscopic intravascular transfusions. AM J OBSTET GYNECOL; 1984:150:769.
6. Grannum PA, Copel JA, Plaxe SC, Scioscia AL, Hobbins JC. In utero exchange transfusion by direct intravascular injection in severe erythroblastosis fetalis. N Engl J Med 1986;314:1431.
7. Berkowitz RL, Chitkara J, Goldberg JD, Wilkins I, Chervenak FA. Intravascular transfusion in utero: the percutaneous approach. AM J OBSTET GYNECOL 1986;154:622.
8. Seeds JW, Bowes WA Jr. Ultrasound-guided fetal intravascular transfusion in severe rhesus immunization: AM J OBSTET GYNECOL 1986;154:1105.

Reply

To the Editors:

We appreciate the interest of Drs. Voto and Margulies in our article (Percutaneous umbilical transfusion in severe rhesus isoimmunization: Resolution of fetal hydrops. AM J OBSTET GYNECOL 1987;157:1369-75). We agree fetal hydrops secondary to severe isoimmunization is an ominous sign, particularly if at <26 weeks' gestation. However, we disagree with the statement that hydrops is irreversible in most cases. To the contrary, we believe ultrasonographically guided intravascular transfusions have the capability to considerably improve the prognosis for these pregnancies. A larger series published by Grannum et al. supports our con-

tention.[1] Sixteen of 20 (80%) hydropic fetuses survived and hydrops was completely reversed in 13 of the 16 (81%) fetuses. Consequently, although there are risks associated with fetal blood sampling and intravascular therapy, we continue to believe the ability to gain access to the fetal circulation enhances effective in utero treatment.

Michael L. Socol, MD
Scott N. MacGregor, DO
Bruce W. Pielet, MD
Ralph K. Tamura, MD
Rudy E. Sabbagha, MD
Section of Maternal-Fetal Medicine
Department of Obstetrics and Gynecology
Northwestern University Medical School
333 E. Superior St.
Chicago, IL 60611

REFERENCE

1. Grannum PA, Copel JA, Moya FR, et al. The reversal of hydrops fetalis by intravascular intrauterine transfusion in severe isoimmune fetal anemia. AM J OBSTET GYNECOL 1988;158:914.

Basic reference gambles recommended for utility assessment

To the Editors:

We wish to question Silver and Minogue's analysis (When does a statistical fact become an ethical imperative? AM J OBSTET GYNECOL 1987;157:229-33) with utility scores of elective repeat cesarean section and trial of scar. They asked eight perinatologists to assign utility scores of 0 to 100 (worst = 100) to 11 outcomes that ranged from the minor (the inconvenience of awaiting spontaneous labor and the discomfort of labor) to the major (adverse outcomes of neonatal and maternal death). This is essentially the same as asking the respondents to mark the outcomes on a linear analog scale and is flawed because people always bunch their replies away from the ends of the scale, even when their utilities differ 100-fold, 1000-fold, or more.

The correct method of utility assessment is the basic reference gamble, which in its most widely used form requires subjects to choose between a certainty of an intermediate outcome and a gamble between two extreme outcomes. For example, to measure the relative utility of maternal death and obstetric hysterectomy, subjects would be asked to choose between a certain hysterectomy and a gamble between maternal death and full health. At gambles with high risks of death, say 1 in 10, almost all people would choose hysterectomy, whereas at low risks, say 1 in 10,000, most would choose to risk death. At some risk of death each subject would be unable to decide and that level is defined as the level of indifference.

We are not aware of any surveys of the basic reference gamble between hysterectomy and maternal death but suspect that most North American women (a population not notably averse to hysterectomy) would choose hysterectomy in preference to very low (e.g., much less than 1 in 100) risks of death. Silver and Minogue, however, value hysterctomy at 62 and maternal death at 100. If these values were correct the average woman would choose a 50:50 gamble between full health and death in preference to hysterectomy. Similar ludicrous conclusions are implied by all of the utilities they quote. For example, they value the discomfort of labor as 6 on the same scale, which would imply that women would take a 1 in 20 risk of death to avoid it. We had a similar problem with a recent unpublished survey of patient utilities in relation to surgical management of microinvasive carcinoma of the cervix, in which 30 young women marked their values on a linear analog scale. On a scale with full health and fertility valued 100 and death valued 0, iatrogenic infertility was valued 50. We do not pretend to know what a reasonable utility for infertility should be, but we are fairly certain that 50 is much too low. The problems with linear analog scales and the theoretic and practical aspects of the basic reference gamble are well covered in textbooks of decision analysis.[1]

There is another weakness in their study. They have failed to measure the utilities of elective and emergency cesarean section. These are of crucial importance because, as several authors have argued,[2,3] by avoidance of the risks of emergency operation elective cesarean section may be equally as safe as trial of labor for the mother. In other words, the relatively greater dangers of intrapartum, compared with elective operation, means that the utility of trial of vaginal delivery falls as the chance of success decreases. If this is accepted, the crucial decision is often between risk of fetal death and loss of the satisfaction of normal delivery. At low avoidable risks of fetal death this may be important for some women. Whereas some who value normal delivery relatively low in relation to fetal health will want all possible intervention, others who value the process of natural childbirth relatively high will refuse the same intervention. We are attempting to measure these values, but even with the use of reference gambles it is difficult to obtain internally consistent results. In the meantime, facile acceptance of clearly untenable utilities will bring clinical decision analysis into disrepute. If utilities are used they should be subjected to sensitivity analyses in the same way as probabilities.

J. G. Thornton, MD
R. J. Lilford, PhD
Departments of Obstetrics and Gynecology
Leeds General Infirmary and St. James' Hospital
Leeds, England

REFERENCES

1. Weinstein MC, Fineberg HV, eds. Clinical decision analysis. Philadelphia: WB Saunders, 1980:184-227.
2. Feldman GB, Freiman JA. Prophylactic cesarean section at term? N Engl J Med 1985;312:1264-7.
3. Bingham P, Lilford RJ. Breech delivery: a decision analysis based on the intended method of delivery. Obstet Gynecol 1987;69:965-78.

Reply

To the Editors:

We would be in agreement with the respondents that our method of assignment of utility scores on a linear scale does not provide the most accurate sensitivity of outcome ranking. Though sensitivity may be reduced by this method, outcome priority (worst to least offensive) is maintained by this method. Inasmuch as priority of outcome is maintained, the various nodes of the decision tree do accurately reflect the originally assigned priorities. Thus the end point comparison of cesarean section versus trial of labor remains valid (i.e., from purely outcome-based analysis, trial of labor provides more benefit and less risk than does repeat cesarean section). Because sensitivity is diminished by our method, the "point spread" between the various nodes of the decision tree may not reflect an appropriate (more sensitive) magnitude of difference.

Certainly, improvement in sensitivity would enhance any decision analysis. But decision analysis, as such, was not our point. In fact, a key objective of the article was to expose a fatal flaw in utility analysis as the *sole* arbiter of ethical decisions. Even with the assumption of a decision analysis method with 100% sensitivity in utility ranking, such ranking would remain highly subjective. Despite the seeming quantitative objectivity of decision analysis, both the ranking and order of magnitude assigned to any outcome are on the basis of personal, subjective preferences. Utility ethics, by making the most good for the most people an absolute, ignores the claims and rights of the few. Translated into quantitative terms, any utility assignment, no matter how sensitive, would have to be on the basis of an average of respondents' scoring. This averaging supports the majority and disenfranchises the minority. Thus the alleged objectivity of utility analysis is actually more a reflection of common opinion than of scientific fact.

Richard Silver, MD
Jack Minogue, DMin
Department of Obstetrics and Gynecology
Northwestern University Medical School
Evanston Hospital
2650 Ridge Avenue
Evanston, IL 60201

Detection of fetal blood

To the Editors:

We read with interest the article, "Ultrasound diagnosis of abruptio placentae with fetomaternal hemorrhage," by Dr. Michael S. Cardwell (Am J Obstet Gynecol 1987;157:358-9). We noted an omission by the author in that he did not consider that 25% of women produce hemoglobin F during pregnancy.[1] This may account for some positive results of Kleihauer-Betke tests. About 1.7% of a population of white Americans had increased numbers of hemoglobin F cells.[2] Moreover, 1.4% of all black Americans have β-thalassemia minor and therefore might have elevated levels of hemoglobin F cells.[3] In a British work 3% of a population was found to have inherited elevated percentages of hemoglobin F cells.[4] The important conclusion is that some patients may have elevated numbers of hemoglobin F cells without fetomaternal hemorrhage. As an alternative to the Kleihauer-Betke test it was recommended to measure α-fetoprotein in maternal blood. This test is more sensitive but it takes more time. We have to point out that maternal serum α-fetoprotein may not be so specific of fetal hemorrhage because it is normally diffused in maternal blood by passage from amniotic fluid. Some authors[5] found that a baseline prehemorrhage sample was necessary; of course, this would be unavailable in maternal trauma patients. Nevertheless, we can overcome these inconveniences in nonurgent cases by repeat blood samples. There are also other tests to detect fetal blood, such as hemoglobin electrophoresis[6] and glycerol lysis time,[7] but most prefer to measure α-fetoprotein in maternal blood as an indicator of fetomaternal bleeding.

Joseph R. Leiberman, MD
Moshe Mazor, MD
Adolph Cohen, MD
Department of Obstetrics and Gynecology
Soroka Medical Center
Faculty of Health Sciences
Ben Gurion University of the Negev
Beer Sheva, POB 151, Israel

REFERENCES

1. Mollison PL. Blood transfusion in clinical medicine. 7th ed. Springfield, Illinois: Charles C Thomas, 1983:368-9.
2. Wood WG., Stomatoyannopoulos G, Lim G, et al. F-cells in the adult: normal values and levels in individuals with hereditary and acquired elevations of HbF. Blood 1975; 46:671-82.
3. Pierce HI, Kurachi S, Sofroniadou K, et al. Frequencies of thalassemia in American blacks. Blood 1977;49:981-6.
4. Zago MA, Wood WG, Clegg JB, et al. Genetic control of F-cells in human adults. Blood 1979;53:977-86.
5. Rose PG, Strohm P, Zuspan FP. Assessment of fetomaternal hemorrhage with Kleihauer-Betke test [Letter Reply]. Am J Obstet Gynecol 1986;155:1146.
6. Richards SR, Miller MM. Bloody tap amniocentesis: discrimination between fetal and maternal blood by means of hemoglobin electrophoresis. Am J Obstet Gynecol 1983;145:837-40.
7. Bar-David J, Piura B, Leiberman JR. et al. Discrimination between fetal and maternal blood [letter]. Am J Obstet Gynecol 1984;148:352.

Reply

To the Editors:

I wish to thank Drs. Leiberman, Mazor, and Cohen for their interest in my article. They are correct in stating that some patients may have elevated fetal hemoglobin levels associated with β-thalassemia minor or inherited elevated levels of hemoglobin F cells. However, I must point out that elevated α-fetoprotein values are associated with a myriad of conditions, including ventral wall defects, neutral tube defects, and fetal death.[1] Moreover, to reliably detect fetomaternal hemorrhage by α-fetoprotein levels, prehemorrhage and posthemorrhage specimens must be available, which is

usually not the case in patients with suspected abruptio placentae.[2] Most hospital blood banks have around-the-clock facilities to run Kleihauer-Betke tests; α-fetoprotein specimens are usually saved for "batches." Quantitations of the fetomaternal hemorrhage demonstrated by the Kleihauer-Betke stain can be accomplished with the formula derived by Mollison[3]: Fetomaternal hemorrhage (milliliters of fetal red blood cells = 2400 × Fetal red blood cells ÷ Adult red blood cells. Quantitation of the bleeding is not possible with α-fetoprotein values. Although other tests are available to detect fetal blood, the Kleihauer-Betke stain (fetal cell stain) remains the standard test in the United States and is widely used to screen Rh-negative mothers for evaluation and quantitation of the $Rh_o(D)$ immune globulin administration.[4]

Michael S. Cardwell, MD
Director of Maternal-Fetal Medicine
University of Missouri—Columbia
Columbia, MO 65212

REFERENCES

1. Pritchard JA, McDonald PC, Gant NF. Williams' Obstetrics. 17th ed. Norwalk, Connecticut: Appleton-Century-Crofts, 1985:277-8.
2. Rose PG, Strohm P, Zuspan FP. Assessment of fetomaternal hemorrhage with Kleihauer-Betke test [Letter Reply]. AM J OBSTET GYNECOL 1986;155:1146.
3. Mollison PL. Quantitation of transplacental hemorrhage. Br Med J 1972;3:31.
4. Scott JR, Warenski JC. Tests to detect and quantitate fetomaternal bleeding. Clin Obstet Gynecol 1982;25:277-82.

Role of ferritin supported in diagnosis of anemias of pregnancy

To the Editors:

I believe the article by Schifman et al. (Schifman RB, Thomasson JE, Evers JM. Red blood cell zinc protoporphyrin testing for iron-deficiency anemia in pregnancy. AM J OBSTET GYNECOL 1987;157:304-7) repeats a mistake that most American investigators have made in the study of anemias of pregnancy. They fail to consider plasma volume expansion and total red blood cell mass (body hematocrit). I predict that the women with the lowest hematocrit in the third trimester actually have the most red blood cells and the biggest infants.[1] Indeed, the bigger the newborn the lower the hematocrit and the higher the incidence of apparent iron deficiency.[2] Studies have reconfirmed that ferritin is indeed the gold standard for diagnosis of iron deficiency anemias of pregnancy and I believe that Schifman's et al. article has only confused the issue.[3]

Robert C. Goodlin, MD
Professor
Obstetrics and Gynecology
University of Colorado Health Sciences Center
School of Medicine
4200 E. Ninth Ave.
Denver, CO 80262

REFERENCES

1. Goodlin RC. Why treat "physiologic" anemias of pregnancy? J Reprod Med 1982;27:639.
2. Higgins AC, Pencharz PB, Strawbridge JE, Maughan GB, Moxley JE. Maternal haemoglobin changes and their relationship to infant birth weight in mothers receiving a program of nutritional assessment and rehabilitation. Nutr Res 1982;2:641.
3. McGarity WJ. Protection of maternal iron stores in pregnancy. J Reprod Med 1987;32(suppl):475.

Reply

To the Editors:

Mild anemia, secondary to increased plasma volume, is a normal physiologic consequence of pregnancy and, as Dr. Goodlin points out, the degree of plasma expansion relates to infant birth weight. Red blood cell mass likewise expands during pregnancy by about 250 ml to 500 ml. This factor, together with fetal growth, places a heavy demand on iron stores, produces a state of negative iron balance, and increases the risk of iron depletion and anemia.[1]

Iron depletion may be absolute (i.e., absent iron stores) or relative, a condition in which reserves are sufficient but are ineffectively used because of increased demands or inflammation. In either case, the ability to expand or maintain red blood cell mass is compromised. Whereas serum ferritin measurement is the gold standard for absolute iron depletion, it does not necessarily identify those with dysfunctional erythropoiesis as a result of excessive or ineffective use of iron.[2]

The red blood cell zinc protoporphyrin test is a functional measure of iron use that detects both absolute and relative iron depletion.[3] In our study, red blood cell zinc protoporphyrin measurements predicted a state of declining red blood cell mass and anemia in third trimester more reliably than did ferritin, presumably because of its sensitivity for the detection of iron depletion erythropoiesis. By the third trimester, most women with elevated red blood cell zinc protoporphyrin values showed hemoglobin values below physiologic anemia levels. If the differences we observed in hemoglobin concentrations were primarily caused by disparate plasma volumes and infant birth weights, rather than by iron depletion erythropoiesis, then the significant correlations we observed between hemoglobin and red blood cell zinc protoporphyrin should also relate to plasma volume differences. However, I am unaware of data that support this supposition, nor would this hypothesis be plausible on the basis of what is known about the cause of elevated red blood cell zinc protoporphyrin concentrations.

Ron B. Schifman, MD
Associate Professor, Pathology
University of Arizona College of Medicine
1501 N. Campbell
Tucson, AZ 85724

REFERENCES

1. Bentley DP. Iron metabolism and anaemia in pregnancy. Clinics in Haematol 1985;14:613-28.

2. Finch CA, Huebers H. Perspectives in iron metabolism. N Engl J Med 1982;306:1520-8.
3. Langer EE, Haining RG, Labbe RF, et al. Erythrocyte protoporphyrin. Blood 1972;40:112-28.

Does terbutaline cause uterine atony and increase intraoperative blood loss?

To the Editors:

I read with interest the study of Patriarco et al. (A study on intrauterine fetal resuscitation with terbutaline. AM J OBSTET GYNECOL 1987;157:384-7). My experience with this therapeutic modality would support its usefulness. However, when terbutaline precedes cesarean section within a short period of time (≤15 minutes) I have, on occasion, encountered troublesome uterine atony with a resultant increase in intraoperative blood loss. I have not studied this problem scientifically. Do the authors have any data to suport or refute this clinical impression?

Mark S. Brown, MD
Central Ohio Medical Group
497 E. Town St.
Columbus, OH 43215

Reply

To the Editors:

Dr. Brown had mentioned in his letter to the Editors that occasionally after he gave terbutaline for fetal resuscitation, he had encountered uterine atony with an increase in intraoperative blood loss. We, too, have occasionally encountered uterine atony after terbutaline injection; however, this seems to respond most readily to intravenous oxytocin (Pitocin, Parke Davis). My personal experience is that this additional blood loss is minimal. I have no way to quantitate this blood loss, but it does not appear to be substantially increased. I wonder whether some of this uterine atony may be secondary to the general anesthetics that occasionally are used for emergency cesarean sections, which is usually the case with fetal distress.

Michael S. Patriarco, DO
College Heights Obstetrical and Gynecologic Professional Corporation
3131 College Heights Blvd.
Allentown, PA 18104

Oxytocin may explain neonatal seizures

To the Editors:

As I have tried to point out before,[1] there is an apparent association between the use of oxytocin in labor and neonatal seizures. This perhaps relates both to the changes in newborn sodium levels as well as to the increased incidence of stronger uterine contractions with oxytocin than with natural labor. With regard to the article by Spellacy et al. (Neonatal seizures after cesarean delivery: Higher risk with labor. AM J OBSTET GYNECOL 1987;157:377-9), I presume that the women delivered before labor by cesarean section did not receive oxytocin, whereas a high percentage of women delivered by cesarean section after trial of labor did receive oxytocin. Perhaps the authors have considered this factor in their analysis. If not, I believe oxytocin might be the explanation for the higher incidence of seizures in the laboring group.

Robert C. Goodlin, MD
Professor
Obstetrics and Gynecology
University of Colorado Health Sciences Center
4200 E. Ninth Ave.
Denver, CO 80262

REFERENCE

1. Goodlin RC. Is oxytocin the culprit? [Letter.] AM J OBSTET GYNECOL 1985;153:928-9.

ITEMS

Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60.00 U.S. per insertion and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, The C.V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318.

International Perinatal Doppler Society (I.P.D.S.). Paris, France, September 13-15, 1989 For more information contact: Dr. Ph. Arbeille, Secretary of the I.P.D.S., Unité INSERM 316, C.H.U. Bretonneau, 37044 Tours, France.

Eleventh Practical Course in Colposcopy and Cervico-Vaginal Histopathology (In English). Demonstrations of the use of diathermy loop, with Dr. René Cartier, Paris, France, September 11 to 15, 1989. For further information contact: Dr. René Cartier, 20 Rue des Cordelières, F. 75 013 Paris, France. Tel.: (1) 43.36.04.46.

Two Postgraduate Gynecology Courses: Comprehensive Review of Gyn for a Clinician, Sept. 12-15, 1989, and **Reproductive Endocrinology,** Sept. 15-18, 1989, Massachusetts General Hospital, Boston, Massachusetts. For information contact: Harvard Medical School, Department of Continuing Education, 25 Shattuck St., Boston, MA 02115. Tel.: (617) 732-1525.

The First International Symposium for the Treatment of Post Surgical Adhesions, September 15-17, 1989, the Ritz-Carlton, Phoenix, Arizona. For further information/formal brochure, contact: Symposium Medicus, 2815 Mitchell Dr., Suite 128, Walnut Creek, CA 94598-1622. Tel.: (415) 935-7889.

American Uro-Gynecologic Society, Tenth Annual Meeting, October 4-7, 1989, Marriott's Camelback Inn, Scottsdale, Arizona. **Post Graduate course in Uro-Gynecology,** October 4, 1989. For further information contact: Jeanne Boyea, Office of the Secretary-Treasurer, c/o The University of Michigan Medical Center, Department of Ob/Gyn, 1500 E. Medical Center Dr., MPB D2239 0718, Ann Arbor, MI 48109. Tel.: (313) 764-8429.

Postgraduate Course in Obstetrics and Gynecology, September 24-27, 1989, Topnotch at Stowe, Vermont. For further information contact: Maureen E. Hanagan, Director, Continuing Medical Education, University of Vermont, 233 Rowell Building, Burlington, VT 05405. Tel.: (802) 656-2292.

Bound volumes available to subscribers

Bound volumes of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY are available to subscribers (only) for the 1989 issues from the Publisher, at a cost of $56.00 ($79.00 international) for Vol. 160 (January-June) and Vol. 161 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue in the volume. The binding is durable buckram with the JOURNAL name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact The C.V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318, USA; phone (800) 325-4177, ext. 351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular JOURNAL subscription.

COLUMBIA UNIVERSITY
The College of Physicians & Surgeons

Sloane Hospital for Women 1888-1989 a division of The Presbyterian Hospital

29th ANNUAL POSTGRADUATE COURSE IN OBSTETRICS & GYNECOLOGY
October 30 through November 3, 1989

at the New York Academy of Medicine, New York, New York

39 Credit Hours, Category 1, A.M.A.'s Physician's Recognition Award; 37 Cognates, Formal Learning, American College of Obstetricians & Gynecologists; and 37 Elective Hours, American Academy of Family Physicians

FEE: $795 (Residents: $475); includes syllabus, luncheons and refreshments.

Conference Directors: **RALPH J. HESSEKIEL, M.D. & MARVIN H. TERRY GRODY, M.D.**

HAROLD E. FOX, M.D.	ROBERT S. NEUWIRTH, M.D.	JOHN D. THOMPSON, M.D.
RAPHAEL JEWELEWICZ, M.D.	RALPH M. RICHART, M.D.	ILAN E. TIMOR-TRITSCH, M.D.
DANIEL R. MISHELL, JR.; M.D.	MORTIMER G. ROSEN, M.D.	ALAN J. WABREK, M.D.
JAMES H. NELSON, JR., M.D.	DANIEL H. SMITH, M.D.	STERLING B. WILLIAMS, M.D.

This comprehensive program is offered in order to update the practitioner to the current state of the art. We hope to accomplish this through an overview of the major segments of obstetrics & gynecology by a visiting guest faculty of established experts and a select group of specialists and authorities from the faculty of the College of Physicians & Surgeons of Columbia University. The main thrust of the course will examine problems in clinical practice and their resolution through the application of current data, treatment options and surgical techniques. Subspecialties of maternal fetal medicine, oncology and reproductive endocrinology will be covered as they apply to the practice of our specialty by the generalist for whom this course is specifically designed. Lectures on the latest technology, panel discussions with audience participation and evaluation of ethical and medico-legal problems affecting health care delivery should help provide the highest and broadest professional competence in each of the registrants.

Contact: Center for Continuing Education, 630 West 168th Street, N.Y., N.Y. 10032; Telephone: (212) 305-3682.

Medical Genetics Medical Staff Fellowship
The Interinstitute Medical Genetics Program
National Institutes of Health

The Interinstitute Medical Genetics Program of the National Institutes of Health offers fellowships providing a comprehensive clinical and laboratory experience in medical genetics. The fellowships begin July, 1991 and are offered for two years. Applications are invited from individuals of the highest caliber with the M.D. or M.D.-Ph.D. degree who have completed at least two years of post-graduate clinical training and who are seriously interested in pursuing careers in academic medicine and research. Clinical training involves inpatient and outpatient services at the NIH 540-bed research and teaching hospital, which, with collaborating medical facilities, provides a broad exposure to clinical genetics, diagnosis and counseling, cytogenetics, biochemical and cancer genetics, and prenatal diagnosis. Exceptional research opportunities exist in the institutes' multiple laboratories. The program staff includes scientists and physicians of international stature.

As a medical staff fellow you will receive an initial salary of $32,000 with increments of $2,000 per year up to a maximum of $36,000. Moving, travel expenses and Federal health care benefits are available. Interested candidates should submit their *curriculum vitae* to or request further information from.

The Fellowship Program
National Institutes of Health
Public Health Service
Building 10, Room 1C292
9000 Rockville Pike
Bethesda, MD 20892
Attn: Vicki Malick
Telephone: (301) 496-2427

Deadline for applications is November 10, 1989

NIH is an Equal Opportunity Employer

NATIONAL INSTITUTES OF HEALTH

An Invitation to Visit the People's Republic of China and Join the

INTERNATIONAL CONGRESS ON OBSTETRICS & GYNECOLOGY, NEONATOLOGY AND MATERNAL-FETAL MEDICINE

SHANGHAI, CHINA • SEPTEMBER 24-29,1989

CONGRESS OFFICIALS

Congress President
CHIH-LUNG CHOW, M.D.,
Professor Shanghai Second Medical University Head and Chairman Department of Obstetrics & & Gynecology. Xin Hua Hospital and Medical Center Shanghai, China

Congress Vice President
MORTON A. STENCHEVER, M.D.
Professor and Chairman, Department of Obstertrics and Gynecology School of Medicine University of Washington Seattle, Seattle, Washington, U.S.A.

Congress Vice President
RICHARD L. SWEET, M.D.,
Professor and Vice Chairman Departmei Obstetrics and Reproductive Science San Francisco General Hospital, Chief Obstetrics and Gynecology University California at San Francisco, San Francis San Francisco, California, USA

CONGRESS ORGANIZERS

SHANGHAI SECOND MEDICAL UNIVERSITY, Shanghai, China • XIN HUA HOSPITAL AND MEDICAL CENTER, Shanghai, China
CHINESE MEDICAL ASSOCIATION, Shanghai Branch–Obstetrics and Gynecology Society • Shanghai Municipal Health Bureau

PROGRAM COMMITTEES

SHANGHAI

LIU DI-LIN, M.D., *CHAIRMAN*
Vice Chairman, Perinatal Division Pediatric Research Institute of Shanghai. Department of Obstetrics & Gynecology, Xin Hua Hospital and Medical Center, Shanghai, China

PAN ZHU-RU, M.D., *VICE CHAIRMAN*
Professor,Shanghai Second Medical University, Vice Chairman, Department of Obstetrics and Gynecology, Xin Hua Hospital and Medical Center, Shanghai, China

LILLY RI-CHUNG CHANG
Visiting Physician, Department of Obsterics & Gynecology, Xin Hua Hospital and MedicalCenter, Visiting Scholar Division of Neonatal-Perinatal Medicine Women's Hospital University of Southern California, Los Angeles, California, USA

INTERNATIONAL

STEWART CAMPBELL, M.D.,
London, England

ALLAN CHANG, M.D.,
Shatin, N.T., Hong Kong

SHEILA E. COHEN, M.B., ChB,
Stanford, California, USA

MANFRED HANSMANN, M.D.,
Bonn, West Germany, FRG

EDWARD A. LYONS, M.D.,
Winnepeg, Manitoba, Canada

MICHAEL G. ROSS, M.D., M.P.H.,
Torrance, California, USA

MASAKUNI SUZUKI, M.D.,
Tokyo, Japan

ILAN E. TIMOR, M.D.,
New York, New York, USA

BRIAN J. TRUDINGER, M.D.,
Westmead, NSW, Australia

JOSEF ZANDER, M.D.,
Munich, West Germany, FRG

INVITED GUEST FACULTY

JOHN I. BREWER, M.D.,
Editor, American Journal of Obstetrics and Gynecology, Chicago, Illinois, USA

ERICH BURGHARDT, M.D.,
Professor, Department of Obstetrics, and Gynecology, Univeristy of Graz, Graz, Austria

LOUIS BURKE, M.D.,
Associate Professor, Department of Obstetrics and Gynecology, Harvard Medical School, Boston, Massachusetts, USA

STEWART CAMPBELL, M.D.,
Professor, Department of Obstetrics and Gynecology, Kings Hospital Medical School, London, England, UK

ALLAN CHANG, M.D.,
Professor, Head and Chairman, Department of Obstetrics and Gynecology, Chinese University of Hong Kong, Shatin, N.T., Hong Kong

LILLY RI-CHUNG CHANG, M.D.,
Visiting Physician, Department of Obsterics & Gynecology, Xin Hua Hospital and Medical Center, Visiting Scholar, Division of Neonatal-Perinatal Medicine Women's Hospital University of Southern California, Los Angeles, California, U.S.A

LUIS A. CIBILS, M.D.,
Mary Campau Ryerson Professor, Department of Obstetrics and Gynecology, Chicago Lying-in Hospital, University of Chicago, Chicago, Illinois U.S.A

SHEILA E. COHEN, M.B. ChB.,
Professor of Anesthesia (Clinical), Director, Obstetrical Anesthesia Stanford University Medical Center, Stanford, California, USA

PHILIP J. DiSAIA, M.D.,
Professor and Chairman, Department of Obstetrics and Gynecology, Univeristy of California at Irvine Medical Center, Irvine, California, USA

HAROLD E. FOX, M.D.,
Professor and Director of Obstetrics Division of Maternal-Fetal Medicine, Department of Obstetrics & Gynecology, Columbia-Presbyterian Medical Center, New York, New York, USA

MANFRED HANSMANN, M.D.,
Professor, Department of Obstetrics & Gynecology Head, Division of Perinatal Diagnostics & Therapy, Universitats-Frauenklinik Bonn, Bonn, West Germany FRG

R. HAROLD HOLBROOK, Jr. M.D.,
Assistant Professor, Chief, Section of Maternal-Fetal Medicine, Department of Obstetrics & G cology, Stanford University Medical Ce Stanford, California, USA

OTTO KASER, M.D.,
Professor, Department of Obstetrics & Gyne ogy, University of Basel, Basel, Switzerland

MARSHALL D. LINDHEIMER, M.D.,
Professor, Department of Obstetrics & Gyne ogy and Medicine, Chicago Lying-in Hosp University of Chicago, Chicago, Illinois, USA

EDWARD A. LYONS, M.D.,
Head Section of Obsteetric Ultraso General Hospital, University of Manitc Winnipeg, Manitoba, Canada

JEAN MELCHIOR, M.D.,
Professor, Department of Obstetrics & Gyne ogy, Centre Medic-Chirugical FO Suresnes Cedex, France

ROY M. PITKIN, M.D.,
Editor, Obstetrics & Gynecology, Los Angeles, California, USA

MICHAEL G. ROSS, M.D. M.P.H.,
Assistant Professor, Department of Obstetrics Gynecology, Division of Maternal-Fetal Medi

For Congress Registration and Information, Contact:

INTERNATIONAL CONGRESS SECRETARIAT
P. O. Box 91581,
Pasadena, California 91109-1581, USA
Telephone: (213) 254-3239

This Continuing Education Offering Meets The Requirements of the Internal Revenue Service For Maintaining Professional Skills for Income Tax Purposes. Please contact your tax accountant for your individual situation.

, Harbor-UCLA Medical Center,
ance, California, USA
CHI SAKAMOTO, M.D.,
essor & Director, Maternal & Perinatal Center,
/o Women's Medical College, Tokyo, Japan
FREY SHER, M.D.,
essor & Director, Pacific Fertility Center,
Francisco, California, USA
TON A. STENCHEVER, M.D.,
essor & Chairman, Department of Obstetrics &
ecology, University of Washington School of
licine, Seattle, Washington, USA
AKUNI SUZUKI, M.D.,
essor, Department of Obstetrics & Gynecol-
, Tokyo Women's Medical College,
yo, Japan
ARD L. SWEET, M.D.,
essor & Vice Chairman, Department of Obstet-
& Repoductive Sciences, San Francisco Gen-
eral Hospital, Chief of Obstetrics & Gynecology, University of California at San Fransico, San Francisco, California, USA
H. WILLIAM TAEUSCH, M.D.,
Professor, Department of Pediatrics, King/Drew Medical Center, Los Angeles, California, USA
KENNETH J. W. TAYLOR, M.D., PhD.,
Professor, Diagnostic Radiology, Director Ultrasound Division, Department of Diagnostic Radiology, Yale University School of Medicine, New Haven, Connecticut, USA
ILAN E. TIMOR, M.D.,
Professor & Director, OB/GYN Ultrasound & Obstetrical Services, Sloane Hospitals for Women, The Presbyterian Hospitals in the City of New York, College of Pysicians & Surgeons of Columbia University, New York, New York, USA
BRIAN TRUDINGER, M.D.,
Associate Professor, Department of Obstetrics & Gynecology, Westmead Hospital, Westmead, NSW, Australia
FRANS WALTHER, M.D., PhD.,
Associate Professor, Division of Neonatal-Perinatal Medicine, Department of Pediatrics, Women's Hospital, University of Southern California School of Medicine, Los Angeles, California, USA
M. LYNN YONEKURA, M.D.,
Professor & Chief, Division of Obstetrics, Department of Obstetrics & Gynecology, Harbor-UCLA Medical Center, Torrance, California, USA
JOSEF ZANDER, M.D.,
Professor & Chairman, Department of Obstetrics & Gynecology, University of Munich, Munich, West Germany, FRG
HARRY K. ZIEL, M.D.,
Director, Department of Obstetrics and Gynecology, Kaiser-Permanente Medical Center, Los Angeles, California, USA

LL FOR ABSTRACT OF PAPERS AND SCIENTIFIC POSTERS SESSIONS • DEADLINE: JULY 15, 1989

CONGRESS PROGRAM

ıternational Faculty of Distinguished Spe-
ɜt, together with renowned specialist from
ıghout China, will present the current status
ɜ clinical practice of Obstetrics and Gyne-
gy, Neonatology and Maternal-Fetal
icine as it is practiced around the world.
five-day congress will be presented in
ɜres during the plenary sessions, specialty
ɔosiums, workshops and product exhibi-
ɜ and demonstrations. There will be daily
heon panels with discussions and audi-
ɜ participation by questions and answers.
congress special attractions will be indus-
ɜechnical exhibits, special seminars on:
l Monitoring and Resuscitation, Surfactant
Lung Maturity, Laser Surgery, Laproscopy/
ɔoscopy, Ultrasound/Mamography and
ɜition.

ITINERARY

ay, September 22;
delegates, participants and guest should
ɜe in Shanghai by this date.

ɜrday and Sunday, September 23-24;
ɜtseeing and touring of Shanghai. Sunday
ning, welcome reception for all congress
ɜgates, participants and guests.

ıday through Friday, September 25-29;
rnational Congress program. Friday eve-
ɜ farewell banquet.

Saturday, September 30;
Departures for foreign destinations or optional post-congress tours of Xi'an and Beijing.

Optional two-day Hong Kong stop-over following departure from China.

CONGRESS FEES

Registration:
US $500.00 Received Before August 1, 1989
US $600.00 Received After August 1, 1989

Registration Fee includes: Congress Information Kit and Portfolio, Welcome Reception, Congress Opening Ceremonies, Admission to all Scientific Sessions, Symposiums and Workshops, Technical Exhibits and Daily Luncheon Panels, Transportation between hotel and congress meetings (daily), Closing Ceremonies and Farewell Banquet, Certificate of Attendance and 40 hours category 1 CME credits.

HOSPITALITY PROGRAM FOR SPOUSES

U.S. $125.00 A daily program of entertaining and cultural events have been arranged for spouses and guests. The daily program will begin at 9:00 AM and end at 3:00 PM. Fee include transportation, professional guide, admission to all events and Luncheons. Program is Monday through Friday.

SHANGHAI LAND COST

U.S. $863.00 Based on two persons sharing the same hotel room September 24 through 29, 1989. Shanghai Land Cost Includes: Transfers from airport to hotel and return by motorcoach, porterage in-and-out of hotel, deluxe room accomodations for six nights, full breakfast and dinner in hotel each day, all tips and gratuities.

INTERNATIONAL AIRFARE

Special roundtrip airfares from the West Coast to Shanghai have been arranged for this congress. For reservations and details contact the Congress Secretariat.

Optional Pre & Post Congress Events:

Pre-Congress Sightseeing of Shanghai:
US $90.00. Two full days of sightseeing and shopping in Shanghai, Saturday and Sunday, September 23-24. Includes; professional guides, all admissions to events, lunch, buses and gratuities.

Post-Congress Tour of Two Cities in China:

U.S. $600.00 Professional Guided tours Specially arranged for the delegates, participants and guests of the International Congress. Visit Xi'an and Beijing. Depart Shanghai on Saturday, September 30 for Xi'an. Visit Xi'an, Saturday - Monday, visit Beijing Monday - Thursday. Depart Beijing for international destinations or Hong Kong stop-over, Friday, October 6.

U.S. $260.00 Hong Kong stop-over following International Congress: Two nites deluxe hotel accommodations, daily breakfast, transfers and porterage. Optional sightseeing and touring of Hong Kong and nearby Islands and New Territories.

FACULTY POSITION OB/GYN GENERAL SPECIALIST

The Knoxville Unit of The University of Tennessee College of Medicine is currently accepting applications for a General Specialist in the Department of Obstetrics and Gynecology at The University of Tennessee Medical Center at Knoxville.

This position requires board certification or eligibility in Obstetrics and Gynecology. Responsibilities include: resident and medical student education; participation in Regionalization Program; and clinical or basic research.

Specialized areas of interest such as benign GYN surgery and/or reproductive immunology are encouraged.

Academic rank is commensurate with both qualifications and experience.

Interested persons should sent curriculum vitae:

Michael R. Caudle, M.D.
Associate Professor and Chairman
Department of Obstetrics and Gynecology

THE UNIVERSITY OF TENNESSEE
Medical Center at Knoxville
1924 ALCOA HIGHWAY
KNOXVILLE, TENNESSEE 37920
EEO/An Affirmative Action/Title IX/Section 504 Employer

FELLOWSHIP
MATERNAL & FETAL MEDICINE

The University of Medicine and Dentistry of New Jersey announces a Fellowship available in Maternal & Fetal Medicine beginning July 1, 1990. This is a 2-year, accredited, academically-oriented Fellowship at the UMDNJ-New Jersey Medical School with broad clinical experience at the UMDNJ-University Hospital. Clinical and research training are emphasized. Please send curriculum vitae to: **Joseph J. Apuzzio, M.D., Director, Maternal and Fetal Medicine, UMDNJ-New Jersey Medical School, (AJOB), 185 South Orange Avenue, Newark, NJ 07103-2757.**

The UMDNJ is an Affirmative Action/
Equal Employment Opportunity Employer M/F/H/V.

UMD
NEW JERSEY

FACULTY POSITION CHAIRMAN WEST VIRGINIA UNIVERSITY SCHOOL OF MEDICINE

West Virginia University School of Medicine is seeking a CHAIRMAN, DEPARTMENT OF OBSTETRICS AND GYNECOLOGY at Charleston Area Medical Center—the 1000 bed clinical campus of WVU Health Sciences Center. This person will also be Associate Chairman, Department of OB/GYN, WVU School of Medicine. CAMC has a newly opened ultramodern Women and Children's Hospital. CAMC has full resident programs and one third of the WVU medical students in permanent residence for their clinical years at the Charleston Division. Applicants should be accomplished clinicians interested in an academic setting emphasizing teaching and be eligible for a rank of Professor or Associate Professor. Please send CV and letter to Shawn Chillag, M.D., Associate Professor and Chairman, Department of Medicine/CAMC, 3110 MacCorkle Avenue, S.E., Charleston, WV 25304. Closing deadline October 30, 1989. WVU is an affirmative action equal opportunity employer.

Obstetrics and Gynecology Maternal/Fetal Medicine Specialist

The Western Pennsylvania Hospital is seeking a second BC/BE specialist in Maternal/Fetal Medicine to join a rapidly growing division of Maternal/Fetal Medicine in a 550 bed tertiary care center. This is a unique opportunity in that major teaching, clinical and research opportunities are available in this university affiliated center. A fully accredited independent Ob/Gyn Residency training program is in place. This is a level III tertiary care center with a 30 bed neonatal intensive care unit. The Maternal/Fetal Medicine service accepts high risk maternal transports from throughout the tri-state area. A full range of genetic services are also performed at the institution, including chorionic villi sampling, amniocentesis, and genetic counseling. A research building is currently being built, and opportunities are available for both basic and clinical research. This position offers a state of the art practice opportunity, an attractive compensation package, and the chance for one to be creative. Send your CV to:

Mark Caine, M.D.
Director, Maternal/Fetal Medicine
Department of Obstetrics and Gynecology
West Penn Hospital
4800 Friendship Avenue
Pittsburgh, PA 15224

DIRECTOR OF MATERNAL AND FETAL MEDICINE

University of Illinois
College of Medicine at Chicago
and
Michael Reese Hospital and Medical Center

The University of Illinois College of Medicine at Chicago, and Michael Reese Hospital and Medical Center invite applications and nominations for the position of Director of Maternal and Fetal Medicine in the Department of Obstetrics and Gynecology. Academic rank dependent upon qualifications. The Director will lead the Obstetric program of two of Chicago's most important medical institutions, both with a long history of academic excellence and outstanding patient care.

Candidates should be certified in Maternal/Fetal Medicine, have substantial scholarly productivity in the field, and have ability to provide dynamic leadership in administering a comprehensive program of patient care, education, and research in Obstetrics.

Interested individuals should send a curriculum vitae to: Antonio Scommegna, M.D., Professor and Head, Department of Obstetrics and Gynecology, University of Illinois College of Medicine at Chicago, 840 South Wood Street, Chicago, IL 60612. The University of Illinois at Chicago is an affirmative action/equal opportunity employer.

Request for Participation in Maternal Autoimmune Thrombocytopenia Study

We have plans for a treatment study of Maternal Autoimmune Thrombocytopenia. Intravenous Gamma Globulin will be given at 32 + 37 weeks of pregnancy. This will be compared to a similar group receiving no gamma globulin. This study will be done on a randomized basis. History of well documented autoimmune thrombocytopenia prior to present pregnancy will be required for patient entry. Other therapy (steroids, C-section, treatment of infant) will be at local option. We are looking for 10+ centers to participate in such a study. Each center should be able to enroll 5 patients or more for 1 year. If interested in receiving protocol and specifics, please write Dr. William Krivit, Professor of Pediatrics, University of Minnesota Department of Pediatrics, 1252 Ingerson Road, St. Paul, MN 55112.

UNIVERSITY OF MIAMI SCHOOL OF MEDICINE
DEPARTMENT OF OBSTETRICS & GYNECOLOGY

ASSISTANT PROFESSOR OF CLINICAL OBSTETRICS & GYNECOLOGY

Opportunity available for M.D. who is board certified or board eligible in Maternal Fetal Medicine. Special interest in infectious disease is desirable but not required.

Applicant must be licensed in State of Florida, and have sufficient clinical experience to qualify for a full time faculty appointment.

Salary will be commensurate with training and experience. Compensation includes excellent fringe benefit and financial incentive plan.

Apply to: Mary J. O'Sullivan, M.D.
Professor
Obstetrics and Gynecology R-136
University of Miami
P.O. Box 016960
Miami, Florida 33101

AN EQUAL OPPORTUNITY/AFFIRMATIVE ACTION EMPLOYER

PERSONALIZED LIBRARY CASES

Protect your personal copies of
AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY
in custom made, titled library cases.

Constructed of durable, reinforced binder's board covered in a rich simulated leather, each is designed to hold an entire year's issues. The Journal name is stamped in gold leaf, making the cases attractive additions to any decor, whether on your bookshelf, desk or any location in your home or office.

Only $7.95 each: 3 for $21.95; 6 for $39.95, and $1 per unit postage and handling ($2.50 per unit outside the U.S.A.). U.S. funds only.

To charge your order using American Express, Visa, MasterCard or Diners Club, call toll-free: 1-800-972-5858.

Please send me _____ library cases for
AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY
at $7.95 each: 3 for $21.95; 6 for $39.95, and $1 per unit postage and handling ($2.50 per unit outside the U.S.A.).

Name _____
(please print legibly or type)

Address _____
(no P.O. boxes, please)

City/State/Zip _____

PA residents add 6% sales tax

Jesse Jones Industries
499 East Erie Avenue
Philadelphia, PA 19134
Department Code: Mosby

UNIVERSITY OF CINCINNATI COLLEGE OF MEDICINE

The Department of Obstetrics and Gynecology at the University of Cincinnati College of Medicine offers academic opportunities for faculty in **General Obstetrics and Gynecology, Maternal/Fetal Medicine, Oncology and Reproductive Endocrinology and Infertility.** All positions offer candidates the opportunity to teach, maintain clinical skills and perform basic and clinical research at a major tertiary care, regional referral teaching hospital. All divisions within the department have experienced faculty and continue to develop.

Applicants must be board certified or candidates for examination, have teaching experience, have a strong background in clinical care and be eligible for an Ohio license. Candidates will be active participants in all departmental and divisional teaching conferences, seminars and lectures.

Academic rank is commensurate with the candidate's qualifications and experience. Compensation includes an excellent fringe benefit package as well as competitive salary. Positions will remain open until filled.

Interested candidates should direct their curriculum vitae to:

Robert W. Rebar, M.D.
Professor and Chairman
Department of Obstetrics and Gynecology
University of Cincinnati College of Medicine
Cincinnati, Ohio 45267-0526

An equal opportunity/affirmative action employer

OBSTETRICIAN-GYNECOLOGIST

Opening for qualified Obstetrician/Gynecologist in the Department of Ob/Gyn in a newly established affiliate clinic of a large multispecialty group in western Kentucky.

Competitive salary and attractive benefit package including malpractice insurance. Partnership eligibility in two years and no buy-in is required.

Opportunity to practice state-of-the-art medicine in a semi-rural setting. Large lakes nearby which offer recreational opportunities—metropolitan areas easily accessible. Community is family oriented with relaxed lifestyle not available in large cities.

Interested parties send curriculum vitae to:

Medical Director
TROVER CLINIC
Madisonville, Kentucky 42431
Phone: 502-825-7470

ACADEMIC FACULTY POSITION:

University of Florida College of Medicine, Jacksonville Division, announces the availability of faculty positions in the Department of Obstetrics/Gynecology. Board eligible or certified in Maternal-Fetal Medicine or Gynecologic Oncology. The positions involve high risk obstetrical practice or oncology, consultative responsibility and administrative duties. Academic rank Asst Prof/Assoc Prof/Professor dependent upon experience. Salary negotiable. Application deadline of 07/31/89, anticipated start date 08/31/89. Interested applicants are invited to submit a CV plus names/addresses of 3 references to: Chairman, Search Committee, Department of Ob/Gyn, University Hospital, 655 W. 8th Street, Jacksonville, FL 32209.

AFFIRMATIVE ACTION/EQUAL OPPORTUNITY EMPLOYER

UCLA-INTERCAMPUS MEDICAL GENETICS TRAINING PROGRAM

The UCLA Inter-campus post-doctoral research training program in Medical Genetics utilizes the research and clinical resources of its affiliated campuses and teaching hospitals. This program is open to academically oriented applicants with the M.D., Ph.D., D.D.S. or equivalent degrees. A wide variety of research training opportunities in molecular, biochemical, immuno-, cancer, cyto-, somatic cell, population and clinical genetics are available. The program also meets all the requirements of the American Board of Medical Genetics. Clinical genetics fellowships are available at each of the affiliated hospitals. Application forms are available from David Rimoin, M.D., Ph.D., Department of Pediatrics, Cedars-Sinai Medical Center, 8700 Beverly Blvd., Los Angeles, CA 90048.

KAISER PERMANENTE

OBSTETRICIAN-GYNECOLOGIST: BC/BE physician wanted full time or part time for satellite medical office practice in Gilroy, California, as part of The Permanente Medical Group, Inc., of San Jose, California. Excellent general Ob/Gyn outpatient skills required plus willingness to work with experienced Family Practice Physicians and Ob/Gyn nurse practitioners. Hospital call optional. Gilroy is located twenty minutes south of San Jose and shares excellent access to the Bay Area recreational activities. Salary is competitive and benefits include full coverage malpractice insurance.

Contact:
Jerome L. Nehemiah, M.D.
Chief
Department of OB/GYN
The Permanente Medical Group, Inc.
260 International Circle
San Jose, CA 95119

Or call: 408-972-6210 or 408-972-6113

PERINATOLOGIST
Denver, Colorado

Rose Medical Center, Denver, Colorado is seeking a second BE/BC perinatologist to work full-time in a 400-bed private community hospital adjacent to the University of Colorado Health Sciences Center. With 3,600 deliveries per year, and 27 University Residents rotating through the department annually, Rose is the major private teaching affiliate of the University.

Responsibilities include private consultation, physician, resident, and nursing education, clinical research, and a limited private practice in perinatology. This individual can expect frequent interaction with the Maternal/Fetal Medicine Division at the University of Colorado, and will be eligible for an appropriate clinical appointment at the University. All clinical responsibilities will be shared with the current full-time perinatologist at Rose.

A competitive salary guarantee is being offered, with excellent fringe benefits including malpractice insurance.

Qualified applicants, including individuals completing fellowship in June, 1989, should forward CV's to:

Robert E. Wall, M.D., Chairman
Department of Obstetrics & Gynecology
Rose Medical Center
4567 East Ninth Avenue
Denver, Colorado 80220
Telephone: (303) 320-2077

3RD YEAR RESIDENCY POSITION
OBSTETRICS/GYNECOLOGY
Providence Hospital
Southfield, Michigan

Providence is a 462 bed private teaching hospital located in Southfield, pop. 80,000, in Southeastern Michigan. New LD, perinatology, and neonatal facilities. Approximately 4,500 deliveries annually.

Program is fully accredited, offers 16 positions with 4 chiefs, and is affiliated with Wayne State University School of Medicine.

Position available July 1, 1989.

For further information, contact:

Department of Medical Education
Providence Hospital
16001 West Nine Mile Road
Southfield, Michigan 48075
(313) 424-3216

MATERNAL-FETAL MEDICINE
(Board eligible or Certified)

600 Bed University-affilited Level III Hospital with 3,600 deliveries/year, 24-hour in-house Anesthesiology and Neonatology/large NICU, active maternal transfer service, perinatal outreach programs, and fully-approved OB/GYN residency program has immediate opening for a full-time position in Perinatology. Duties to include residency teaching, maternal transfer care, and private practice incentive. Competitive guaranteed salary plus incentive. Excellent public school system, housing, and Lake St. Clair in very close proximity.

CALL COLLECT: 313-343-3495
MINU KASHEF, M.D.
ST. JOHN HOSPITAL
DEPT. OB/GYN
DETROIT, MI 48236

Maternal-Fetal Medicine

The Department of Obstetrics and Gynecology at the University of Tennessee Medical Center at Knoxville, Tennessee is currently accepting applications for an additional Maternal-Fetal Medicine Specialist.

This position requires Board certification or eligibility in Maternal-Fetal Medicine. Responsibilities include resident and medical student education, participation in Regionalization Program and clinical or basic research. Specialized areas of interest such as critical care, ultrasonography and Doppler studies, and prenatal diagnosis are encouraged. A private practice opportunity is available.

Academic rank is commensurate with qualifications and experience.

Interested persons should send curriculum vitae to:

Michael R. Caudle, M.D.
Associate Professor and Gynecology
Department of Obstetrics and Gynecology
For more information call (615) 544-9306

THE UNIVERSITY OF TENNESSEE
Medical Center at Knoxville
1924 ALCOA HIGHWAY
KNOXVILLE, TENNESSEE 37920
EEO/An Affirmative Action/Title IX/Section 504 Employer

Join a Leader
We're The Ohio Permanente Medical Group, Inc.

and we are looking for board certified/board eligible **Obstetrician/Gynecologists** to help us keep up with rapid growth of the Kaiser Permanente Program in Northeastern Ohio. OPMG is the multispecialty group practice that provides health care services to the more than 185,000 Kaiser members in the Cleveland-Akron area.

Our wealth of experience of over 40 plus years (25 in Ohio) makes Kaiser Permanente a mature, solid, leader in the managed care sector of the health care industry. The rewards of practice with us are substantial—excellent salary and benefit packages, company-paid retirement plan, full malpractice coverage, a stimulating, collegial environment in which to practice quality medicine, and more.

Kaiser Permanente's Ohio Region is located in the heart of the dynamic, resurgent industrial Midwest. The area offers the best of big city sophistication and culture in an affordable, accessible living area.

Please send your resume to: Ronald G. Potts, M.D., Medical Director, Ohio Permanente Medical Group, Inc., 1300 E. 9th Street, Suite 1100, Cleveland, OH 44114. Or you may call us collect at (216) 623-8780.

KAISER PERMANENTE®

CHIEF OF MATERNAL FETAL MEDICINE
UNIVERSITY OF TEXAS MEDICAL BRANCH AT GALVESTON

Faculty position in the Division of Maternal-Fetal Medicine in the Department of Obstetrics and Gynecology at the University of Texas Medical Branch at Galveston. A highly qualified individual is sought to become the Chief of the Maternal Fetal Medicine Division. Successful candidates should be board certified or eligible in maternal fetal medicine. They should have a proven record of research and commitment to an academic career. Academic rank is commensurate with qualification and experience. Interested candidates please send curriculum vitae to Garland D. Anderson, M.D., Professor and Chairman, Department of Obstetrics and Gynecology, University of Texas Medical Branch at Galveston, Galveston, Texas 77550.

UTMB is an equal opportunity M/F/H/V Affirmative Action employer. UTMB hires only individuals authorized to work in the United States.

PERINATAL GENETICIST

The Department of Obstetrics and Gynecology at Pennsylvania Hospital is seeking an obstetrician with genetic fellowship training to join an active perinatal genetics program.

Skills in second trimester amniocentesis, chorionic villus sampling, and Level II ultrasound are desired.

Responsibilities include resident and medical student education and active clinical practice with a University Appointment at the level of Assistant Professor.

Please send resumes to:

Ronald J. Bolognese, M.D.
Department of Obstetrics
and Gynecology
Pennsylvania Hospital
8th & Spruce Streets
Philadelphia, PA 19107

OBSTETRICIAN GYNECOLOGIST

Expanding Western Massachusetts staff model Health Maintenance Organization is seeking a full-time Board Certified/Board Eligible Obstetrician/Gynecologist to join a staff of eight (8) physicians and six (6) midwives.

Our multi-specialty group practice employs seventy (70) full-time physicians and provides care for a growing membership of over 70,000 from modern suburban facilities, in-patient care is provided at 900-bed Tufts affiliated teaching hospital.

Attractive Pioneer Valley location with fine educational, cultural and recreational opportunities. Excellent salary and comprehensive benefit program. Please send curriculum vitae to: Oded Rencus, M.D., Chief of OB/GYN, 305 Bicentennial Highway, Springfield, MA 01118.

Medical West
Community Health Plan
A BLUE CROSS & BLUE SHIELD HMO

We are an equal opportunity/affirmative action employer

HAVE YOUR MEDICAL SCHOOL LOANS REPAID

Practice obstetrics in a rural medical center. Receive attractive compensation package, PLUS have your medical school loans repaid! Modern, fully-equipped facilities offering full spectrum of obstetrical services regardless of ability to pay. Federal or state funding with full-time administrator and local governing board.

Call Joe Robbins or Judi Ashbaugh collect to discuss opportunities, the advantages of living in North Carolina, and our sensitive placement services. If your professional and personal needs match those of the communities, the State will subsidize your interview expenses.

(919) 733-2040
NORTH CAROLINA STATE
OFFICE OF HEALTH RESOURCES
DEVELOPMENT

OBSTETRICIAN/ GYNECOLOGIST
KAISER PERMANENTE

Kaiser Permanente's Kansas City Region is experiencing continuous membership growth and is seeking an additional BC/BE OB-GYN Physician. Our six medical offices are strategically located throughout the city, and two area hospitals are utilized. Kaiser Permanente is a nation-wide health care program that has enjoyed success since its inception in 1942. We offer excellent salary and benefits, fully-paid malpractice insurance, and are academically affiliated. Send CV or contact Larry McDonald, M.D., 6900 Squibb Road, Suite 201, Shawnee Mission, Kansas 66202. (913) 384-9090.

Michael Reese Health Plan

The **Michael Reese Health Plan,** a large well-established HMO serving its members in 20 Health Centers throughout the Chicago Metro area, is seeking additional Board Certified/Eligible physicians in the following specialty:

Obstetrics Gynecology

Physicians with the Health Plan enjoy a full practice with modern facilities and equipment as well as opportunities for teaching.

A highly-competitive salary and excellent benefits complement a rewarding practice and lifestyle.

Interested applicants should address inquiries to:

Scott P. Smith, M.D.
MICHAEL REESE HEALTH PLAN
2545 Dr. Martin Luther King Dr.,
Chicago 60616
or call (312) 808-4551

an equal opportunity employer m/f/h

Perinatologist

The Department of Obstetrics and Gynecology of Long Island Jewish Medical Center is recruiting a third Perinatologist to join the Division of Maternal/Fetal Medicine. The Medical Center is an 825-bed institution with approximately 4,000 deliveries each year, 40% of which are "High Risk." An established antepartum testing unit operated by the Division offers sophisticated ultrasonographic facilities and doppler technologies for fetal blood flow assessment. Our neonatal ICU is one of the largest and most advanced in the entire metropolitan area.

Responsibilities include student and resident teaching, prenatal diagnosis, perinatal consultation and a strong interest in research.

Excellent benefit package includes competitive salary, pension plan and ample time for research and scientific meetings. Contact: **Dr. Adiel Fleischer, Dept. Ob/Gyn, (718) 470-7660. LIJMC, 270-05 76th Avenue, Room 1100, New Hyde Park, NY 11042.**

Long Island Jewish Medical Center

Long Island Campus for the Albert Einstein College of Medicine

Equal Opportunity Employer

Obstetrics/ Gynecology

WISCONSIN: Marshfield Clinic, a multi-specialty group practice with 300 physicians, is seeking BE/BC obstetrician/gynecologists to join expanding regional centers. Positions available in three locations: Two in northwestern Wisconsin within 90 miles of Minneapolis and one, a group practice of 25 physicians, in north central Wisconsin, about 1 1/2 hours from Lake Superior. Communities offer exceptional recreational, cultural, and educational opportunities. An emphasis on quality practice and life-style is combined with a total compensation package on reaching membership in excess of $200,000. Starting salary negotiable. No start-up expense. Send C.V. and references to:

David L. Draves
Director Regional Development

1000 North Oak Avenue
Marshfield, WI 54449

or call collect at (715) 387-5376

Marshfield Clinic

OB/GYN

46-physician multi-specialty group located 40 miles north of Sacramento seeks a physician to join our OB/GYN Department. Excellent compensation package the first year. Partnership opportunity thereafter. Contact:

Warren J. Boyer, Jr., M.D.
Marysville Medical Group
800 Third Street
Marysville, CA 95901

MARYSVILLE MEDICAL GROUP

OBSTETRICS AND GYNECOLOGY WISCONSIN

We are looking for a Board Certified/ Board Eligible obstetrician/gynecologist to join a 7 physician OB/GYN Department in a large multi-specialty group practice located near Milwaukee, Wisconsin, to share coverage at two clinic locations and two hospitals. Competitive salary and fringe benefits.

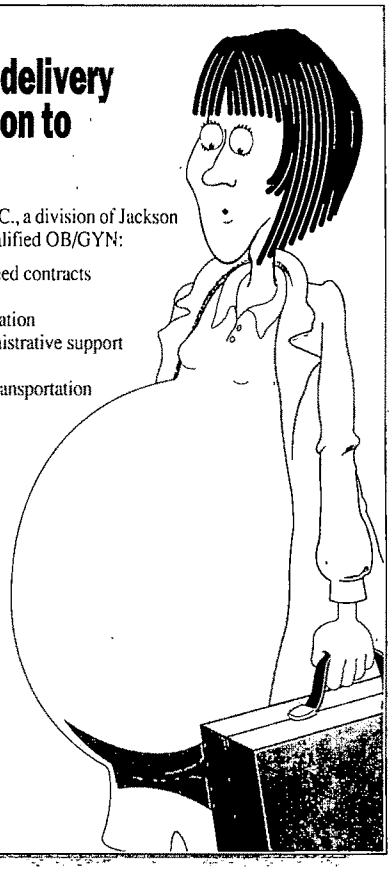
Forward inquiries and CV to:

Administrator
P.O. Box 427
Menomonee Falls, WI 53051

PHYSICIANS
Department of OB/GYN

We are currently seeking several full-time board certified/eligible OB/GYN Physicians. Academic appointment to the faculty of Columbia University is anticipated at either the level of Instructor or Asst. Clinical Professor, contingent upon experience and credentials. Candidates may apply to: Sterling B. Williams, MD, Director, Dept. of OB/GYN, Harlem Hospital Center, 506 Lenox Avenue, New York, NY 10037. We take affirmative action to ensure equal opportunity.

Be in on the delivery of the solution to OB staffing.



LOCUM TENENS, INC., a division of Jackson & Coker, offers the qualified OB/GYN:

- 3 to 9 month guaranteed contracts
- competitive daily rate
- licensing and coordination
- knowledgeable administrative support
- round trip airfare
- on-site housing and transportation
- permanent practice opportunities

Call 1-800-544-1987
or write to:
Locum, Tenens, Inc.
400 Perimenter
Center Terrace
Suite 760 OGLT9
Atlanta, Georgia 30346

LOCUM TENENS, INC.
A Division of Jackson and Coker

SPECIAL DELIVERIES. SPECIAL CHALLENGES.

The Southern California Permanente Medical Group invites you to join a unique prepaid health care program. Our multispecialty group practice is a partnership composed of and managed by SCPMG physicians.

Board eligible/certified **PERINATOLOGISTS** are needed for our high-volume obstetric services which handles 3,000-6,000 deliveries per year. Opportunities exist both to join and create high risk services.

There are positions available to practice Perinatology exclusively. Perinatologists who are interested in performing other aspects of obstetrics and gynecology may do so. We also have positions available for Endocrinologists and General Obstetrician-Gynecologists. University affiliation and clinical research are encouraged.

Our compensation is competitive and benefits are outstanding. They include: professional liability, medical and dental coverage, vacation and sick leave, continuing education, life insurance and retirement plans. **After two years full-time employment, physicians are eligible to be considered for partnership.**

For a physician application, please call or write to: William G. McCormick, M.D., Physician Recruitment, Dept. 006, Walnut Center, Pasadena, CA 91188-8013. (714) 829-5365.

Equal Opportunity Employer M/F/H

KAISER PERMANENTE
Good People. Good Medicine.

DIRECTOR OF PERINATOLOGY SERVICES

The Irvine Medical Center, located in Irvine, California, is seeking a full-time, Board Certified Perinatologist to oversee the perinatology services of a 177-bed hospital with a current Medical Staff of 600 physicians. The Irvine Medical Center is currently under construction and is the only hospital in Irvine, a rapidly growing, well established community located in Orange County. The Perinatology Director will have the opportunity to develop the perinatology services for the Medical Center complex.

Please send CV to: Perinatology Search Committee, Irvine Medical Center, 4605 Barranca Parkway, Suite 200, Irvine, CA 92714.

BC/BE OBSTETRICIAN/GYNECOLOGISTS to join an expanding and progressive 200 member multispecialty clinic with a multisatellite referral system encompassing a service population of 250,000. Clinic and adjoining 210 bed hospital contains all aspects of Ob/Gyn office and hospital care including a Level III nursery, laser surgery, and a superb GIFT and infertility program. All practice costs paid. Tri-College community located 40 miles from beautiful Minnesota lake district. Please send CV to:

John L. Weir
Associate Administrator
Dakota Clinic, Ltd.
P. O. Box 6001
Fargo, ND 58108

Life.

It's our life's work.

If your mission in life is to practice the ideals of medicine without administrative demands, we invite your inquiry.

Group Health Plan in St. Louis is a staff model HMO that promotes the ideals of private practice while freeing doctors from the financial baggage of the private practitioner.

Group Health Plan isn't for every physician. Just for those few who want to practice medicine without compromise.

For an interview please talk with Tim Walton at (314) 993-5955. Or write Group Health Plan, 11475 Olde Cabin Road, St. Louis, Mo. 63141. An equal opportunity employer.

GROUP HEALTH PLAN

REPRODUCTIVE ENDOCRINOLOGIST

Fargo Clinic—MeritCare is seeking a BE/BC Reproductive Endocrinologist to join our staff of seven Obstetrician-Gynecologists in the Department of Ob-Gyn. Fargo Clinic is a 240 physician multi-specialty group practice located next to St. Luke's Hospital. St. Luke's is a 400 + bed level III hospital with a designated Children's Hospital. The department of Ob/Gyn is active in donor and husband insemination. Excellent opportunity exists to develop Invitro and GIFT Program.

Fargo-Moorhead is a tri-college community of 130,000. The sister cities and surrounding area offer a wide range of cultural and recreational activities.

Guaranteed first year salary with attractive long term compensatory arrangements. Reply to Robert C. Montgomery, M.D., 737 Broadway, Fargo, ND 58102 or call 1-800-437-4010 ext. 2151.

Fargo Clinic
MeritCare

OBSTETRICS/ GYNECOLOGY

This 175 physician, multi-specialty group seeks an Obstetrician and Gynecologist desiring 1989 employment opportunities. The group is hospital based in Northeastern Pennsylvania at the New York border. Our state-of-the-art facility offers medical school teaching affiliations as well as clinical research opportunities. Residency programs are conducted in Internal Medicine and General Surgery. Physicians seeking challenging and rewarding practice opportunities, excellent salaries and benefits, combined with rural living and endless recreational opportunities are encouraged to submit a letter and curriculum vitae to:

Guthrie Clinic
Sayre, PA 18840
(717) 888-5858
ATTN: G. V. Ippolito
Assistant Vice President

BRONSON
Management Services Corporation

OBSTETRICIAN-GYNECOLOGIST
Kalamazoo, Michigan

Bronson Methodist Hospital is actively recruiting board eligible/board certified OB/GYNs for two group practices. Competitive salaries, malpractice insurance, and moving expenses are among the many benefits that these practices offer.

Bronson Methodist Hospital, the Perinatal Center for southwestern Michigan, has a 500 member medical staff which includes 23 obstetrician/gynecologists, six neonatologists and two perinatologists. An average of 3,200 deliveries are performed every year. Bronson's Level-3 expertise and capability in perinatal and neonatal intensive care is regionally and nationally recognized.

Kalamazoo, located midway between Chicago and Detroit, offers diverse cultural opportunities. Lake Michigan's sandy beaches and dunes are only minutes away and numerous lakes, hills, and forests provide boundless recreational activities. Excellent schools, several colleges, and a major university further contribute to a high quality life style.

For more information, in confidence and no cost or obligation, respond with curriculum vitae or call:

Debra C. Hartman
Bronson Management Services Corporation
210 East Vine Street
Kalamazoo, Michigan 49001
(616) 344-6444

Central Minnesota
Group Health Plan

OB/GYN

Rapidly growing, innovative primary care clinic has openings for two board eligible/certified OB/GYN physicians. New clinic facility in the fastest growing urban area in Minnesota. St. Cloud is a community of 45,000 located on the upper Mississippi River 70 miles from Mpls./St. Paul. Be close to lakes, ski areas and excellent schools, including three universities. Join a supportive primary care staff and receive competitive salary, outstanding benefits, liberal vacation and educational leave, malpractice coverage, etc. Contact Physician Services, Central Minnesota Group Health Plan, 1245 15th Street North, St. Cloud, MN 56303. 612/253-5220. EOE.

COLUMBIA UNIVERSITY
The College of Physicians & Surgeons

Sloane Hospital for Women 1888-1989 a division of The Presbyterian Hospital

INFECTIOUS DISEASES IN OBSTETRICS & GYNECOLOGY: CURRENT CONCEPTS & NEW GOALS

November 9 & 10, 1989

Grand Hyatt, New York, New York

15 Credit Hours, Category 1, A.M.A.'s Physician's Recognition Award; and
13 Cognates, Formal Learning, American College of Obstetricians & Gynecologists

FEE: $540; Residents & Fellows in Training: $350. Tuition fee includes a syllabus, luncheons and refreshments.

Conference Director: **MARVIN H. TERRY GRODY, M.D.** *Conference Co-Director:* **ANITA PARNES LaSALA, M.D.**

DAVID A. ESCHENBACH, M.D.
RONALD S. GIBBS, M.D.
MARVIN H. TERRY GRODY, M.D.
ANITA PARNES LaSALA, M.D.
JOAN A. REGAN, M.D.
RALPH M. RICHART, M.D.
JOHN L. SEVER, M.D.
RICHARD L. SWEET, M.D.
STERLING B. WILLIAMS, M.D.

Of all the various problems we encounter in obstetrics and gynecology, the most ubiquitous and seemingly ever increasing, is infection. When we're almost convinced we are in control of a problem, resistant strains appear, or a new infectious agent is discovered. To help solve this dilemma, we are offering this comprehensive course in infectious diseases in ob/gyn. Experts in this new subspecialty have been assembled from across the nation to provide current diagnostic and therapeutic information. This is a practical course for the practitioner, and the goal is to send each participant home far better equipped than he or she ever was before to combat the myriad of infectious diseases in the general practice of ob/gyn.

Contact: Center for Continuing Education, 630 West 168th Street, N.Y., N.Y. 10032; Telephone: (212) 305-3682.

BRIDGEPORT HOSPITAL
Bridgeport Connecticut

DEPARTMENT OB-GYN
is seeking a full time

DIRECTOR OF AMBULATORY SERVICES

Bridgeport Hospital, located on Long Island Sound 50 miles from New York, is a 550 bed community hospital affiliated with Yale University.

The hospital has accredited residency training programs in Internal Medicine, Primary Care, Surgery, Pediatrics, Radiology, and Ob-Gyn.

The Ob-Gyn Dept. has 22 BC/BE attendings, including 4 full-time faculty, 2 P.A.'s, and 8 residents.

We are a Level III unit with approx. 3000 deliveries and 8000 clinic visits annually.

Primary responsibilities of this position include supervision and teaching of residents and students in the ambulatory clinics.

For further information contact:

Philip M. LaMastra M.D.
Chairman, Dept. Ob-Gyn
Bridgeport Hospital
Bridgeport, Connecticut 06610

203-384-3042

OPPORTUNITY AVAILABLE
DISPLAY ADVERTISING

1989 Rates Per Insertion

	1/4 page	1/2 page
1 time rate	$550.00	$775.00
3 time rate	$545.00	$765.00

CLOSING DATE: 10th of month preceding month of issue

Forward ads to:

THE AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY
Journal Advertising Department
The C.V. Mosby Company
11830 Westline Industrial Drive
St. Louis, MO 63146
(314)-872-8370

Mosby

A TRADITION OF PUBLISHING EXCELLENCE

Please call the Journal Advertising Department for advertising production charges (i.e. type, film from camera ready art.)

Healthline

helping keep well people well

"Good reading for doctor, nurse and patient"*

HEALTHLINE is a monthly, 16-page publication offering up-to-date information on a wide variety of health issues of concern to your patients.

Presented in an attractive two-color format, HEALTHLINE features a concise, easy-to-read style that's ideal for patient education. HEALTHLINE makes excellent waiting room literature.

- Provides the latest health news on disease prevention, nutrition, exercise, mental health, aging, child health, and other topics of interest.
- Alerts your patients to warning signs and symptoms requiring professional attention.
- Presents reliable, *readable* articles written by a national network of doctors, nurses, psychologists, and other health professionals. Paul M. Insel, Ph.D., of the Stanford University School of Medicine serves as Editor in Chief.

HEALTHLINE is timely, inviting, enlightening—and *affordable!*

Regular annual subscription rate: $19.00 U.S.
$31.00 International

Mosby offers a *special rate* on multiple-copy orders of HEALTHLINE for waiting room use or for individual handouts. (See chart.) Use the coupon on this page to place an order or to request a sample issue.

Or, in the U.S., call us toll-free at 1-800-325-4177, ext. 351. Outside the U.S., call (314) 872-8370, ext. 351.

Number of Subscriptions	Price Per Subscription
10–25	$17.25
26–50	$16.00
51–100	$15.25
101–500	$13.75
501–1000	$12.35
Over 1000	Contact Publisher

Mosby

*Louis Chardon, MD, Redwood City, California
Just one of the positive comments from physicians concerning the contents of HEALTHLINE.

YES!

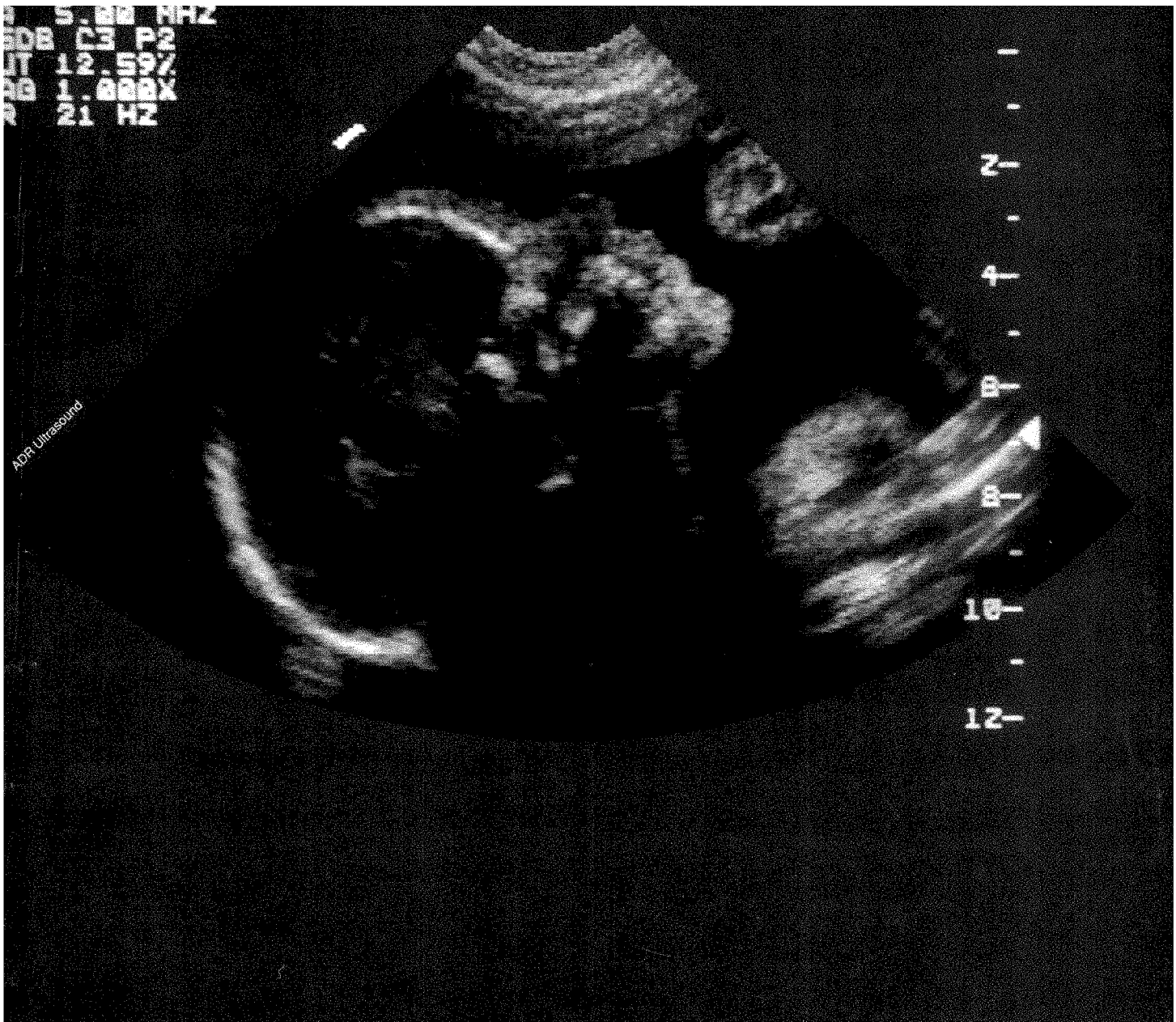
☐ Start my individual subscription to HEALTHLINE (12 issues for one year).
Annual rate: $19.00 U.S., $31.00 International
☐ I'm placing a multiple-copy order for HEALTHLINE.
No. of subscriptions: _____
Price per subscription: _____
☐ Please send me a sample issue of HEALTHLINE.

Payment Method:
☐ MasterCard ☐ VISA ☐ Check Enclosed ☐ Bill Me

Card # _____ Exp. date _____
Signature (if using MC/VISA) _____
Name (print legibly) _____
Address _____
City _____ State _____ ZIP _____
Specialty _____

Mail to: The C.V. Mosby Company
Journal Circulation Department
11830 Westline Industrial Drive
St. Louis, MO 63146

HEA-013



ALREADY HE'S PICKING UP HER BAD HABITS.

Maybe if his mother knew in advance that cocaine use during pregnancy could result in spontaneous abortion, premature delivery, retarded fetal growth, or birth defects, cocaine would never enter the picture.

Don't allow him to pick up her bad habits. Make it your habit to warn her in time.

PARTNERSHIP FOR A DRUG-FREE AMERICA

INDEX TO ADVERTISERS

Berlex Laboratories, Inc.
Tri-Levlen 18A,19A,20A

Bridgeport Hospital
Opportunity Available 58A

Bronson Management Services Corporation
Opportunity Available 57A

Central Minnesota Group Health Plan
Opportunity Available 57A

College of Physicians & Surgeons
Course 45A,57A

Cook County Graduate School of Medicine, The
Course 29A

Dakota Clinic, Ltd.
Opportunity Available 56A

Fargo Clinic
Opportunity Available 56A

Group Health Plan
Opportunity Available 56A

Guthrie Clinic
Opportunity Available 56A

Harlem Hospital Center
Opportunity Available 55A

IdenTechs
Right Light 29A

Institute for International Medical Exchange
International Congress 46A

Irvine Medical Center
Opportunity Available 55A

Kaiser Permanente
Opportunity Available 55A

Kaiser Permanente, Kansas City Region
Opportunity Available 53A

Locum Tenens, Inc.
Opportunity Available 55A

Long Island Jewish Medical Center
Opportunity Available 54A

Marshfield Clinic
Opportunity Available 54A

Marysville Medical Group
Opportunity Available 54A

Medical West
Opportunity Available 53A

Michael Reese Health Plan
Opportunity Available 53A

National Institutes of Health
Fellowship 45A

North Carolina State Office of Health Resources
Opportunity Available 53A

Ohio Permanente Medical Group, Inc.
Opportunity Available 52A

Ortho Pharmaceutical Corporation
All-Flex 6A
Ortho-Novum 7/7/7 ..... Third Cover, Fourth Cover

Pennsylvania Hospital
Opportunity Available 52A

Permanente Medical Group
Opportunity Available 51A

Providence Hospital
Opportunity Available 51A

Rose Medical Center
Opportunity Available 51A

Searle & Co.
Demulen 1/35 ..... 33A,34A,35A,36A

Serono Laboratories, Inc.
Serophene 3A,4A

St. John Hospital
Opportunity Available 51A

Syntex Laboratories, Inc.
Femstat 38A,39A,40A
Tri-Norinyl ..... Second Cover,1A,2A

Trover Clinic
Opportunity Available 50A

2V Medtech International Corp.
2V Stat 37A

U.S. Air Force
Opportunity Available 22A,23A

University of California Los Angeles
Opportunity Available 50A

University of Cincinnati College of Medicine
Opportunity Available 50A

University of Florida College of Medicine
Opportunity Available 50A

University of Illinois College of Medicine at Chicago
Opportunity Available 49A

University of Medicine and Dentistry of New Jersey
Fellowship 48A

University of Miami School of Medicine
Opportunity Available 49A

University of Minnesota
Opportunity Available 49A

University of Tennessee Medical Center at Knoxville
Opportunity Available 48A,52A

University of Texas Medical Branch at Galveston
Opportunity Available 52A

West Penn Hospital
Opportunity Available 48A

West Virginia University School of Medicine
Opportunity Available 48A

Wyeth-Ayerst Laboratories
Lo/Ovral 30A,31A,32A
Triphasil 41A,42A,43A,44A

While every precaution is taken to insure accuracy, we cannot guarantee against the possibility of an occasional change or omission in the preparation of this index.

21- and 28-day regimens

ORTHO-NOVUM® 7/7/7 Tablets

norethindrone/ethinyl estradiol

ach white tablet contains 0.5 mg norethindrone and 0.035 mg ethinyl estradiol. Each light peach tablet ontains 0.75 mg norethindrone and 0.035 mg ethinyl estradiol. Each peach tablet contains 1 mg nore- hindrone and 0.035 mg ethinyl estradiol. Each green tablet in 28-day regimen contains inert ingredients.

MPORTANT NOTE—This information is a BRIEF SUMMARY of the complete prescribing information rovided with the product and therefore should not be used as the basis for prescribing the product. his summary was prepared by deleting from the complete prescribing information certain text, tables nd references. The physician should be thoroughly familiar with the complete prescribing information efore prescribing the product.

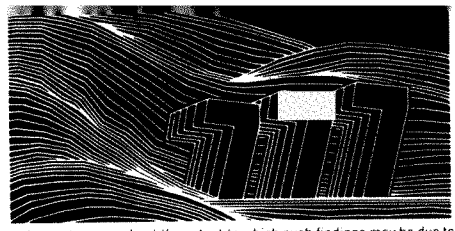
NDICATIONS AND USAGE: PREVENTION OF PREGNANCY.

:ONTRAINDICATIONS: Oral contraceptives should not be used in women who currently have the following onditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophle- itis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease.4. Known or suspected arcinoma of the breast. 5. Carcinoma of the endometrium or other known or suspected estrogen-depen- lent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Cholestatic jaundice of pregnancy or jaundice vith prior pill use. 8. Hepatic adenomas or carcinomas. 9. Known or suspected pregnancy.

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contracep- tive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia, and gallbladder disease, although the risk of serious morbidity or mortality is very small in healthy women without underlying risk factors. The risk of morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, obesity and diabetes. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks. The information contained in this brief summary is principally based on studies carried out in patients who used oral contraceptives with higher formulations of estrogens and progestogens than those in common use today. The effect of long term use of the oral contraceptives with lower formulations of both estrogens and progestogens remains to be determined. Throughout this brief summary epidemiological studies reported are of two types: retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of a disease, namely a *ratio* of the incidence of a disease among oral contra- ceptive users to that among nonusers. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide a measure of attributable risk, which is the *difference* in the incidence of disease between oral contraceptive users and nonusers. The attributable risk does pro- vide information about the actual occurrence of a disease in the population. For further information, the reader is referred to a text on epidemiological methods. 1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. a. Myocardial Infarction. An increased risk of myocardial infarction has been associated with oral contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary artery disease such as hypertension, hypercholesterolemia, morbid obesity, and diabetes. The relative risk of heart attack for current oral contraceptive users has been estimated to be two to six. The risk is very low under the age of 30. Smoking in combination with oral contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women in their mid-thirties or older with smoking accounting for the majority of excess cases. Mortality rates associated with circulatory disease have been shown to increase substantially in smokers, especially in those 36 years of age and older among women who use oral contraceptives. Oral contraceptives may compound the effects of well-known risk factors, such as hypertension, diabetes, hyperlipidemias, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and cause glucose intolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see section 9 in WARNINGS). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors. b. Thromboembolism. An increased risk of thromboem- bolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to non-users to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. Cohort studies have shown the relative risk to be somewhat lower, about 3 for new cases and about 4.5 for new cases requiring hospital- ization. The risk of thromboembolic disease associated with oral contraceptives is not related to length of use and disappears after pill use is stopped. A two- to four-fold increase in relative risk of post-operative thromboembolic complications has been reported with the use of oral contraceptives. The relative risk of venous thrombosis in women who have predisposing conditions is twice that of women without such medical conditions. If feasible, oral contraceptives should be discontinued at least four weeks prior to and for two weeks after elective surgery of a type associated with an increase in risk of thromboembolism and during and following prolonged immobilization. Since the immediate postpartum period is also associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than four weeks after delivery in women who elect not to breast feed. c. Cerebrovascular diseases. Oral contracep- tives have been shown to increase both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes), although, in general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and non-users, for both types of strokes, and smoking interacted to increase the risk of stroke. In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for non- smokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hyper- tension. The attributable risk is also greater in older women. d. Dose-related risk of vascular disease from oral contraceptives. A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high density lipoproteins (HDL) has been reported with many progestational agents. A decline in serum high density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estro- gens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the activity of the progestogen used in the contraceptive. The activity and amount of both hormones should be considered in the choice of an oral contraceptive. Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral contraceptive agents should be started on prepara- tions containing 0.035 mg or less of estrogen. e. Persistence of risk of vascular disease. There are two studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contracep- tives persists for at least 9 years for women 40-49 years who had used oral contraceptives for five or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. However, both studies were performed with oral contraceptive formulations containing 50 micrograms or higher of estrogens. 2. ESTIMATES OF MORTALITY FROM CONTRACEPTIVE USE. One study gathered data from a variety of sources which have estimated the mortality rate associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attribu- table to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is low and below that associated with childbirth. However, smokers 35 and older and non-smokers 40 and older who use oral contraceptives have a significant increase in mortality higher than those using other methods of birth control. These facts must be weighed in conjunction with failure rates for other methods and the risk associated with subsequent pregnancy. 3. CARCINOMA OF THE REPRODUCTIVE ORGANS. Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. While there are conflicting reports the overall evidence in the literature suggests that use of oral contraceptives is not associated with an increase in the risk of developing breast cancer. The Cancer and Steroid Hormone (CASH) study also showed no latent effect on the risk of breast cancer for at least a decade following long term use. Some studies have shown a slightly increased relative risk of developing breast cancer. Some studies suggest that oral contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of



women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors. In spite of many studies of the relationship between oral contraceptive use and breast and cervical cancers, a cause and effect relationship has not been estab- lished. 4. HEPATIC NEOPLASIA. Benign hepatic adenomas are associated with oral contraceptive use, although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases/100,000 for users, a risk that increases after four or more years of use especially with oral contraceptives of higher dose. Rupture of benign, hepatic adenomas may cause death through intra-abdominal hemorrhage. Studies from Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (>8 years) oral contraceptive users. However, these cancers are rare in the U.S. and the attributable risk (the excess incidence) of liver cancers in oral contraceptive users approaches less than one per million users. 5. OCULAR LESIONS. There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contracep- tives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia, papilledema, or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately. 6. ORAL CONTRACEPTIVE USE BEFORE OR DURING EARLY PREG- NANCY: RISK OF BIRTH DEFECTS. Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. The majority of recent studies also do not indicate a teratogenic effect, particularly in so far as cardiac anomalies and limb reduction defects are concerned, when taken inadvertently during early pregnancy. The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion. It is recom- mended that for any patient who has missed two consecutive periods pregnancy should be ruled out before continuing oral contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral contraceptive use should be discontinued until pregnancy is ruled out. 7. GALLBLADDER DISEASE. Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral contraceptive formulations containing lower hormonal doses of estrogens and progestogens. 8. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. Oral contraceptives have been shown to cause a decrease in glucose tolerance in a significant percentage of users. This effect has been shown to be directly related to estrogen dose. Progestogens increase insulin secretion and create insulin resist- ance, this effect varying with different progestational agents. However, in the non-diabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects prediabetic and diabetic women in particular should be carefully monitored while taking oral contracep- tives. A small proportion of women will have persistent hypertriglyceridemia while on the pill. As dis- cussed earlier (see WARNINGS 1a and 1d), changes in serum triglycerides and lipoprotein levels have been reported in oral contraceptive users. 9. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in women taking oral contraceptives, and this increase is more likely in older oral contra- ceptive users and with extended duration of use. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing progestational activity. Women with a history of hypertension or hypertension-related diseases, or renal disease, should be encouraged to use another method of contraception. If women elect to use oral contraceptives, they should be monitored closely and if significant elevation of blood pressure occurs, oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives, and there is no difference in the occurrence of hypertension between former and never users. 10. HEADACHE. The onset or exacerbation of migraine or development of headache with a new pattern which is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause. 11. BLEEDING IRREGULARITIES. Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first three months of use. Non-hormonal causes should be considered and adequate diagnostic measures taken to rule out malig- nancy or pregnancy in the event of breakthrough bleeding as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out. Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was preexistent. 12. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. **PRECAUTIONS:** 1. PHYSICAL EXAMI- NATION AND FOLLOW UP. A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology, and relevant laboratory tests. In case of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care. 2. LIPID DISORDERS. Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult. 3. LIVER FUNCTION. If jaundice develops in any woman receiving such drugs, the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function. 4. FLUID RETENTION. Oral contracep- tives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention. 5. EMO- TIONAL DISORDERS. Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 6. CONTACT LENSES. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist. 7. DRUG INTERACTIONS. Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irreg- ularities have been associated with concomitant use of rifampin. A similar association, though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines. 8. INTERACTIONS WITH LABORATORY TESTS. Certain endocrine and liver function tests and blood components may be affected by oral contraceptives: a. Increased prothrombin and factors VII, VIII, IX, and X; decreased anti-thrombin 3; increased norepinephrine-induced platelet aggregability. b. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radio-immunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. c. Other binding proteins may be elevated in serum. d. Sex-binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged. e. Triglycerides may be increased. f. Glucose tolerance may be decreased. g. Serum folate levels may be depressed by oral contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives. 9. CARCINOGENESIS. See WARNINGS section. 10. PREGNANCY. Pregnancy Category X. See CONTRAINDICATIONS and WARNINGS sections. 11. NURSING MOTHERS. Small amounts of oral contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlarge- ment. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child. **INFORMATION FOR THE PATIENT: See Patient Package Insert. ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS section). Thrombophlebitis and venous thrombosis with or without embolism. Arterial throm- boembolism. Pulmonary embolism. Myocardial infarction. Cerebral hemorrhage. Cerebral thrombosis. Hypertension. Gallbladder disease. Hepatic adenomas or benign liver tumors. The following adverse reac- tions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea. Vomiting. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Amenorrhea. Temporary infertility after discontinuation of treatment. Edema. Melasma which may persist. Breast changes: tenderness, enlargement, secretion. Change in weight (increase or decrease). Change in cervical erosion and secretion. Diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intoler- ance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted: Pre-menstrual syndrome. Cataracts. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Ery- thema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal func- tion. Hemolytic uremic syndrome. Acne. Changes in libido. Colitis. **OVERDOSAGE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea and withdrawal bleeding may occur in females.

ORTHO PHARMACEUTICAL CORPORATION
Raritan, N.J. 08869-0602

Revised 11/88

Low-impact norethindrone

No significant change in mean values for total cholesterol, HDL, and LDL†



21- and 28-day regimens

ORTHO-NOVUM® 777 Tablets

norethindrone/ethinyl estradiol

†Serious as well as minor side effects have been reported with the use of oral contraceptives. The physician should remain alert to the earliest manifestations of any symptoms of serious disease and discontinue oral contraceptive therapy when appropriate.

Please see brief summary of complete Prescribing Information on the preceding page.

ORTHO PHARMACEUTICAL CORPORATION
Raritan, New Jersey 08869-0602

ORTHO

August 1989
volume 161, *number* 2

American Journal of OBSTETRICS AND GYNECOLOGY

Copyright © 1989 by The C.V. Mosby Company

Editor in Chief
JOHN I. BREWER

Editors
FREDERICK P. ZUSPAN • E. J. QUILLIGAN

Associate Editor
ALBERT B. GERBIE

Official Publication

AMERICAN GYNECOLOGICAL AND OBSTETRICAL SOCIETY
CENTRAL ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS
SOUTH ATLANTIC ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS
PACIFIC COAST OBSTETRICAL AND GYNECOLOGICAL SOCIETY
AMERICAN BOARD OF OBSTETRICS AND GYNECOLOGY
SOCIETY FOR GYNECOLOGIC INVESTIGATION
SOCIETY OF PERINATAL OBSTETRICIANS

Published by

THE C.V. MOSBY COMPANY
St. Louis, Missouri 63146-3318

ISSN 0002-9378





Even at midcycle, she wears white with confidence

Only Tri-Norinyl provides a full 1 mg of progestin at the midcycle (days 8–16), when clinical data indicate that breakthrough bleeding and spotting are most likely to occur.[1]

To help control midcycle breakthrough bleeding: make the midcycle the high point of protection.

Extra midcycle progestin to reduce midcycle breakthrough bleeding

9 DAYS
1.0 mg norethindrone

7 DAYS
0.5 mg norethindrone

5 DAYS
0.5 mg norethindrone

21 DAYS *0.035 mg ethinyl estradiol*

TRI-NORINYL®

(NORETHINDRONE AND ETHINYL ESTRADIOL) TABLETS

1. Data available from Syntex Laboratories, Inc., document #92363.

Serious as well as minor side effects have been reported following the use of all oral contraceptives. These include thromboembolic disease. Before prescribing Tri-Norinyl, please see brief summary of prescribing information on following page.

© 1989 Syntex Laboratories, Inc.

SYNTEX

SYNTEX LABORATORIES, INC.
PALO ALTO, CA 94304

CALCUTTA CENTRAL LIBRARY

TRI-NORINYL®

(NORETHINDRONE AND ETHINYL ESTRADIOL) TABLETS

TRI-NORINYL® 21-Day Tablets (7 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg, 9 green tablets of norethindrone 1.0 mg with ethinyl estradiol 0.035 mg, 5 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg)
TRI-NORINYL® 28-Day Tablets (7 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg, 9 green tablets of norethindrone 1.0 mg with ethinyl estradiol 0.035 mg, 5 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg, 7 inert tablets)
BREVICON® 21-Day Tablets (norethindrone 0.5 mg with ethinyl estradiol 0.035 mg)
BREVICON® 28-Day Tablets (21 norethindrone 0.5 mg with ethinyl estradiol 0.035 mg tablets followed by 7 inert tablets)
NORINYL® 1 + 35 21-Day Tablets (norethindrone 1 mg with ethinyl estradiol 0.035 mg)
NORINYL® 1 + 35 28-Day Tablets (21 norethindrone 1 mg with ethinyl estradiol 0.035 mg tablets followed by 7 inert tablets)
NORINYL® 1 + 50 21-Day Tablets (norethindrone 1 mg with mestranol 0.05 mg)
NORINYL® 1 + 50 28-Day Tablets (21 norethindrone 1 mg with mestranol 0.05 mg tablets followed by 7 inert tablets)
NORINYL® 1 + 80 21-Day Tablets (norethindrone 1 mg with mestranol 0.08 mg)
NORINYL® 1 + 80 28-Day Tablets (21 norethindrone 1 mg with mestranol 0.08 mg tablets followed by 7 inert tablets)
NORINYL® 2 mg Tablets (norethindrone 2 mg with mestranol 0.1 mg)
NOR-Q.D.® (norethindrone) Tablets 0.35 mg

ORAL CONTRACEPTIVE (O.C.) AGENTS

Indications Prevention of pregnancy. *DOSE-RELATED RISK OF THROMBOEMBOLISM.* Because studies have shown a positive association between OC estrogen dose and risk of thromboembolism, it is prudent to minimize estrogen exposure. Prescribe an OC with the least amount of estrogen compatible with an acceptable pregnancy rate and patient acceptance. Start new users on OCs containing 0.05 mg or less of estrogen.

Contraindications 1. Known or suspected pregnancy (see Warning #5). 2. Thrombophlebitis or thromboembolic disorders. 3. Past history of deep vein thrombophlebitis or thromboembolic disorders. 4. Undiagnosed abnormal genital bleeding. 5. OCs should not be used by women who have or have had any of the following: a. cerebral vascular or coronary artery disease, including myocardial infarction. b. known or suspected carcinoma of the breast. c. known or suspected estrogen dependent neoplasia. d. benign or malignant liver tumor that developed during use of OCs or other estrogen containing products.

WARNINGS: Cigarette smoking increases the risk of serious cardiovascular side effects from OC use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use OCs should be strongly advised not to smoke.

The use of OCs is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, liver tumor, gall bladder disease, visual disturbances, fetal abnormalities, and hypertension. Practitioners prescribing OCs should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with OC use is established. One study demonstrated an increased relative risk for fatal venous thromboembolism and several studies demonstrated it for non-fatal venous thromboembolism. They estimate that OC users are 4-11 times more likely than nonusers to develop these diseases without evident cause. One British study reported an excess death rate of 40% in OC users, most of which resulted from cardiovascular disease. Another British study showed a lower death rate in OC users than controls; an increase in cardiovascular deaths was seen but was not statistically significant. A U.S. prospective study failed to disclose increased mortality rates from cardiovascular disorders, but a subset analyzed as a retrospective, case-control study showed significant increases in venous thromboembolism. CEREBROVASCULAR DISORDERS. Two American studies demonstrated an increased relative risk for stroke not shown in prior British studies. In an American study of cerebrovascular disorders in women with and without predisposing causes, relative risk of hemorrhagic stroke was estimated as 2.0 times greater and thrombotic stroke as 4-9.5 times greater in users than nonusers. A British long-term follow-up study reported in 1976 a highly significant association between OC use and stroke. Another study had suggested an association in 1974, but the number of cases was too small to estimate the risk. Subarachnoid hemorrhage has been shown to be increased by OC use in British and American studies. Smoking alone increases incidence of these accidents; smoking and pill use appear to increase risk more than either alone.

MYOCARDIAL INFARCTION (MI). Increased relative risk of MI associated with OC use has been reported. One British study found that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing MI, regardless of OC use. OCs were an additional risk factor. In terms of relative risk, it has been estimated that nonsmoking OC users (smoking is considered a major predisposing condition to MI) are twice as likely to have a fatal MI as nonsmoking nonusers. OC users who are smokers have a 5-fold increased risk of fatal infarction compared to nonsmoking users, and a 10-12-fold increased risk compared to nonsmoking nonusers. The number of cigarettes smoked is important. In determining the importance of these relative risks, baseline rates for various age groups must be considered. (Estimates are based on British vital statistics which show acute MI death rates 2-3 times less than in the U.S. so U.S. death rates could be higher.) Importance of other predisposing conditions in determining relative and absolute risks has not been quantified; other synergistic actions may exist. RISK OF DOSE. Using data from several national adverse reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to estrogen dose in OCs. OCs with 0.1 mg or more of estrogen were associated with a higher risk of thromboembolism than those containing 0.05-0.08 mg but quantity of estrogen may not be the sole factor. This was supported by a U.S. study. A British study found a positive association between dose of progestogen or estrogen and certain thromboembolic conditions. Swedish authorities noted decreased reporting of thromboembolic episodes when higher estrogen preparations were no longer prescribed. Careful epidemiological studies to determine degree of thromboembolic disease risk associated with progestogen-only OCs have not been done. Thromboembolic disease has been reported in women using these products, and they should not be considered free of excess risk. PERSISTENCE OF RISK. Two studies have suggested an increased risk may persist for 6 years after discontinuation of OC use for cerebrovascular disease and 9 years for MI; another study suggested the persistence of risk for subarachnoid hemorrhage. ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES. A large British prospective study estimated mortality rate per 100,000 women per year from circulatory system diseases for OC users and nonusers according to age, smoking habits, and duration of use. The overall annual excess death rate for OC users was estimated to be 20/100,000 (ages 15-34 – 5/100,000; ages 35-44 – 33/100,000; ages 45-49 – 140/100,000). Risk is concentrated in long-term users and in smokers, and may persist after OC discontinuation. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all occurred in women 35 or older. An update provided the following rates: ages 15-34 – 1/6700 for nonsmokers and 1/2000 for smokers; ages 45 and over – 1/2500 for nonsmokers and 1/500 for smokers. Risk appeared to increase with parity, but not with duration of use. Until more women under 35 with continuous use for 5 or more years are available, it is not possible to assess relative risk for this age group. Data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. Estimates include combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease for OCs) plus risk attributable to pregnancy or abortion if the method fails (which varies with the effectiveness of the contraceptive method). The study concluded that mortality associated with all contraceptive methods is below that of childbirth, except for OCs in women over 40 who smoke. (Rates given for pill only/smokers for each age are for smokers as a class. For "heavy" smokers (more than 15 cigarettes a day), the rates would be about double; for "light" smokers (less than 15), about half.) The lowest mortality is with the condom or diaphragm backed up by early abortion. The study also concluded that OC users who smoke, especially over 30, have greater mortality risk than OC users who do not smoke.

Risk of thromboembolic and thrombotic disease associated with OCs increases with age after 30 and for MI is further increased by hypertension, hyperlipidemias, obesity, diabetes, or history of preeclamptic toxemia, and especially by smoking. The following chart gives a gross estimate of risk of death from circulatory disorders associated with OC use.

SMOKING HABITS AND OTHER PREDISPOSING CONDITIONS – RISK ASSOCIATED WITH USE OF OCs

Age	Below 30	30-39	40 +
Heavy smokers	C	B	A
Light smokers	D	C	B
Nonsmokers (no predisposing conditions)	D	C,D	C
Nonsmokers (other predisposing conditions)	C	C,B	B,A

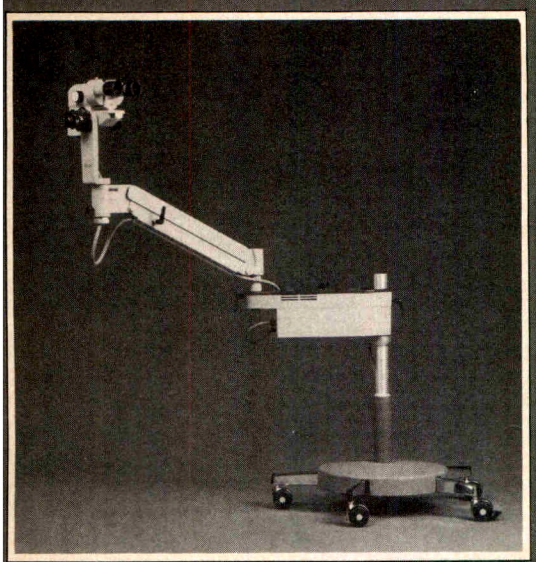
A – Use associated with very high risk.
B – Use associated with high risk.
C – Use associated with moderate risk.
D – Use associated with low risk.

Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, discontinue OC immediately. A 4-6 fold increased risk of post-surgery thromboembolic complications has been reported in OC users. If feasible, discontinue OCs at least 4 weeks before surgery associated with increased risk of thromboembolism or prolonged immobilization. Before resuming OC after major surgery or bedrest, balance risks of post-surgery thromboembolic complications with contraceptive needs. Data suggest varicose veins substantially increase risk of superficial venous thrombosis of the leg, the risk depending on the severity of the varicosities. 2. *Ocular Lesions:* Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OC use. Discontinue OC if there is unexplained, sudden, or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions, and institute appropriate diagnostic and therapeutic measures. 3. *Carcinoma:* Long-term continuous administration of natural or synthetic estrogen in certain animals increases certain tumors, benign or malignant, such as breast, cervix, vagina, uterus, ovary, pituitary and liver. Certain synthetic progestogens, none currently in OCs, increase the incidence of mammary nodules, benign and malignant, in dogs. Several retrospective case-control studies reported an increased relative risk (31-13.9 times) associating endometrial carcinoma with the prolonged use of estrogens in postmenopausal women. One publication reported the first 30 cases submitted to the registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the adenocarcinomas found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding when OCs were first given, polycystic ovaries), nearly all occurred in women who had used sequential OCs, which are no longer marketed. No statistical association has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs, although individual cases have been reported. Studies have shown no increased risk of breast cancer to OC or estrogen users. One study found no overall increased risk of breast cancer in OC users but a greater risk was suggested for OC users with documented benign breast disease and for long-term (2-4 years) users. Another study found a history of breast cancer among grandmothers or aunts was significantly more frequent among breast cancer patients who had used an OC continuously for one or more years than among nonusers with breast cancer. One other study indicated an increasing risk of breast cancer in women taking menopausal estrogens, which increased with duration of follow-up. One author suggests that extended (over 6 years) OC use prior to first full term pregnancy was associated with a significant relative risk of breast cancer, and other studies suggested an increased risk of breast cancer with long-term OC use before age 25. A reduced occurrence of benign breast tumors in OC users has been well documented. One study reported malignant melanoma more frequently in OC users than controls and suggests an increased incidence of urinary tract and thyroid cancers. A prospective study of women with cervical dysplasia found an increase in severity and conversion to cancer *in situ* in OC users compared to nonusers. This became statistically significant after 3-4 years of use. Nonreversal of dysplasia within the first 6 months of pill use was suggested to predict progression after prolonged exposure. One study disclosed an increased risk of cancer of the cervix (largely carcinoma *in situ*) in OC users under 40, particularly those who had used OCs over 4 years. Two other studies suggested an increasing incidence of cervical neoplasms with longer duration of OC use. There have been other reports of microglandular hyperplasia of the cervix in OC users. One study reported an association between OC use and endocervical adenocarcinoma. In summary, there is no confirmed evidence from human studies of increased risk of cancer associated with OCs. Close clinical surveillance of all OC users is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, take appropriate diagnostic measures to rule out malignancy. Monitor OC users with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms with particular care. (See also Warnings, 4. *Liver Tumors.*) 4. *Liver Tumors:* Sudden severe abdominal pain or shock may be due to rupture and hemorrhage of a liver tumor. There have been reports associating benign or malignant liver tumors with short-term and long-term OC use. One study reported use of OCs with high hormonal potency and age over 30 may further increase risk of hepatocellular adenoma. Two studies relate risk with duration of use, risk being much greater after 4 or more years of use. Long-term OC users have an estimated annual incidence of hepatocellular adenoma of 3-4/100,000. Although an uncommon lesion, it should be considered in women presenting with an "acute abdomen". The tumor may cause serious or fatal hemorrhage. Patients with liver tumors have demonstrated variable clinical features which may make preoperative diagnosis difficult. Some cases presented because of right upper quadrant masses, while most had signs and symptoms of acute intraperitoneal hemorrhage. Routine radiological and laboratory studies may not be helpful. Liver scans may show a focal defect. Hepatic arteriography may be useful in diagnosing primary liver neoplasm. Cases of hepatocellular carcinoma have been reported in long-term users of OCs.[see also] An epidemiological study suggested a 4-fold increase in relative risk for hepatocellular carcinoma in OC users for 8 years or more, which increased to 7 in women with markers of hepatitis B. A second study suggested a risk of about 20 for use in excess of 8 years. 5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring:* Use of female sex hormones – estrogenic and progestational agents – during early pregnancy may seriously damage the offspring. Females exposed *in utero* to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1/1000 exposures or less. Although there is no evidence that OCs further enhance the risk of developing this type of malignancy, such OC users should be monitored with particular care. A high percentage of women exposed to diethylstilbestrol (30-90%) have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are a precursor of vaginal malignancy. Male children so exposed may develop urogenital tract abnormalities. Although similar data are not available on other estrogens, it cannot be presumed that they would not induce similar changes. Increased risk of congenital anomalies, including heart and limb defects, has been reported following use of sex hormones, including OCs, in pregnancy. One case-control study estimated a 4.7-fold increased relative risk of limb-reduction defects in infants exposed *in utero* to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some exposures involved only a few days of treatment. Data suggest risk of limb-reduction defects in exposed fetuses is somewhat less than 1/1000 live births. In a large prospective study, cardiovascular defects in children born to women who received female hormones, including OCs, during early pregnancy occurred at 18.2/1000 births, compared to 7.8/1000 for children not so exposed *in utero*. These results are statistically significant. A Welsh study found a statistically significant excess of neural tube defects among offspring of prior OC users (within 3 months) than among controls. The incidence of twin births may be increased for women who conceive shortly after discontinuing OC use. In the past, female sex hormones were used during pregnancy in an attempt to treat threatened or habitual abortion. There is evidence that estrogens are ineffective, and there is no evidence from well controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. If the patient has not adhered to the prescribed schedule, consider possible pregnancy at the first missed period (or 45 days from the last menstrual period if progestogen-only OCs are used) and discontinue OC use until pregnancy has been ruled out. For any patient who has missed two consecutive periods, rule out pregnancy before continuing the OC. If pregnancy is confirmed, tell the patient about potential risks to the fetus and discuss advisability of continuing the pregnancy. Women who discontinue OCs to become pregnant should use an alternate form of contraception for a period of time before attempting to conceive. A 3-month period is supported by a study suggesting increased frequency of neural tube defects in women impregnated during the first 3 months after cessation of OC use. Do not use progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding as a pregnancy test. 6. *Gall Bladder Disease:* Studies report increased risk of gall bladder disease in OC or estrogen users. In one study, an increased risk appeared after 2 years of use and doubled after 4-5 years. In another study, an increased risk was apparent between 6 and 12 months. 7. *Carbohydrate and Lipid Metabolic Effects:* Because a decrease in glucose tolerance has been observed in a significant percentage of patients on OCs, prediabetic and diabetic OC users should be carefully observed. An increase in triglycerides and total phospholipids has been observed in OC users, but its clinical significance is unknown. 8. *Elevated Blood Pressure:* An increase in blood pressure has been reported with OC use. Hypertension may occur within a few months of beginning OCs. In the first year of use, incidence of hypertension may be no higher in OC users than in nonusers. Incidence in users increases with exposure and in the fifth year of use is 2.5-3 times that in the first year. Age is strongly correlated with hypertension in OC users. Women with a history of elevated blood pressure (hypertension), preexisting renal disease, history of toxemia or elevated blood pressure during pregnancy, familial tendency to hypertension or its consequences, or history of excessive weight gain or fluid retention during the menstrual cycle may be more likely to develop elevated blood pressure when given OCs and should be monitored closely. Even though elevated blood pressure may remain within the "normal" range, closely watch elevations, particularly for women with other risk factors for cardiovascular disease or stroke. High blood pressure may or may not persist after OC discontinuation. 9. *Headache:* Discontinue OC and evaluate the cause of onset or exacerbation of migraine or development of a new pattern of headache which is recurrent, persistent, or severe. 10. *Bleeding Irregularities:* Breakthrough bleeding, spotting, and missed menses often make users discontinue OCs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, consider nonfunctional causes. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, use adequate diagnostic measures to rule out pregnancy or malignancy. If pathology has been excluded, time or another formulation may solve the problem. While potentially useful in minimizing menstrual irregularity, change to an OC with a higher estrogen content only if necessary since this may increase the risk of thromboembolic disease. Women with a history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may tend to remain anovulatory or to become amenorrheic after OC discontinuation. Women with these preexisting problems should be advised of this and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may occur in women without previous irregularities. A higher incidence of galactorrhea and of pituitary tumors (e.g., adenomas) has been associated with amenorrhea in former users compared with nonusers. One study reported a 16-fold increased incidence of pituitary prolactin-secreting tumors among patients with postpill amenorrhea when galactorrhea was present. 11. *Infertility:* There is evidence of impairment of fertility in women discontinuing OCs in comparison with other contraceptive methods, which appears to be independent of duration of use. While impairment diminishes with time, there is an appreciable difference in the results in nulliparous women for OC and other groups 30 months after discontinuation of birth control. For parous women the difference is not apparent 30 months after cessation of contraception. One study suggests that the incidence of *Chlamydia trachomatis* infection, the leading cause of nongonococcal PID, is increased by OCs. 12. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in OC failure. In progestogen-only OC failures, the ratio of ectopic to intrauterine pregnancies is higher than in nonusers, since the drugs are more effective in preventing intrauterine than ectopic pregnancies. 13. *Breast Feeding:* OCs in the postpartum period may interfere with lactation by decreasing quantity and quality of breast milk. A small fraction of OC hormonal agents has been identified in milk of mothers receiving OCs. Effects, if any, on the breast-fed child have not been determined. If feasible, defer OC use until the infant has been weaned. **Precautions** *GENERAL* 1. Take a complete medical and family history before starting OCs. Pretreatment and periodic physical exams should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant lab tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may enlarge. 3. Observe patients with a history of psychic depression and discontinue OCs if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the OC and use an alternate method of contraception to determine whether the symptom is drug related. 4. OCs may cause some fluid retention. Prescribe with caution, and only with careful monitoring, in patients with conditions that might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma or cardiac, hepatic or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while using OCs. Discontinue OC if jaundice develops. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism that may result in a relative pyridoxine deficiency. The clinical significance is unknown. 8. Serum folate levels may be depressed by OC use. Since the pregnant woman is predisposed to folate deficiency and incidence of folate deficiency increases with increasing gestation, if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and related complications. 9. Advise the pathologist of OC use when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing OCs. For example: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnaneciol excretion. e. Reduced response to metyrapone test. f. Increased phospholipids and triglycerides. g. Temporarily decreased glucose tolerance. 11. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist and temporary or permanent cessation of wear considered. DRUG INTERACTIONS. OCs may be less effective and there may be increased breakthrough bleeding from interactions with rifampin, isoniazid, ampicillin, tetracycline, neomycin, penicillin V, chloramphenicol, sulfonamides, nitrofurantoin, barbiturates, phenytoin, primidone, analgesics, tranquilizers, antimigraine preparations, antihistamines, and griseofulvin. OCs may alter effectiveness of such other drugs as oral anticoagulants, anticonvulsants, tricyclic antidepressants, antihypertensive agents (e.g., guanethidine), vitamins, hypoglycemic agents, tranquilizers, hypnotic preparations, theophylline, and beta blockers. Toxic liver effects have been reported with a combination of an OC and cyclosporin A. *CARCINOGENESIS* See Warnings. *PREGNANCY* Pregnancy category X. See Contraindications and Warnings. *NURSING MOTHERS* See Warnings. **Adverse Reactions** An increased risk of the following serious adverse reactions has been associated with OC use (see Warnings): thrombophlebitis, thrombosis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, mesenteric thrombosis, liver tumors, cerebral hemorrhage, hypertension, gall bladder disease, congenital anomalies, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis, Budd-Chiari syndrome, Raynaud's disease, arterial thromboembolism. The following adverse reactions have been reported in OC users and are believed to be drug related: bleeding irregularities (breakthrough bleeding, spotting, missed menses during treatment, amenorrhea after treatment), gastrointestinal symptoms (nausea, vomiting, bloating, abdominal cramps, colitis), dysmenorrhea, infertility after discontinuance of treatment, edema, chloasma or melasma which may persist after drug is discontinued, breast changes (tenderness, enlargement, and secretion), intolerance to contact lenses, changes in corneal curvature (steepening), change in weight (increase or decrease), change in cervical erosion and cervical secretion, possible diminution of lactation when given immediately postpartum, cholestatic jaundice, migraine, increase in size of uterine leiomyomata, rash (allergic), mental depression, reduced tolerance to carbohydrates, vaginal candidiasis, prolactin-secreting pituitary tumors, chiblains. The following adverse reactions have been reported in OC users and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of hair, erythema multiforme, erythema nodosum, hemorrhagic eruptions, vaginitis, porphyria, impaired renal function, malignant nephrosclerosis (hemolytic uremic syndrome), malignant hypertension, acute renal failure, premature supraventricular contractions. **Information for the Patient** (See Patient Package Insert).

Rev. April 1987

SYNTEX

COLPOSCOPE 1-FC

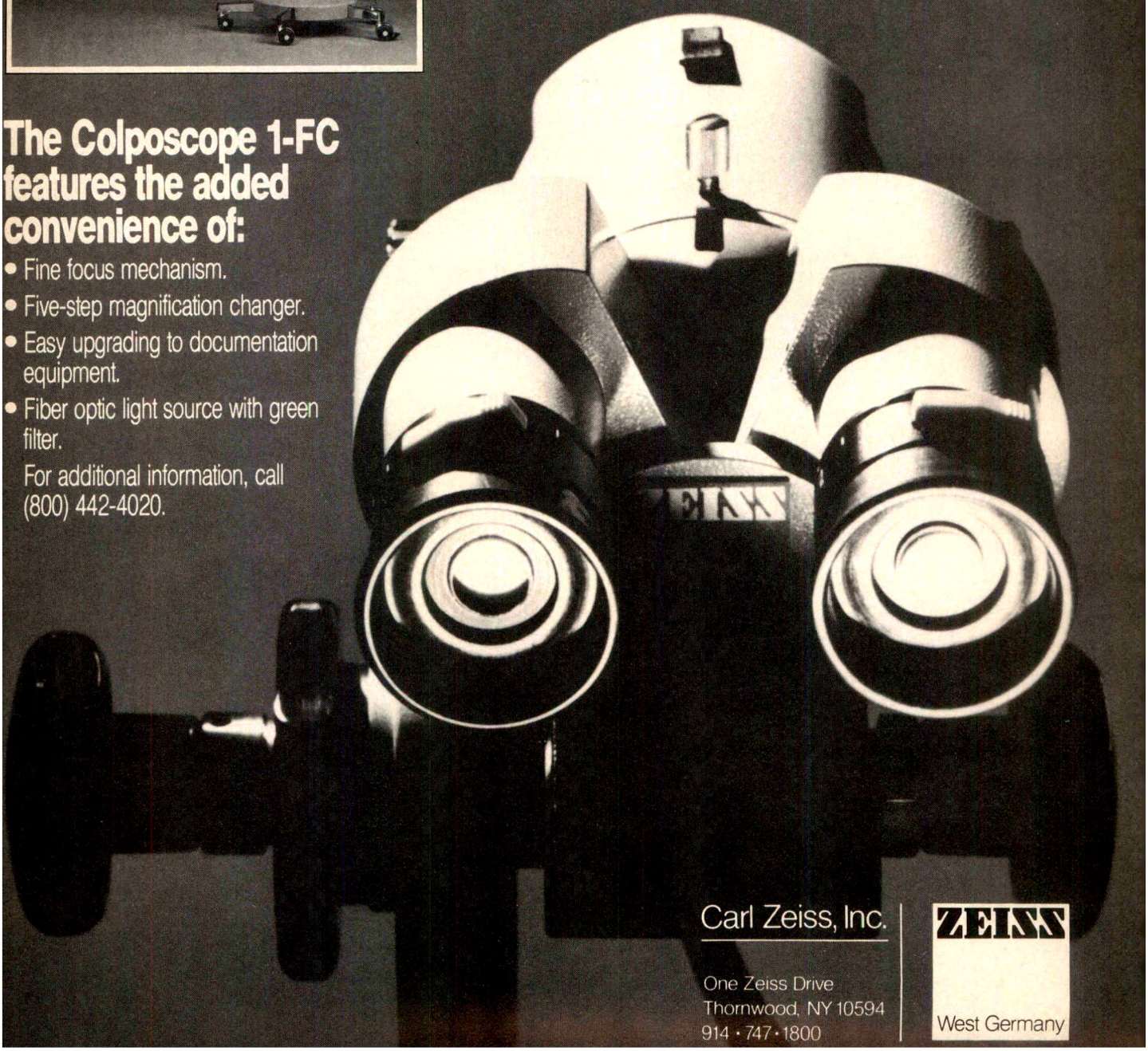


FULLY CO_2 LASER COMPATIBLE, TODAY OR IN THE FUTURE.

The Colposcope 1-FC features the added convenience of:

- Fine focus mechanism.
- Five-step magnification changer.
- Easy upgrading to documentation equipment.
- Fiber optic light source with green filter.

For additional information, call (800) 442-4020.



Carl Zeiss, Inc.

One Zeiss Drive
Thornwood, NY 10594
914 · 747 · 1800

ZEISS
West Germany

Satisfaction realized.



87% patient continuation rate† with Ortho ALL-FLEX* Arcing Spring Diaphragm.

Easy for patient to insert—
the Ortho ALL-FLEX* Diaphragm folds easily.

Easy for patient to correctly position in vagina—
the Ortho ALL-FLEX* Diaphragm can be compressed at any two opposing points around the rim.

Easy to use with **GYNOL II* (2% nonoxynol-9) Contraceptive Jelly**—
greaseless, odorless spermicide.

Easy to clean—one-step molding process eliminates gap between spring and dome so jelly or secretions can't accumulate.

Ortho ALL-FLEX*
DIAPHRAGM

†Percentage of patients continuing the method for 12 consecutive months.

Consult the revised patient brochure for new information on Toxic Shock Syndrome (TSS) and wearing time.

ORTHO PHARMACEUTICAL CORPORATION
Raritan, New Jersey 08869-0602

© OPC 1987 OJ-899 *Trademark

Mosby

American Journal of Obstetrics and Gynecology

Founded in 1920

Copyright © 1989 by The C.V. Mosby Company

Contents

August
1989

CLINICAL SECTION

Clinical Opinion

Missed abortion: Still appropriate terminology? 261
Gabriella Pridjian, MD, and Atef H. Moawad, MD
Chicago, Illinois

In modern obstetrics, timely use of ultrasonography in early pregnancy has made the use of the term *missed abortion* no longer appropriate.

Multidisciplinary team counseling for fetal anomalies 263
Robert P. Lorenz, MD, and Margueritte H. Kuhn, MD
Royal Oak and Detroit, Michigan

When a fetal anomaly occurs, a multidisciplinary team of specialists assists the obstetrician in planning and counseling a family in an effective new program to assist in this family crisis.

Breast disease programs in obstetrics and gynecology: A plea for training in mammography 267
Norbert Gleicher, MD
Chicago, Illinois

The inclusion of mammography training programs within obstetrics and gynecology curricula is proposed as an integral step in the establishment of teaching programs in breast disease.

Intrauterine growth retardation: Standards for diagnosis 271
Robert L. Goldenberg, MD, Gary R. Cutter, PhD, Howard J. Hoffman, MA, Judith M. Foster, MPH, Kathleen G. Nelson, MD, and John C. Hauth, MD
Birmingham, Alabama

The methodology used to develop the current standards for defining intrauterine growth retardation were evaluated, and, on the basis of these findings, recommendations are made to develop a single national standard for the diagnosis of intrauterine growth retardation.

(Contents continued on page 7A)

Vol. 161, No. 2, August 1989. The American Journal of Obstetrics and Gynecology (ISSN 0002-9378) is published monthly (six issues per volume, two volumes per year) by The C.V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318. Second-class postage paid at St. Louis, Missouri, and additional mailing offices. POSTMASTER: Send address changes to The C.V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318, (314) 872-8370, ext. 351. Annual subscription rates for 1989: domestic, $65.50 for individuals and $118.00 for institutions. Printed in the U.S.A. Copyright © 1989 by The C.V. Mosby Company.

SUCCESS

Starts with Serophene®

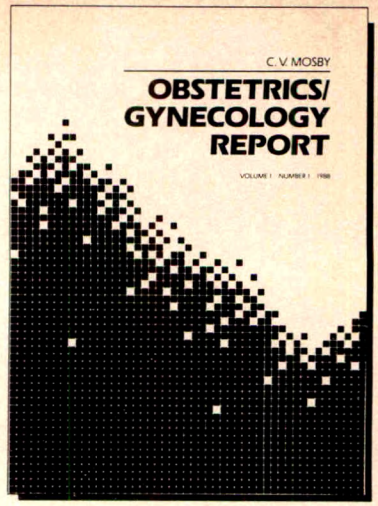
(clomiphene citrate tablets, USP) 50 mg

Serono

Serono Laboratories, Inc.
100 Longwater Circle
Norwell, MA 02061

© 1989, Serono Laboratories, Inc.

An idea whose time has come



OBSTETRICS/GYNECOLOGY REPORT

The depth of texts, the timeliness of journals . . .

OBSTETRICS/GYNECOLOGY REPORT, a **new** resource that works for **you**, is a synthesis of current information on the most important clinical developments in the field. The REPORT makes it **convenient** for the busy practitioner to stay up to date.

- Three issues every 12 months.
- Each issue focuses on two main topics within the specialty:

Vol. 1, No. 1	Infectious diseases Reproductive endo-fertility
Vol. 1, No. 2	Fetal monitoring Human papilloma virus
Vol. 1, No. 3	Breast disease Urinary incontinence

- Comprehensive coverage of the selected topics, from new developments to cases of interest.
- Approximately 150 pages per issue; no advertisements.
- Attractive, two-color format throughout.

Our editorial board of prominent physicians is committed to offering you an insightful, reliable, and practical resource that truly is beneficial to clinical practice.

Editor in Chief: **Frederick P. Zuspan, MD**

Editors:

Richard C. Bump, MD
William T. Creasman, MD
Philip J. DiSaia, MD
Patrick Duff, MD
Steven G. Gabbe, MD
Thomas J. Garite, MD
Ronald S. Gibbs, MD
Mary G. Hammond, MD
Glenn Hurt, MD
Mark B. Landon, MD
Edward J. Quilligan, MD
Luther M. Talbert, MD

Subscribe now at the low rate of $52.50 U.S. and $58.50 Canada and International.

Try the REPORT under our no-risk, money-back guarantee! To place an order or to request more information about OBSTETRICS/GYNECOLOGY REPORT, please contact the Journal Circulation Department ■ The C.V. Mosby Company ■ 11830 Westline Industrial Drive ■ St. Louis, MO 63146. In the U.S., call toll-free: 1-800-325-4177, ext. 394. Or call: (314) 872-8370, ext. 394.

OBR-010

Mosby

Contents continued from page 5A

Clinical Articles

Anal and perianal abnormalities in prepubertal victims of sexual abuse 278
David Muram, MD
Memphis, Tennessee

Perianal abnormalities were observed in one third of children who were sexually abused.

Phosphorus 31 nuclear magnetic resonance examination of female reproductive tissues 282
Elizabeth A. Noyszewski, MSc, Jyothi Raman, MSc, Suzanne R. Trupin, MD, Barbara L. McFarlin, CNM, MS, and M. Joan Dawson, PhD
Urbana, Illinois

Noninvasive nuclear magnetic resonance spectroscopy examination may be a useful addition to magnetic resonance imaging, and it could become a cost-effective method of evaluating biopsy samples biochemically.

A new surgical technique to treat refractory labial fusion in the elderly 289
N. Johnson, MRCOG, FRCS, R. J. Lilford, MRCOG, MRCP, PhD, and D. Sharpe, FRCS, OBE
Leeds and Bradford, England

A new treatment of refractory labial fusion is described, in which a skin flap from the thigh is used to cover the raw area.

(Contents continued on page 8A)

1989 Annual subscription rates	U.S.A.	*International (surface mail)* *All regions*	*International (airmail)** *Canada*	*Mexico*	*Region 1*	*Region 2*	*Region 3*
Institutions†	$118.00	$142.50	$170.00	$176.25	$188.50	$230.25	$272.00
Individuals‡	$ 65.50	$ 90.00	$117.50	$123.75	$136.00	$177.75	$219.50
Students, residents‡	$ 38.00	$ 62.50	$ 90.00	$ 96.25	$108.50	$150.25	$192.00

Subscription rates include supplements. Single copies are $7.00. Remittances should be made by check, draft, or post office or express money order, in U.S. funds drawn through a U.S. bank, payable to this JOURNAL. Claims for missing issues will be serviced only within six months of cover date. Single copy prices will be charged on missing issue claims older than six months from cover date. Back issues generally are available for the previous five years. Contact the Publisher to confirm availability of specific issues.

**Airmail breakdown—Domestic:* First-class and Priority rates for the U.S. and possessions are available upon request. *Canada:* All provinces of Canada. *Mexico:* All localities within Mexico. *Region 1:* Colombia, Venezuela, Central America, Caribbean Islands, Bahamas, Bermuda, and St. Pierre and Miquelon (also from American Samoa to Western Samoa and from Guam to the Philippines). *Region 2:* South America (except Colombia and Venezuela), Europe (except Estonia, Latvia, Lithuania, and U.S.S.R), and North Africa (Morocco, Algeria, Tunisia, Libya, and Egypt). *Region 3:* Estonia, Latvia, Lithuania, U.S.S.R., Asia, Australia, New Zealand, Pacific Ocean Islands, Africa (other than North Africa), Indian Ocean Islands, and the Middle East.

†Institutional (multiple-reader) subscriptions are available to public and private libraries, schools, hospitals, and clinics; city, county, state, provincial, and national government bureaus and departments; and all commercial and private institutions and organizations.

‡Individual subscriptions and all student-rate subscriptions must be in the names of, billed to, and paid by individuals. All student-rate requests must indicate training status and name of institution.

Subscriptions may begin at any time.

Contents continued from page 7A

Predictive values, relative risks, and overall benefits of high and low maternal serum α-fetoprotein screening in singleton pregnancies: New epidemiologic data 291
Aubrey Milunsky, MB, BCh, DSc, Susan S. Jick, MPH, Carol L. Bruell, MS, Dean S. MacLaughlin, PhD, Yean-Kai Tsung, PhD, Hershel Jick, MD, Kenneth J. Rothman, DrPH, and Walter Willett, MD
Boston, Massachusetts

In addition to detection of pregnancies in which the fetus has a neural tube or chromosome defect, maternal serum α-fetoprotein screening is shown in this study to also be useful in the identification of high-risk pregnancy.

The efficacy of fetal sonographic biometry in Down syndrome screening 297
Mark T. Peters, MD, Charles J. Lockwood, MD, and Wayne A. Miller, MD
Lincoln and Boston, Massachusetts

Fetal sonographic biometry cannot be used as a sole criterion for Down syndrome screening.

Pregnancy loss in a small chorionic villus sampling series 301
Brian A. Clark, PhD, MD, John M. Bissonnette, MD, Susan B. Olson, PhD, and R. Ellen Magenis, MD
Portland, Oregon

The pregnancy loss rate in the initial 211 chorionic villus sampling cases does not support the contention that there is an initial high rate of pregnancy loss.

Maternity shelter care for adolescents: Its effect on incidence of low birth weight 303
Katherine D. LaGuardia, MD, MPH, Maurice L. Druzin, MD, and Carol Eades, BA
New York, New York

A retrospective analysis of 225 pregnant adolescents revealed that those living in a maternity shelter yet receiving identical medical care as those living at home were delivered of significantly fewer infants of low birth weight and preterm gestation.

Fetal hyperinsulinism in anencephaly 306
C. Hubinont, MD, PhD, N. M. Fisk, MD, U. Nicolini, MD, and C. H. Rodeck, MD
London, England

In utero hyperinsulinism present in anencephalic fetuses could be responsible for their subnormal growth despite an absent hypothalamohypophysial axis.

Antenatal ultrasonographic diagnosis of fetal seizure activity 308
Helain J. Landy, MD, Alfred N. Khoury, MD, and Peter S. Heyl, MD
Washington, D.C., and Fairfax, Virginia

The ultrasonographic diagnosis of in utero seizures in a chromosomally normal, growth-retarded fetus is reported.

Contents

Phase II trial of ifosfamide and mesna in mixed mesodermal tumors of the uterus (A Gynecologic Oncology Group Study) 309
Gregory P. Sutton, MD, John A. Blessing, PhD, Neil Rosenshein, MD, Guy Photopulos, MD, and Philip J. DiSaia, MD
Indianapolis, Indiana, Buffalo, New York, Baltimore, Maryland, Memphis, Tennessee, and Orange, California

Ifosfamide and mesna produced a 32.2% response rate with a 17.9% complete response rate in advanced or recurrent mixed mesodermal tumors of the uterus.

Ceramide lactoside in amniotic fluid: High concentration in chorioamnionitis and in preterm labor 313
Mikko Hallman, MD, DrMedSci, Kristiina Bry, MD, and Olli Pitkänen, MD
Helsinki, Finland

Cermide lactoside may serve as a sensitive and specific marker of inflammation in fetal membranes during preterm labor.

Cardiac isoenzymes and electrocardiographic changes during ritodrine tocolysis 318
Hamid A. Hadi, MD, and Sabah J. Albazzaz, MD
Augusta, Georgia

Serially determined cardiac isoenzymes and electrocardiographic examinations during ritodrine tocolysis suggest that this drug in the recommended regimen does not produce direct myocardial damage.

Elevated liver enzymes and thrombocytopenia in the third trimester of pregnancy: An unusual case report and a review of the literature 322
Mary E. Hannah, MSc, MDCM, Ron Gonen, MD, Eva J. M. Mocarski, MSc, MD, Ross Cameron, MD, Laurence Blendis, MD, and Michael Glynn, MD
Toronto, Ontario, Canada

Abdominal pain, thrombocytopenia, and elevated liver enzymes, occurring in a twin pregnancy and diagnosed as preeclampsia on liver biopsy, resolved before delivery.

Continuous tissue pH monitoring in the term fetus 323
Mary L. Small, MD, Marie Beall, MD, Lawrence D. Platt, MD, Diane Dirks, RN, and Howard Hochberg, MD
Los Angeles, California

Continuous tissue pH monitoring of 59 patients showed poor correlation with scalp pH; however, knowing the trend of tissue pH is thought to be worthwhile.

Noninvasive management of isolated bilateral fetal hydrothorax 330
Leen Pijpers, MD, PhD, Annette Reuss, MD, Patricia A. Stewart, NDCT, CCPT, and Juriy W. Wladimiroff, MD, PhD
Rotterdam, The Netherlands

Normal neonatal outcome was observed in eight cases of bilateral isolated fetal hydrothorax in which a noninvasive management approach was adopted.

(Contents continued on page 10A)

Contents continued from page 9A

Relapsing polychondritis in pregnancy: A case report and review 332
Martin L. Gimovsky, MD, and Mark Nishiyama, MD
Los Angeles, California

A case of chronic pulmonary insufficiency during gestation is described.

Abscess of allantoic duct remnant 334
I. Cori Baill, MD, G. William Moore, MD, PhD, and Lora A. Hedrick, MD
Baltimore, Maryland

Funicitis and an abscessed allantoic duct remnant were present in an umbilical cord at 22 1/7 weeks' gestation.

Infection and labor. IV. Cachectin–tumor necrosis factor in the amniotic fluid of women with intraamniotic infection and preterm labor 336
Roberto Romero, MD, Kirk R. Manogue, PhD, Murray D. Mitchell, DPhil, Ying King Wu, MD, Enrique Oyarzun, MD, John C. Hobbins, MD, and Anthony Cerami, PhD
New Haven, Connecticut, New York, New York, and Salt Lake City, Utah

Tumor necrosis factor may play a role in the onset of preterm labor in women with intraamniotic infection.

Pulsed Doppler flow-velocity waveforms in the prediction of fetal hematocrit of the severely isoimmunized pregnancy 341
Joshua A. Copel, MD, Peter A. Grannum, MD, Jacqueline J. Green, MS, RDMS, Kathy Belanger, PhD, and John C. Hobbins, MD
New Haven, Connecticut

Two previously reported formulas relating fetal hematocrit to Doppler indices were evaluated prospectively and were found not to predict hematocrit reliably.

Concurrence of mouthing movement and rapid eye movement/non–rapid eye movement phases with advance in gestation of the human fetus 344
Naoki Horimoto, MD, Takashi Koyanagi, MD, Shin Nagata, MD, Hiromasa Nakahara, MD, and Hitoo Nakano, MD
Fukuoka, Japan

By ultrasonographic observation of 26 human fetuses between 28 and 41 weeks of gestation, regular mouthing movement was found to concur with the non–rapid eye movement period from 35 weeks' gestation to term.

Fetal heart rate patterns in red blood cell isoimmunized pregnancies 351
K. H. Nicolaides, BSc, MD, G. Sadovsky, MD, and E. Cetin, MD
London, England

Fetal heart rate patterns in 33 red blood cell isoimmunized pregnancies were related to oxygenation and hemoglobin concentration of fetal blood obtained at cordocentesis.

Contents

Plasma atrial natriuretic peptide in human fetus: Response to intravascular blood transfusion 357
Marios Z. Panos, MD, Kypros H. Nicolaides, MD, John V. Anderson, MD, Demetrios L. Economides, MD, Lesley Rees, MD, and Roger Williams, MD
London, England

Human fetal plasma atrial natriuretic peptide concentration rises in response to intravascular blood transfusion.

Fetal cardiac output in the isoimmunized pregnancy: A pulsed Doppler-echocardiographic study of patients undergoing intravascular intrauterine transfusion 361
Joshua A. Copel, MD, Peter A. Grannum, MD, Jacqueline J. Green, MS, RDMS, Kathy Belanger, PhD, Nagui Hanna, MD, C. Carl Jaffe, MD, John C. Hobbins, MD, and Charles S. Kleinman, MD,
New Haven, Connecticut

Cardiac output was measured in a group of isoimmunized fetuses undergoing intravascular transfusion and was found to be significantly higher than in controls.

Twin-to-twin transfusion syndrome: What are appropriate diagnostic criteria? 365
Fiona H. Danskin, MB, ChB, and James P. Neilson, MD
Glasgow, Scotland

The standard diagnostic criteria for the chronic twin-to-twin transfusion syndrome were reevaluated in a study of 178 consecutive twin pregnancies, and it was concluded that a definitive diagnosis of this syndrome cannot be made with these criteria alone.

The prevalence and biologic significance of lupus anticoagulant and anticardiolipin antibodies in a general obstetric population 369
C. J. Lockwood, MD, R. Romero, MD, R. F. Feinberg, MD, PhD, L. P. Clyne, FIMLS, MS, B. Coster, BS, and J. C. Hobbins, MD
New Haven, Connecticut

In a low-risk obstetric population the prevalence of lupus anticoagulant and antiphospholipid antibodies was 0.27% and 2.2%, respectively, with normal outcomes observed in 25% of affected patients.

Fetal blood gas analysis from cordocentesis for abnormal fetal heart rate patterns 374
Dinesh M. Shah, MD, and Frank H. Boehm, MD
Nashville, Tennessee

Three cases that involve dilemmas from lack of synchrony of fetal heart rate tracing, cordocentesis-obtained blood gas analysis, and postnatal blood gas analysis are presented.

Presence of autoantibodies in women with unexplained infertility 377
Pamela V. Taylor, PhD, James M. Campbell, MRCOG, and James S. Scott, MD
Leeds, England

Unexplained infertility was studied in 41 patients and was shown to be associated with a significantly higher prevalence of autoantibodies to smooth muscle, phospholipid, and some nuclear antigens, compared with normal pregnancy.

(Contents continued on page 12A)

Contents continued from page 11A

Successful twin pregnancy after embryo donation to a patient with XY gonadal dysgenesis 380
Mark V. Sauer, MD, Rogerio A. Lobo, MD, and Richard J. Paulson, MD
Los Angeles, California

A twin pregnancy was accomplished in a patient with XY gonadal dysgenesis with the use of donated oocytes obtained by transvaginal aspiration, fertilized in vitro, and replaced transcervically.

Ovarian hyperstimulation presenting as acute hydrothorax after in vitro fertilization 381
Charles R. Kingsland, MB, ChB, John V. Collins, MD, Botros Rizk, MB, ChB, and Bridgett A. Mason, MB, BS
London, England

This is the first reported case of acute hydrothorax as the only presenting sign of ovarian hyperstimulation after in vitro fertilization.

Normal amniotic fluid volume changes throughout pregnancy 382
Robert A. Brace, PhD, and Edward J. Wolf, MD
La Jolla, California

A total of 705 measured amniotic fluid volumes in normal human pregnancies was used to statistically characterize gestational changes in amniotic fluid volume.

Doppler velocimetry and placental disease 388
Luis A. Bracero, MD, Debra Beneck, MD, Nancy Kirshenbaum, MD, Marianne Peiffer, RDMS, Patricia Stalter, CDMS, and Harold Schulman, MD
Valhalla and Mineola, New York

An elevated umbilical artery systolic-diastolic ratio was found to be associated with a decrease in the number of small muscular arteries within the placenta.

Local methotrexate injection: A nonsurgical treatment of ectopic pregnancy 393
Mordechai Pansky, MD, Ian Bukovsky, MD, Abraham Golan, MD, Rami Langer, MD, David Schneider, MD, Shlomo Arieli, MD, and Eliahu Caspi, MD
Zerifin, Israel

Twenty-seven patients with unruptured tubal pregnancy were treated by local injection of methotrexate at laparoscopy; all but three, who needed laparotomy because of rising titers of β-hCG, recovered uneventfully.

Serial human chorionic gonadotropin determinations by fluoroimmunoassay for differentiation between intrauterine and ectopic gestation 397
Bo Lindblom, MD, PhD, Mats Hahlin, MD, and Peter Sjöblom, PhD
Göteborg, Sweden

Improved analysis of hCG patterns increases accuracy in diagnosis of ectopic pregnancy.

Contents

Dandy-Walker syndrome: A review of fifteen cases evaluated by prenatal sonography 401

Paul D. Russ, MD, Dolores H. Pretorius, MD, and Mark J. Johnson, MD

Denver, Colorado, San Diego, California, and Columbus, Ohio

Fifteen cases of Dandy-Walker syndrome diagnosed in utero were reviewed to determine the spectrum of prenatal sonographic findings, associated congenital anomalies, and clinical outcome.

The frequency and management of uterine perforations during first-trimester abortions 406

Steven G. Kaali, MD, Ivan A. Szigetvari, MD, PhD, and George S. Bartfai, MD, PhD

Dobbs Ferry, New York

First-trimester abortions combined with laparoscopic sterilizations indicate that the rate of unsuspected uterine perforations is manyfold higher than previously believed, and observation rather than further surgery is suggested

Histologic and immunologic study of uterine biopsy tissue of women with incipient abortion 409

Magdy Michel, MB, MRCOG, Jenny Underwood, BSc, David A. Clark, MD, PhD, FRCPC, James F. Mowbray, MD, FRCP, and Richard W. Beard, MD, FRCOG

London, England, and Hamilton, Ontario, Canada

Biopsy specimens of decidua from women with incipient first-trimester spontaneous miscarriage showed a morphologic change in granulated mononuclear cell populations and a decreased ability to release soluble immunosuppressive factors in culture.

Randomized trial of diet versus diet plus cardiovascular conditioning on glucose levels in gestational diabetes 415

Lois Jovanovic-Peterson, MD, Eric P. Durak, MSc, and Charles M. Peterson, MD

Santa Barbara, California, and New York, New York

Arm ergometry is a safe and efficacious form of adjunctive therapy for the gestational diabetic woman.

Thrombosis prophylaxis in pregnancy with use of subcutaneous heparin adjusted by monitoring heparin concentration in plasma 420

Thomas C. Dahlman, MD, Margareta S. E. Hellgren, MD, and Margareta Blombäck, MD

Stockholm, Sweden

Anti–factor Xa activity can be used for monitoring the increasing need for heparin during a low-dose heparin regimen, but the activated partial thromboplastin time method is too insensitive.

(Contents continued on page 14A)

Contents continued from page 13A

Early pregnancy glycosylated hemoglobin, severity of diabetes, and fetal malformations 426
Michael J. Lucas, MD, Kenneth J. Leveno, MD, M. Lynne Williams, RN, Philip Raskin, MD, and Peggy J. Whalley, MD
Dallas, Texas

Malformation outcome among insulin-treated diabetic women was related both to early glycohemoglobin level and to White classification.

Predictive value of increased plasma levels of fibronectin in gestational hypertension 432
Véronique Ballegeer, MD, Bernard Spitz, MD, Lena Kieckens, Huberte Moreau, André Van Assche, MD, and Désiré Collen, MD, PhD
Leuven, Belgium

Increased plasma fibronectin levels (>140% of normal) predict the development of gestational hypertension in primigravid women with a sensitivity and specificity of 95%.

Zinc, cadmium, and hypertension in parturient women 437
Noam Lazebnik, MD, Betty R. Kuhnert, PhD, and Paul M. Kuhnert, PhD, MD
Cleveland, Ohio

Maternal plasma zinc, placental zinc, and the relationship between whole blood cadmium and plasma zinc is altered in preeclamptic toxemia but not in chronic hypertension.

Erythrocyte cation metabolism in preeclampsia 441
James R. Sowers, MD, Michael B. Zemel, PhD, Richard A. Bronsteen, MD, Paula C. Zemel, PhD, Mary F. Walsh, PhD, Paul R. Standley, MS, and Robert J. Sokol, MD
Detroit, Michigan

The relationship between blood pressure and erythrocyte cation metabolism is explored in black women with pregnancy-induced hypertension.

BASIC SCIENCE SECTION

Immunoregulatory activity in supernatants from cultures of normal human trophoblast cells of the first trimester 446
Mrinal K. Sanyal, PhD, Charles J. Brami, MD, Paul Bischof, PhD, Edwina Simmons, MD, Eytan R. Barnea, MD, John M. Dwyer, MD, PhD, and Frederick Naftolin, MD, DPhil
New Haven, Connecticut, and Geneva, Switzerland

Placental trophoblast cells of human first-trimester pregnancy secreted an immunosuppressive factor in culture, and this factor may be associated with the immunoregulation and maintenance of fetal allograft during early gestation.

Contents

Increased myometrial responsiveness to oxytocin during term and preterm labor 454
R. E. Garfield, PhD, and S. Beier, PhD
Hamilton, Ontario, Canada, and Berlin, West Germany

During term and preterm labor in rats, oxytocin dose–myometrial response curves showed increased maximal tension and steeper slopes but no change in pD_2 values (i.e., sensitivity).

CA 125 in the epithelium closely related to the embryonic ectoderm: The periderm and the amnion 462
Yoshihiko Nanbu, MD, Shingo Fujii, MD, Ikuo Konishi, MD, Hirofumi Nonogaki, MD, and Takahide Mori, MD
Kyoto, Japan

In addition to the fetal coelomic epithelium–related tissues, the amnion and the periderm, both of which constitute the epithelia covering the amniotic cavity, produce CA 125.

Power spectrum analysis of myometrial electromyogram and intrauterine pressure changes in the pregnant rhesus monkey in late gestation 467
Hsing Wen Hsu, BS, Jorge P. Figueroa, MD, PhD, M. Barbera O. M. Honnebier, MD, Richard Wentworth, PhD, and Peter W. Nathanielsz, MD, PhD
Ithaca, New York

Power spectrum analysis of myometrial electromyogram and intrauterine pressure is used to distinguish and quantify contracture and contraction-type myometrial activity in pregnant rhesus monkeys in late gestation.

Pattern of human chorionic gonadotropin binding in the polycystic ovary 474
James Brawer, PhD, Martine Richard, MSc, and Riaz Farookhi, PhD
Montreal, Quebec, Canada

This study describes the histologic features and the LH-hCG binding capacity of structures that make up the experimentally induced polycystic ovary.

Effect of a 48-hour intravenous Δ^4-androstenedione infusion on the pregnant rhesus monkey in the last third of gestation: Changes in maternal plasma estradiol concentrations and myometrial contractility 481
Jorge P. Figueroa, MD, PhD, M. Barbera O. M. Honnebier, MD, Zbigniew Binienda, DVM, Jeffrey Wimsatt, DVM, and Peter W. Nathanielsz, MD, PhD
Ithaca, New York

Infusion of androstenedione for 48 hours into pregnant rhesus monkeys in late gestation significantly elevated maternal plasma estrogen levels and was accompanied by increased myometrial activity.

(Contents continued on page 16A)

Contents continued from page 15A

Flow cytometry of normal, hyperplastic, and malignant human endometrium 487
James G. Thornton, MD, Philip Quirke, PhD, and Michael Wells, MD
Leeds, England

Flow cytometric proliferative indices from endometrial hyperplasias and carcinomas correlate with S-phase labeling indices and tissue differentiation.

Atrial natriuretic peptide receptors in guinea pig placentas 493
Christos G. Hatjis, MD, and David M. Grogan, BA
Winston-Salem, North Carolina

In guinea pig placentas and subplacentas, the affinity of atrial natriuretic peptide receptors for atrial natriuretic peptide changes from 45 to 65 days' gestation.

CORRESPONDENCE

Attempted suicide and pregnancy Andrew Czeizel, MD, and Agnes Lendvay, PhD *Budapest, Hungary* 497

Sperm antibodies and infertility Sidney Shulman, PhD *New York, New York* 497

Reply Steven S. Witkin, PhD *New York, New York* 498

Vaginosonographic examination—Routine procedure? Margaret E. Furness, FRACR, DDU *Rose Park, South Australia* 499

Reply Gerhard Bernaschek, MD, and Rudolf Rudelstorfer, MD *Vienna, Austria* 499

Nerve entrapment after Pfannenstiel incision Benjamin Piura, MD, MRCOG *Beer-Sheva, Israel* 499

Reply William C. Sippo, MD *West Point, New York* 500

Unexplained elevated maternal serum α-fetoprotein level associated with third-trimester problems John D. Stephens, MD *San Jose, California* 500

Neurofibromatosis and pregnancy G. Mitterschiffthaler, MD, and L. C. Fuith, MD *Innsbruck, Austria* 501

Reply Isaac Blickstein, MD *Rehovot, Israel* 501

Isolation of human immunodeficiency virus from the placenta or from maternal blood contaminating the placenta? Jeffrey S. Greenspoon, MD, and Robert Hurd Settlage, MD, MPH *Los Angeles, California* 501

Reply Washington C. Hill, MD, James R. Carlson, PhD, and Veronica Bolton *Omaha, Nebraska* 502

Race and gender factors omitted from results Rudi Ansbacher, MD, MS *Ann Arbor, Michigan* 502

Reply Israel Goldstein, MD *New Haven, Connecticut* 502

***Candida* warrants concern** John P. Elliott, MD *Phoenix, Arizona* 503

Reply Carl V. Smith, MD *Omaha, Nebraska* 503

Contents

ITEMS

Items 504

INFORMATION FOR AUTHORS

Information for authors 25A

Index to advertisers on page 55A

Make researching easier with the Ten-Year Cumulative Index (1978-1987) to the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY

The Ten-Year Cumulative Index is a complete guide to more than 30,000 pages of original articles, case reports, letters, and editorials published in the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY from 1978 through 1987 (volumes 130 to 157). Numbering more than 800 pages, the Cumulative Index includes both a subject and an author index. This hard-cover volume is $64.50 ($69.00 international). Prices include shipping. *Payment must accompany all orders and must be in U.S. funds, drawn on a U.S. bank.*

Contact The C.V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318 USA; phone (314) 872-8370, ext. 394. In the U.S., call toll-free: 1-800-325-4177, ext. 394. Please allow six weeks for delivery.

The JOURNAL is abstracted and/or indexed in *Index Medicus, Science Citation Index, Current Contents/Clinical Medicine, Current Contents/Life Sciences, Excerpta Medica, BRS Colleague,* and MEDLINE.

Authorization to photocopy items for internal or personal use, or the internal or personal use of specific clients, is granted by The C.V. Mosby Company for libraries and other users registered with the Copyright Clearance Center (CCC) Transactional Reporting Service, provided that 10¢ per page per copy is paid directly to CCC, 27 Congress St., Salem, MA 01970; phone (508)744-3350. The U.S. Government retains a nonexclusive, royalty-free license in and to any copyright on articles authored by government employees. 0002-9378/89 $00.00 + .10.

Editors

Editors

JOHN I. BREWER, *Editor in Chief*
Sandra Fernandez, *Senior Editorial Assistant*

FREDERICK P. ZUSPAN, *Editor*
Donna Peterson, *Editorial Assistant*

E. J. QUILLIGAN, *Editor*
Sherry A. Merryman, *Editorial Assistant*

ALBERT B. GERBIE, *Associate Editor*

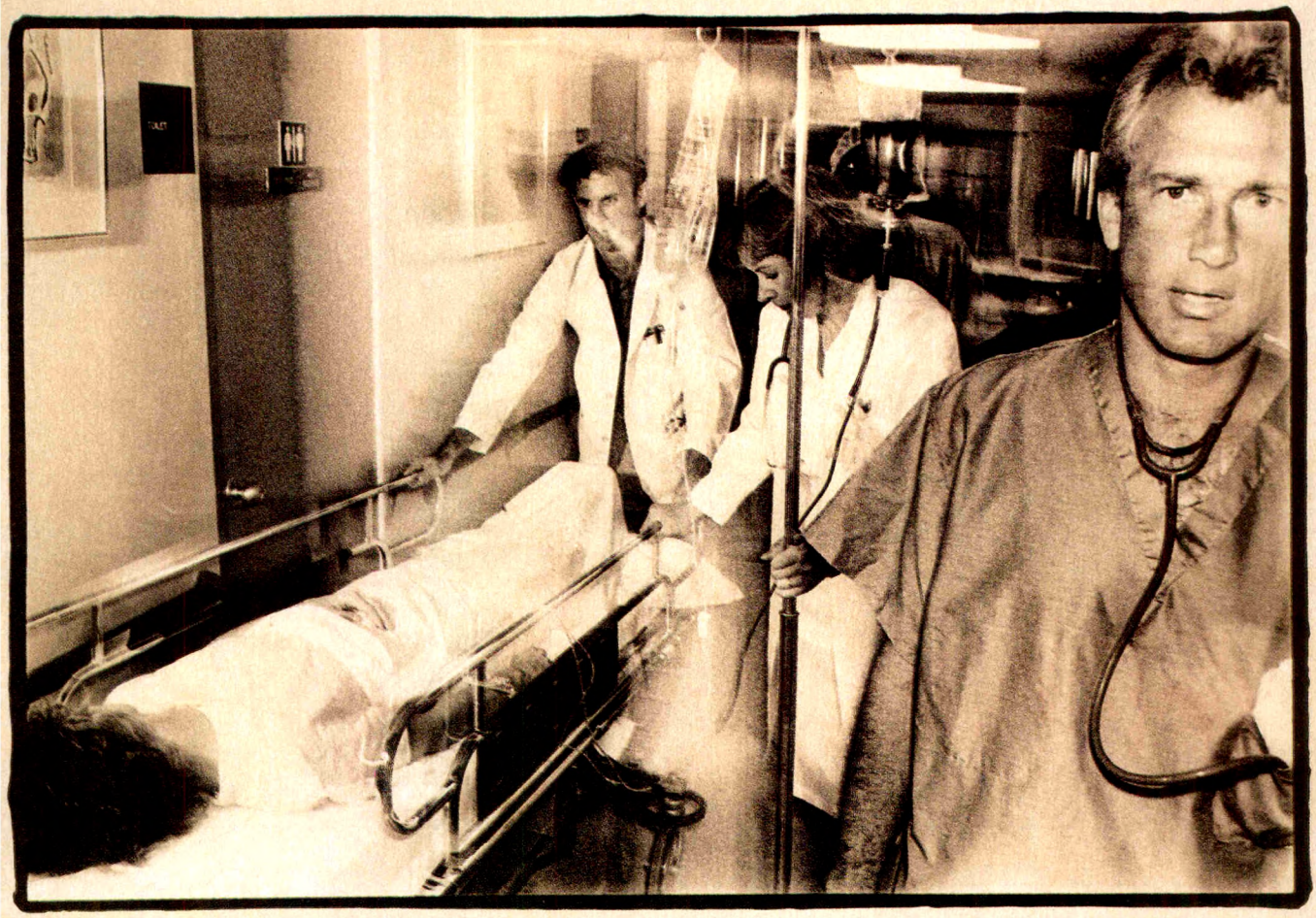
Advisory committee on policy

Garland Anderson
William R. Anderson
Mason C. Andrews
E. Forrest Boyd
David C. Figge
A. Jerome Freeland
Edgar O. Horger III
W. Glenn Hurt
James M. Ingram
Walter S. Keifer
Emmet J. Lamb
Roger A. Lobo
Douglas J. Marchant
James H. Maxwell
Arthur M. McCausland
Allan G. W. McLeod
James A. Merrill
Daniel R. Mishell, Jr.
John C. Morrison
Carl J. Pauerstein
Richard Paul
Karl C. Podratz
John A. Read
Ruth W. Schwartz
Henry A. Thiede
Carol Trumbold

Board of corresponding editors

Stuart Campbell, *England*
Luigi Carenza, *Italy*
Eliahu Sadovsky, *Israel*
Nils-Otto Sjöberg, *Sweden*
Masakuni Suzuki, *Japan*

Publisher
THE C.V. MOSBY COMPANY, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318
Kay G. Goehler, *Senior Manuscript Editor*



"It's still like walking the high wire, but now I have a safety net."

More confidence in presumptive antibiotic therapy. Prescribing UNASYN presumptively is a step you can take with confidence. UNASYN covers gram-positives (even enterococci), prevalent gram-negatives, and anaerobes: The right combination for most patients with common mixed infections.*

And UNASYN minimizes safety concerns with a reliable penicillin-class profile.[1] (However, UNASYN is contraindicated in penicillin-allergic patients.)

Expect strong support from UNASYN in the mixed infections* you face every day.

1.5 or 3g q6h

Unasyn® IM IV

(ampicillin sodium/sulbactam sodium)

An antibiotic for every day

ROERIG Pfizer
A division of Pfizer Pharmaceuticals

*Due to susceptible strains of indicated organisms.

Please see brief summary of prescribing information on adjacent page.

©1989, Pfizer Inc.

Join a Leader
We're The Ohio Permanente Medical Group, Inc.

and we are looking for board certified/board eligible **Obstetrician/Gynecologists** to help us keep up with rapid growth of the Kaiser Permanente Program in Northeastern Ohio. OPMG is the multispecialty group practice that provides health care services to the more than 185,000 Kaiser members in the Cleveland-Akron area.

Our wealth of experience of over 40 plus years (25 in Ohio) makes Kaiser Permanente a mature, solid, leader in the managed care sector of the health care industry. The rewards of practice with us are substantial—excellent salary and benefit packages, company-paid retirement plan, full malpractice coverage, a stimulating, collegial environment in which to practice quality medicine, and more.

Kaiser Permanente's Ohio Region is located in the heart of the dynamic, resurgent industrial Midwest. The area offers the best of big city sophistication and culture in an affordable, accessible living area.

Please send your resume to: Ronald G. Potts, M.D., Medical Director, Ohio Permanente Medical Group, Inc., 1300 E. 9th Street, Suite 1100, Cleveland, OH 44114. Or you may call us collect at (216) 623-8780.

KAISER PERMANENTE®

PERINATAL GENETICIST

The Department of Obstetrics and Gynecology at Pennsylvania Hospital is seeking an obstetrician with genetic fellowship training to join an active perinatal genetics program.

Skills in second trimester amniocentesis, chorionic villus sampling, and Level II ultrasound are desired.

Responsibilities include resident and medical student education and active clinical practice with a University Appointment at the level of Assistant Professor.

Please send resumes to:

Ronald J. Bolognese, M.D.
Department of Obstetrics
and Gynecology
Pennsylvania Hospital
8th & Spruce Streets
Philadelphia, PA 19107

Unasyn® IM IV
(ampicillin sodium/sulbactam sodium)

1.5 or 3g q6h

References:
1. Data available on request from Roerig. 2. Aldridge KE, Sanders CV, Marier RL: Variation in the potentiation of β-lactam antibiotic activity by clavulanic acid and sulbactam against multiply antibiotic-resistant bacteria. *J Antimicrob Chemother* 1986;17:463-469. 3. Crombleholme WR, Ohm-Smith M, Robbie MO, et al: Ampicillin/sulbactam versus metronidazole-gentamicin in the treatment of soft tissue pelvic infections. *Am J Obstet Gynecol* 1987;156(Feb):507-512. 4. Cucharal G, Snydman D, Jacobus N, et al: Comparison of new antimicrobial agents against the *Bacteroides fragilis* group. Abstract presented at the Annual Meeting of the American Society of Microbiology, Miami Beach, Fla, May 1988. 5. D'Amato RF, Hochstein L, Pavia M: *In vitro* susceptibilities of aerobic and anaerobic clinical isolates to ampicillin/sulbactam. Presented as a Scientific Exhibit at the 87th Annual Meeting of the American Society of Microbiology, Atlanta, March 1987. 6. Finegold SM: Anaerobic bacteria: Their role in infection and their management. *Postgrad Med* 1987;81(8):141-147. 7. Jenkins SG: Susceptibility patterns of *Bacteroides fragilis* group isolates to ampicillin/sulbactam and other selected antibiotics. Presented at a symposium entitled *Ampicillin/Sulbactam: New Strategy for the Treatment of Infectious Disease*, Montreux, Switzerland, July 16-17, 1987, pp 19-20. 8. Tally FP, Jacobus NV: Activity of sulbactam against recent isolates of aerobic and anaerobic bacteria, abstract 36. Presented at the 23rd International Congress of Antimicrobial Agents and Chemotherapy, Las Vegas, Oct 24-26, 1983 (abs). 9. Wasilauskas B: Susceptibility of anaerobes to ampicillin-sulbactam and 6 other antimicrobial agents, abstract. Presented at the Annual Meeting of the American Society for Microbiology, Miami Beach, Fla, May 8-13, 1988. 10. Wexler HM, Harris B, Carter WT, et al: In vitro efficacy of sulbactam combined with ampicillin against anaerobic bacteria. *Antimicrob Agents Chemother* 1985;27:876-878. 11. Ampicillin/sulbactam (Unasyn). *Med Lett Drugs Ther* 1987;29(August 28):79-81. 12. Hemsell DL, Wendel GD, Hemsell PG: Combating beta-lactamase enzyme—principal defense mechanism of many pelvic infection pathogens. Presented as a scientific exhibit at the Thirty-Sixth Annual Clinical Meeting of the American College of Obstetricians and Gynecologists, Boston, May 2-4, 1988. 13. Senft H-H, Stiglmayer R, Eibach HW, et al: Sulbactam/ampicillin versus cefoxitin in the treatment of obstetric and gynaecological infections. *Drugs* 1986;31(suppl 2):18-21. 14. Newton ER, Gibbs RS: Treatment of postpartum endometritis: A comparison of ampicillin/sulbactam vs. gentamicin plus clindamycin. Presented as a scientific exhibit at the Thirty-Sixth Annual Clinical Meeting of the American College of Obstetricians and Gynecologists, Boston, May 2-4, 1988. 15. Gunning J: A comparison of parenteral sulbactam/ampicillin versus clindamycin/gentamicin in the treatment of pelvic inflammatory disease. *Drugs* 1986;31(suppl 2):14-17. 16. Stromberg BV, Reines HD, Hunt P: Comparative clinical study of sulbactam and ampicillin and clindamycin and tobramycin in infections of soft tissues. *Surg Gyn Obstet* 1986;162:575-578. 17. Kager L, Malmborg AS, Nord CE, et al: A randomized controlled trial of ampicillin plus sulbactam vs. gentamicin plus clindamycin in the treatment of intraabdominal infections: A preliminary report. *Rev Infect Dis* 1986;8(suppl 5):S583-S588. 18. Bruhat MA, Pouly JL, Le Boedec G, et al: Treatment of acute salpingitis with sulbactam/ampicillin: Comparison with cefoxitin. *Drugs* 1986;31(suppl 2):7-10. 19. Faro S: Experience with ampicillin/sulbactam for OB/GYN infections. Presented at a symposium entitled *β-Lactamase Inhibitors: Progress with Ampicillin/Sulbactam*, Atlanta, December 6, 1987, p 14 (abs).

INDICATIONS AND USAGE
UNASYN is indicated for the treatment of infections due to susceptible strains of the designated microorganisms in the conditions listed below.
Skin and Skin Structure Infections caused by beta-lactamase producing strains of *Staphylococcus aureus*, *Escherichia coli*,* *Klebsiella* spp.* (including *K. pneumoniae**), *Proteus mirabilis*,* *Bacteroides fragilis*,* *Enterobacter* spp.,* and *Acinetobacter calcoaceticus*.*
Intra-Abdominal Infections caused by beta-lactamase producing strains of *Escherichia coli*, *Klebsiella* spp. (including *K. pneumoniae**), *Bacteroides* spp. (including *B. fragilis*), and *Enterobacter* spp.*
Gynecological Infections caused by beta-lactamase producing strains of *Escherichia coli*,* and *Bacteroides* spp.* (including *B. fragilis**).
*Efficacy for this organism in this organ system was studied in fewer than 10 infections.
While UNASYN* (ampicillin sodium/sulbactam sodium) is indicated only for the conditions listed above, infections caused by ampicillin-susceptible organisms are also amenable to treatment with UNASYN due to its ampicillin content. Therefore, mixed infections caused by ampicillin-susceptible organisms and beta-lactamase producing organisms susceptible to UNASYN should not require the addition of another antibiotic.

CONTRAINDICATIONS
The use of UNASYN is contraindicated in individuals with a history of hypersensitivity reactions to any of the penicillins.

WARNINGS
SERIOUS AND OCCASIONALLY FATAL HYPERSENSITIVITY (ANAPHYLACTIC) REACTIONS HAVE BEEN REPORTED IN PATIENTS ON PENICILLIN THERAPY. THESE REACTIONS ARE MORE APT TO OCCUR IN INDIVIDUALS WITH A HISTORY OF PENICILLIN HYPERSENSITIVITY AND/OR HYPERSENSITIVITY REACTIONS TO MULTIPLE ALLERGENS. THERE HAVE BEEN INDIVIDUALS WITH A HISTORY OF PENICILLIN HYPERSENSITIVITY WHO HAVE EXPERIENCED SEVERE REACTIONS WHEN TREATED WITH CEPHALOSPORINS. BEFORE THERAPY WITH A PENICILLIN, CAREFUL INQUIRY SHOULD BE MADE CONCERNING PREVIOUS HYPERSENSITIVITY REACTIONS TO PENICILLINS, CEPHALOSPORINS, AND OTHER ALLERGENS. IF AN ALLERGIC REACTION OCCURS, UNASYN SHOULD BE DISCONTINUED AND THE APPROPRIATE THERAPY INSTITUTED.
SERIOUS ANAPHYLACTOID REACTIONS REQUIRE IMMEDIATE EMERGENCY TREATMENT WITH EPINEPHRINE, OXYGEN, INTRAVENOUS STEROIDS, AND AIRWAY MANAGEMENT, INCLUDING INTUBATION, SHOULD ALSO BE ADMINISTERED AS INDICATED.

PRECAUTIONS
General: A high percentage of patients with mononucleosis who receive ampicillin develop a skin rash. Thus, ampicillin class antibiotics should not be administered to patients with mononucleosis. In patients treated with UNASYN the possibility of superinfections with mycotic or bacterial pathogens should be kept in mind during therapy. If superinfections occur (usually involving *Pseudomonas* or *Candida*), the drug should be discontinued and/or appropriate therapy instituted.
Drug Interactions: Probenecid decreases the renal tubular secretion of ampicillin and sulbactam. Concurrent use of probenecid with UNASYN may result in increased and prolonged blood levels of ampicillin and sulbactam. The concurrent administration of allopurinol and ampicillin increases substantially the incidence of rashes in patients receiving both drugs as compared to patients receiving ampicillin alone. It is not known whether this potentiation of ampicillin rashes is due to allopurinol or the hyperuricemia present in these patients. There are no data with UNASYN and allopurinol administered concurrently. UNASYN and aminoglycosides should not be reconstituted together due to the *in vitro* inactivation of aminoglycosides by the ampicillin component of UNASYN.
Drug/Laboratory Test Interactions: Administration of UNASYN will result in high urine concentrations of ampicillin. High urine concentrations of ampicillin may result in false positive reactions when testing for the presence of glucose in urine using Clinitest™, Benedict's Solution or Fehling's Solution. It is recommended that glucose tests based on enzymatic glucose oxidase reactions (such as Clinistix™ or Testape™) be used. Following administration of ampicillin to pregnant women, a transient decrease in plasma concentration of total conjugated estriol, estriolglucuronide, conjugated estrone, and estradiol has been noted. This effect may also occur with UNASYN.
Carcinogenesis, Mutagenesis, Impairment of Fertility: Long-term studies in animals have not been performed to evaluate carcinogenic or mutagenic potential.
Pregnancy
Pregnancy Category B: Reproduction studies have been performed in mice, rats, and rabbits at doses up to ten (10) times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to UNASYN. There are, however, no adequate and well controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed. (See—Drug/Laboratory Test Interactions.)
Labor and Delivery: Studies in guinea pigs have shown that intravenous administration of ampicillin decreased the uterine tone, frequency of contractions, height of contractions, and duration of contractions. However, it is not known whether the use of UNASYN in humans during labor or delivery has immediate or delayed adverse effects on the fetus, prolongs the duration of labor, or increases the likelihood that forceps delivery or other obstetrical intervention or resuscitation of the newborn will be necessary.
Nursing Mothers: Low concentrations of ampicillin and sulbactam are excreted in the milk; therefore, caution should be exercised when UNASYN is administered to a nursing woman.
Pediatric Use: The efficacy and safety of UNASYN have not been established in infants and children under the age of 12.

ADVERSE REACTIONS
UNASYN is generally well tolerated. The following adverse reactions have been reported:
Local Adverse Reactions
Pain at IM injection site—16% Pain at IV injection site—3%
Thrombophlebitis—3%
Systemic Adverse Reactions
The most frequently reported adverse reactions were diarrhea in 3% of the patients and rash in less than 2% of the patients.
Additional systemic reactions reported in less than 1% of the patients were itching, nausea, vomiting, candidiasis, fatigue, malaise, headache, chest pain, flatulence, abdominal distension, glossitis, urine retention, dysuria, edema, facial swelling, erythema, chills, tightness in throat, substernal pain, epistaxis and mucosal bleeding.
Adverse Laboratory Changes
Adverse laboratory changes without regard to drug relationship that were reported during clinical trials were:
Hepatic: Increased AST (SGOT), ALT (SGPT), alkaline phosphatase, and LDH.
Hematologic: Decreased hemoglobin, hematocrit, RBC, WBC, neutrophils, lymphocytes, platelets and increased lymphocytes, monocytes, basophils, eosinophils, and platelets.
Blood Chemistry: Decreased serum albumin and total proteins.
Renal: Increased BUN and creatinine.
Urinalysis: Presence of RBC's and hyaline casts in urine.
The following adverse reactions have been reported with ampicillin-class antibiotics and can also occur with UNASYN.
Gastrointestinal: Gastritis, stomatitis, black "hairy" tongue, enterocolitis and pseudomembranous colitis.
Hypersensitivity Reactions: Urticaria, erythema multiforme, and an occasional case of exfoliative dermatitis have been reported. These reactions may be controlled with antihistamines and, if necessary, systemic corticosteroids. Whenever such reactions occur, the drug should be discontinued, unless the opinion of the physician dictates otherwise. Serious and occasional fatal hypersensitivity (anaphylactic) reactions can occur with a penicillin (see WARNINGS).
Hematologic: In addition to the adverse laboratory changes listed above for UNASYN, agranulocytosis has been reported during therapy with penicillins. All of these reactions are usually reversible on discontinuation of therapy and are believed to be hypersensitivity phenomena.

ROERIG Pfizer
A division of Pfizer Pharmaceuticals



"I used to think only <u>two</u> things in life were certain."

Nothing comes closer to 100% activity against anaerobes in vitro. It's a fact. Studies have proved no other antibiotic superior to UNASYN in overall antianaerobic activity.[1-10] Not clindamycin. Not metronidazole. Nothing.

And that's nothing to overlook—given the importance of anaerobes in today's mixed infections.

Though clinical results may not always mirror those obtained in vitro, there's a world of confidence in choosing UNASYN for presumptive therapy of the mixed infections* you see every day.

1.5 or 3g q6h

Unasyn® IM IV

(ampicillin sodium/sulbactam sodium)

An antibiotic for every day

ROERIG Pfizer
A division of Pfizer Pharmaceuticals

*Due to susceptible strains of indicated organisms

Please see brief summary of prescribing information on adjacent page.

©1989, Pfizer Inc.

CALCUTTA CENTRAL

OBSTETRICIAN GYNECOLOGIST

Expanding Western Massachusetts staff model Health Maintenance Organization is seeking a full-time Board Certified/Board Eligible Obstetrician/Gynecologist to join a staff of eight (8) physicians and six (6) midwives.

Our multi-specialty group practice employs seventy (70) full-time physicians and provides care for a growing membership of over 70,000 from modern suburban facilities, in-patient care is provided at 900-bed Tufts affiliated teaching hospital.

Attractive Pioneer Valley location with fine educational, cultural and recreational opportunities. Excellent salary and comprehensive benefit program. Please send curriculum vitae to: Oded Rencus, M.D., Chief of OB/GYN, 305 Bicentennial Highway, Springfield, MA 01118.

MedicalWest
Community Health Plan
A BLUE CROSS & BLUE SHIELD HMO

We are an equal opportunity/affirmative action employer

Michael Reese Health Plan

The **Michael Reese Health Plan,** a large well-established HMO serving its members in 20 Health Centers throughout the Chicago Metro area, is seeking additional Board Certified/Eligible physicians in the following specialty:

Obstetrics Gynecology

Physicians with the Health Plan enjoy a full practice with modern facilities and equipment as well as opportunities for teaching.

A highly-competitive salary and excellent benefits complement a rewarding practice and lifestyle.

Interested applicants should address inquiries to:

Scott P. Smith, M.D.
MICHAEL REESE HEALTH PLAN
2545 Dr. Martin Luther King Dr.,
Chicago 60616
or call (312) 808-4551

an equal opportunity employer m/f/h

Unasyn IM IV

(ampicillin sodium/sulbactam sodium)

1.5 or 3g q6h

References:
1. Data available on request from Roerig. **2.** Aldridge KE, Sanders CV, Marier RL: Variation in the potentiation of β-lactam antibiotic activity by clavulanic acid and sulbactam against multiply antibiotic-resistant bacteria. *J Antimicrob Chemother* 1986;17:463-469. **3.** Crombleholme WR, Ohm-Smith M, Robbie MO, et al: Ampicillin/sulbactam versus metronidazole-gentamicin in the treatment of soft tissue pelvic infections. *Am J Obstet Gynecol* 1987;156(Feb):507-512. **4.** Cucharal G, Snydman D, Jacobus N, et al: Comparison of new antimicrobial agents against the *Bacteroides fragilis* group. Abstract, presented at the Annual Meeting of the American Society of Microbiology, Miami Beach, Fla, May 1988. **5.** D'Amato RF, Hochstein L, Pavia M: *In vitro* susceptibilities of aerobic and anaerobic clinical isolates to ampicillin/sulbactam. Presented as a Scientific Exhibit at the 87th Annual Meeting of the American Society of Microbiology, Atlanta, March 1987. **6.** Finegold SM: Anaerobic bacteria: Their role in infection and their management. *Postgrad Med* 1987;81(8):141-147. **7.** Jenkins SG: Susceptibility patterns of *Bacteroides fragilis* group isolates to ampicillin/sulbactam and other selected antibiotics. Presented at a symposium entitled *Ampicillin/Sulbactam: New Strategy for the Treatment of Infectious Disease*, Montreux, Switzerland, July 16-17, 1987, pp 19-20. **8.** Tally FP, Jacobus NV: Activity of sulbactam against recent isolates of aerobic and anaerobic bacteria. abstract 36. Presented at the 23rd International Congress of Antimicrobial Agents and Chemotherapy, Las Vegas, Oct 24-26, 1983 (abs). **9.** Wasilauskas B: Susceptibility of anaerobes to ampicillin-sulbactam and 6 other antimicrobial agents. abstract. Presented at the Annual Meeting of the American Society for Microbiology, Miami Beach, Fla, May 8-13, 1988. **10.** Wexler HM, Harris B, Carter WT, et al: In vitro efficacy of sulbactam combined with ampicillin against anaerobic bacteria. *Antimicrob Agents Chemother* 1985;27:876-878. **11.** Ampicillin/sulbactam (Unasyn). *Med Lett Drugs Ther* 1987;29(August 28):79-81. **12.** Hemsell DL, Wendel GD, Hemsell PG: Combating beta-lactamase enzyme—principal defense mechanism of many pelvic infection pathogens. Presented as a scientific exhibit at the Thirty-Sixth Annual Clinical Meeting of the American College of Obstetricians and Gynecologists, Boston, May 2-4, 1988. **13.** Senft H-H, Stiglmayer R, Eibach HW, et al: Sulbactam/ampicillin versus cefoxitin in the treatment of obstetric and gynaecological infections. *Drugs* 1986;31(suppl 2):18-21. **14.** Newton ER, Gibbs RS: Treatment of postpartum endometritis: A comparison of ampicillin/sulbactam vs. gentamicin plus clindamycin. Presented as a scientific exhibit at the Thirty-Sixth Annual Clinical Meeting of the American College of Obstetricians and Gynecologists, Boston, May 2-4, 1988. **15.** Gunning J: A comparison of parenteral sulbactam/ampicillin versus clindamycin/gentamicin in the treatment of pelvic inflammatory disease. *Drugs* 1986;31(suppl 2):14-17. **16.** Stromberg BV, Reines HD, Hunt P: Comparative clinical study of sulbactam and ampicillin and clindamycin and tobramycin in infections of soft tissues. *Surg Gyn Obstet* 1986;162:575-578. **17.** Kager L, Malmborg AS, Nord CE, et al: A randomized controlled trial of ampicillin plus sulbactam vs. gentamicin plus clindamycin in the treatment of intraabdominal infections: A preliminary report. *Rev Infect Dis* 1986;8(suppl 5):S583-S588. **18.** Bruhat MA, Pouly JL, Le Boedec G, et al: Treatment of acute salpingitis with sulbactam/ampicillin: Comparison with cefoxitin. *Drugs* 1986;31(suppl 2):7-10. **19.** Faro S: Experience with ampicillin/sulbactam for OB/GYN infections. Presented at a symposium entitled *β-Lactamase Inhibitors: Progress with Ampicillin/Sulbactam*, Atlanta, December 6, 1987, p 14 (abs).

INDICATIONS AND USAGE
UNASYN is indicated for the treatment of infections due to susceptible strains of the designated microorganisms in the conditions listed below.
Skin and Skin Structure Infections caused by beta-lactamase producing strains of *Staphylococcus aureus*, *Escherichia coli*,* *Klebsiella* spp.* (including *K. pneumoniae**), *Proteus mirabilis*,* *Bacteroides fragilis*,* *Enterobacter* spp.,* and *Acinetobacter calcoaceticus*.*
Intra-Abdominal Infections caused by beta-lactamase producing strains of *Escherichia coli*, *Klebsiella* spp. (including *K. pneumoniae**), *Bacteroides* spp. (including *B. fragilis*), and *Enterobacter* spp.*
Gynecological Infections caused by beta-lactamase producing strains of *Escherichia coli*,* and *Bacteroides* spp.* (including *B. fragilis**).
*Efficacy for this organism in this organ system was studied in fewer than 10 infections.
While UNASYN* (ampicillin sodium/sulbactam sodium) is indicated only for the conditions listed above, infections caused by ampicillin-susceptible organisms are also amenable to treatment with UNASYN due to its ampicillin content. Therefore, mixed infections caused by ampicillin-susceptible organisms and beta-lactamase producing organisms susceptible to UNASYN should not require the addition of another antibiotic.

CONTRAINDICATIONS
The use of UNASYN is contraindicated in individuals with a history of hypersensitivity reactions to any of the penicillins.

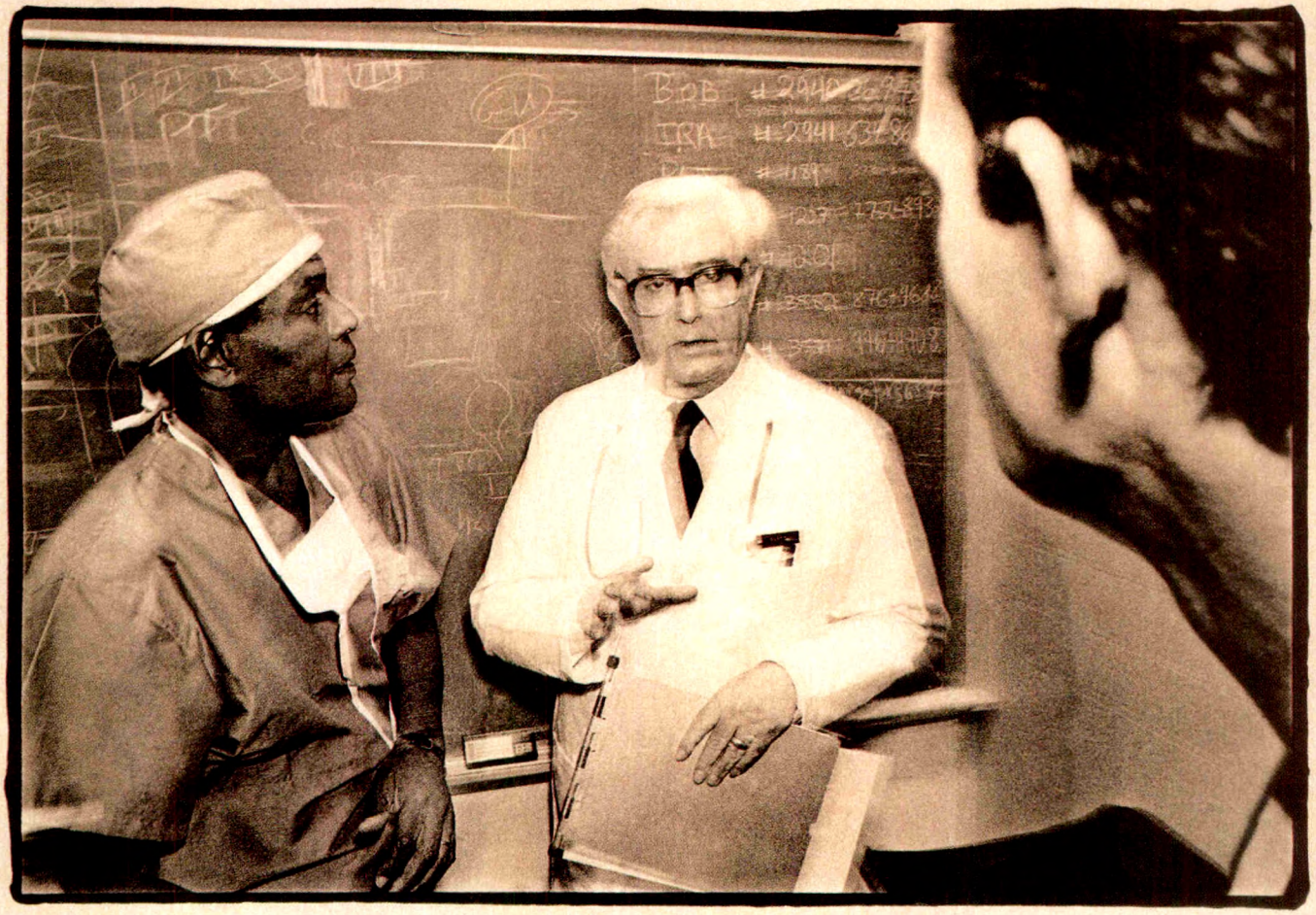
WARNINGS
SERIOUS AND OCCASIONALLY FATAL HYPERSENSITIVITY (ANAPHYLACTIC) REACTIONS HAVE BEEN REPORTED IN PATIENTS ON PENICILLIN THERAPY. THESE REACTIONS ARE MORE APT TO OCCUR IN INDIVIDUALS WITH A HISTORY OF PENICILLIN HYPERSENSITIVITY AND/OR HYPERSENSITIVITY REACTIONS TO MULTIPLE ALLERGENS. THERE HAVE BEEN INDIVIDUALS WITH A HISTORY OF PENICILLIN HYPERSENSITIVITY WHO HAVE EXPERIENCED SEVERE REACTIONS WHEN TREATED WITH CEPHALOSPORINS. BEFORE THERAPY WITH A PENICILLIN, CAREFUL INQUIRY SHOULD BE MADE CONCERNING PREVIOUS HYPERSENSITIVITY REACTIONS TO PENICILLINS, CEPHALOSPORINS, AND OTHER ALLERGENS. IF AN ALLERGIC REACTION OCCURS, UNASYN SHOULD BE DISCONTINUED AND THE APPROPRIATE THERAPY INSTITUTED.
SERIOUS ANAPHYLACTOID REACTIONS REQUIRE IMMEDIATE EMERGENCY TREATMENT WITH EPINEPHRINE. OXYGEN, INTRAVENOUS STEROIDS, AND AIRWAY MANAGEMENT, INCLUDING INTUBATION, SHOULD ALSO BE ADMINISTERED AS INDICATED.

PRECAUTIONS
General: A high percentage of patients with mononucleosis who receive ampicillin develop a skin rash. Thus, ampicillin class antibiotics should not be administered to patients with mononucleosis. In patients treated with UNASYN the possibility of superinfections with mycotic or bacterial pathogens should be kept in mind during therapy. If superinfections occur (usually involving *Pseudomonas* or *Candida*), the drug should be discontinued and/or appropriate therapy instituted.
Drug Interactions: Probenecid decreases the renal tubular secretion of ampicillin and sulbactam. Concurrent use of probenecid with UNASYN may result in increased and prolonged blood levels of ampicillin and sulbactam. The concurrent administration of allopurinol and ampicillin increases substantially the incidence of rashes in patients receiving both drugs as compared to patients receiving ampicillin alone. It is not known whether this potentiation of ampicillin rashes is due to allopurinol or the hyperuricemia present in these patients. There are no data with UNASYN and allopurinol administered concurrently. UNASYN and aminoglycosides should not be reconstituted together due to the *in vitro* inactivation of aminoglycosides by the ampicillin component of UNASYN.
Drug/Laboratory Test Interactions: Administration of UNASYN will result in high urine concentrations of ampicillin. High urine concentrations of ampicillin may result in false positive reactions when testing for the presence of glucose in urine using Clinitest™, Benedict's Solution or Fehling's Solution. It is recommended that glucose tests based on enzymatic glucose oxidase reactions (such as Clinistix™ or Testape™) be used. Following administration of ampicillin to pregnant women, a transient decrease in plasma concentration of total conjugated estriol, estriolglucuronide, conjugated estrone, and estradiol has been noted. This effect may also occur with UNASYN.
Carcinogenesis, Mutagenesis, Impairment of Fertility: Long-term studies in animals have not been performed to evaluate carcinogenic or mutagenic potential.
Pregnancy
Pregnancy Category B: Reproduction studies have been performed in mice, rats, and rabbits at doses up to ten (10) times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to UNASYN. There are, however, no adequate and well controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed. (See—Drug/Laboratory Test Interactions.)
Labor and Delivery: Studies in guinea pigs have shown that intravenous administration of ampicillin decreased the uterine tone, frequency of contractions, height of contractions, and duration of contractions. However, it is not known whether the use of UNASYN in humans during labor or delivery has immediate or delayed adverse effects on the fetus, prolongs the duration of labor, or increases the likelihood that forceps delivery or other obstetrical intervention or resuscitation of the newborn will be necessary.
Nursing Mothers: Low concentrations of ampicillin and sulbactam are excreted in the milk; therefore, caution should be exercised when UNASYN is administered to a nursing woman.
Pediatric Use: The efficacy and safety of UNASYN have not been established in infants and children under the age of 12.

ADVERSE REACTIONS
UNASYN is generally well tolerated. The following adverse reactions have been reported:
Local Adverse Reactions
Pain at IM injection site—16%; Pain at IV injection site—3%; Thrombophlebitis—3%.
Systemic Adverse Reactions
The most frequently reported adverse reactions were diarrhea in 3% of the patients and rash in less than 2% of the patients.
Additional systemic reactions reported in less than 1% of the patients were: itching, nausea, vomiting, candidiasis, fatigue, malaise, headache, chest pain, flatulence, abdominal distension, glossitis, urine retention, dysuria, edema, facial swelling, erythema, chills, tightness in throat, substernal pain, epistaxis and mucosal bleeding.
Adverse Laboratory Changes
Adverse laboratory changes without regard to drug relationship that were reported during clinical trials were:
Hepatic: Increased AST (SGOT), ALT (SGPT), alkaline phosphatase, and LDH.
Hematologic: Decreased hemoglobin, hematocrit, RBC, WBC, neutrophils, lymphocytes, platelets and increased lymphocytes, monocytes, basophils, eosinophils, and platelets.
Blood Chemistry: Decreased serum albumin and total proteins.
Renal: Increased BUN and creatinine.
Urinalysis: Presence of RBC's and hyaline casts in urine.
The following adverse reactions have been reported with ampicillin-class antibiotics and can also occur with UNASYN:
Gastrointestinal: Gastritis, stomatitis, black "hairy" tongue, enterocolitis and pseudomembranous colitis.
Hypersensitivity Reactions: Urticaria, erythema multiforme, and an occasional case of exfoliative dermatitis have been reported. These reactions may be controlled with antihistamines and, if necessary, systemic corticosteroids. Whenever such reactions occur, the drug should be discontinued, unless the opinion of the physician dictates otherwise. Serious and occasional fatal hypersensitivity (anaphylactic) reactions can occur with a penicillin (see WARNINGS).
Hematologic: In addition to the adverse laboratory changes listed above for UNASYN, agranulocytosis has been reported during therapy with penicillins. All of these reactions are usually reversible on discontinuation of therapy and are believed to be hypersensitivity phenomena.

ROERIG Pfizer
A division of Pfizer Pharmaceuticals
New York, New York 10017

© 1988, Pfizer Inc



"We need gold-standard results that don't cost a fortune."

Lower antibiotic costs without lowering your standards. UNASYN costs at least 40% less per day than clindamycin/gentamicin or cefoxitin.[11] Yet UNASYN is at least as effective in a variety of mixed infections.*[3,12-19]

And in some studies versus combination therapy, patients treated with UNASYN spent fewer days in the hospital.[3,16,17]

So, look to UNASYN for highly effective, highly affordable therapy in mixed infections* every day.

1.5 or 3g q6h

Unasyn® IM IV

(ampicillin sodium/sulbactam sodium)

An antibiotic for every day

ROERIG Pfizer
A division of Pfizer Pharmaceuticals

*Due to susceptible strains of indicated organisms.

Please see brief summary of prescribing information on adjacent page.

© 1989, Pfizer Inc.

Societies

in addition to those listed on the front cover
the Journal is the official publication
of the following societies

NEW YORK OBSTETRICAL SOCIETY

OBSTETRICAL SOCIETY OF PHILADELPHIA

BROOKLYN GYNECOLOGICAL SOCIETY

ST. LOUIS GYNECOLOGICAL SOCIETY

NEW ORLEANS GYNECOLOGICAL AND OBSTETRICAL SOCIETY

THE OBSTETRICAL AND GYNECOLOGICAL SOCIETY OF MARYLAND

CHICAGO GYNECOLOGICAL SOCIETY

CINCINNATI OBSTETRICAL AND GYNECOLOGICAL SOCIETY

WASHINGTON GYNECOLOGICAL SOCIETY

PITTSBURGH OBSTETRICAL AND GYNECOLOGICAL SOCIETY

BOSTON OBSTETRICAL SOCIETY

LOUISVILLE OBSTETRICAL AND GYNECOLOGICAL SOCIETY

SEATTLE GYNECOLOGICAL SOCIETY

ALABAMA ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS

AKRON OBSTETRICAL AND GYNECOLOGICAL SOCIETY

KANSAS CITY GYNECOLOGICAL SOCIETY

CENTRAL NEW YORK ASSOCIATION OF GYNECOLOGISTS
AND OBSTETRICIANS

NEW JERSEY OBSTETRICAL AND GYNECOLOGICAL SOCIETY

IOWA OBSTETRIC AND GYNECOLOGIC SOCIETY

THE TEXAS ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS

OKLAHOMA CITY OBSTETRICAL AND GYNECOLOGICAL SOCIETY

MEMPHIS OBSTETRICAL AND GYNECOLOGICAL SOCIETY

UTAH OBSTETRICAL AND GYNECOLOGICAL SOCIETY

ROCHESTER OBSTETRICAL AND GYNECOLOGICAL SOCIETY

ARKANSAS OBSTETRICAL AND GYNECOLOGICAL SOCIETY

TENNESSEE STATE OBSTETRICAL AND GYNECOLOGICAL SOCIETY

NEW YORK GYNECOLOGICAL SOCIETY

PACIFIC NORTHWEST OBSTETRICAL AND GYNECOLOGICAL ASSOCIATION

BUFFALO OBSTETRICAL AND GYNECOLOGICAL SOCIETY

SAN FRANCISCO GYNECOLOGICAL SOCIETY

JACKSON GYNECIC SOCIETY

INDIANA OBSTETRICAL AND GYNECOLOGICAL SOCIETY

THE MINNESOTA OBSTETRICAL AND GYNECOLOGICAL SOCIETY

COLUMBUS OBSTETRIC AND GYNECOLOGIC SOCIETY

GREATER HARTFORD OBSTETRICAL AND GYNECOLOGICAL SOCIETY

American Journal of Obstetrics and Gynecology

Copyright © 1989 by The C.V. Mosby Company

Information for Authors

Editors

John I. Brewer, *Editor in Chief*
710 North Fairbanks Court, Suite 8300,
Chicago, IL 60611

Sandra Fernandez, *Senior Editorial Assistant*
Tel.: (312) 908-5266

Frederick P. Zuspan, *Editor*
The Ohio State University, Fifth Floor, Means Hall,
1654 Upham Dr., Columbus, OH 43210

Donna Peterson, *Editorial Assistant*
Tel.: (614) 293-8798

E. J. Quilligan, *Editor*
University of California, Irvine, California College of Medicine, Med Surge 1, Room 109H, Irvine, CA 92717

Sherry A. Merryman, *Editorial Assistant*
Tel.: (714) 856-5092

Albert B. Gerbie, *Associate Editor*
710 North Fairbanks Court, Suite 8300,
Chicago, IL 60611

Publisher
THE C.V. MOSBY COMPANY, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318

Kay G. Goehler, *Senior Manuscript Editor*
Tel.: (314) 872-8370 (ext. 591)

INFORMATION FOR AUTHORS

Editorial policies

The requirements for manuscripts submitted to the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY conform to the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" established by the International Committee of Medical Journal Editors and published in *Annals of Internal Medicine* 1988;108:258-65. Certain requirements unique to our JOURNAL are provided in Information for Authors, published in each issue of the JOURNAL, and in more detail in the *Guide to Writing for the American Journal of Obstetrics and Gynecology*. The latter may be obtained from The C.V. Mosby Company or the Editors on request.

Manuscript submission. Manuscripts should be submitted to one of the three Editors as follows:

1. **Dr. Brewer**—all manuscripts originating from the southeastern quadrant of the United States or Canada and those presented before one of the official sponsoring societies, except the Society for Gynecologic Investigation and The Society of Perinatal Obstetricians.

2. **Dr. Zuspan**—manuscripts from the northeastern quadrant of the United States and from Japan, Israel, Italy, and England, manuscripts written for Clinical Opinion and Current Development, and Letters to the Editors. Dr. Zuspan is responsible for manuscripts from The Society of Perinatal Obstetricians. Manuscripts from The Society of Perinatal Obstetricians should be submitted to Dr. John A. Read, 9102 Lake Steilacoom Point Road, S.W., Tacoma, WA 98498.

3. **Dr. Quilligan**—manuscripts from the north central states (including Ohio), states west of the Mississippi River, Hawaii, Alaska, and abroad (except Japan, Israel, Italy, and England). Dr. Quilligan is responsible for manuscripts from the Society for Gynecologic Investigation. Manuscripts from the Society for Gynecologic Investigation should be submitted to Dr. Roger A. Lobo, Women's Hospital, 1240 North Mission Road, Room 1M2, Los Angeles, CA 90033.

Author's designation of reviewers. When authors submit their manuscripts, they may provide the names and addresses of three reviewers for consideration by the Editors.

Copyright statement. Effective July 1, 1988, all manuscripts must be accompanied by the following written statement, signed by *all* authors: "The undersigned author(s) transfers all copyright ownership of the manuscript [title of article] to The C.V. Mosby Company in the event the work is published. The undersigned author(s) warrants that the article is original; is not under consideration by another publication; and its essential substance, tables, or figures have not been previously published. This restriction does not apply to abstracts or press reports published in connection with scientific meetings. The author(s) confirms the final manuscript has been read and each author's contribution has been approved by the appropriate author. The author(s) responsible for the manuscript must be identified."

Previous publication. If a report by the same author(s) has been previously published in any medium that deals in any respect whatever with the same patients, same animals, same laboratory experiments, or same data, in part or in full, as those reported in the manuscript being submitted, two reprints of the article or two copies of the manuscript, be it a full-length report or an abstract, must be submitted with the manuscript. The author(s) should inform the Editor of the circumstances of the two reports. This requirement also applies to the submission of a manuscript in which a few different patients, animals, laboratory experiments, or data were added to those reported in a previous publication or in a submitted or accepted manuscript. Articles previously published in another language will not be considered.

Human and animal experimentation. It is assumed by the Editors that manuscripts emanating from a particular

institution are submitted with the approval of the requisite authority. Human experimentation that requires local institutional approval must have this approval *before the experiment is started* and approval must be so indicated in the Methods section of the submitted manuscript. Reports of experiments on animals must *state in the Methods section of the manuscript* that the guidelines for the care and use of the animals *approved by the local institution* were followed.

Authorship. For manuscripts with two or more authors, each author must qualify by having participated actively and sufficiently in the study that is being performed and reported. The inclusion of each author in the authorship list of a report is based only (1) on substantial contributions to (a) concept and design, or analysis and interpretation of data and (b) drafting the manuscript or revising it critically for important intellectual content; and (2) on final approval by each author of the version of the manuscript. Conditions 1 (a and b) and 2 must both be met. Others contributing to the work should be recognized separately in an Acknowledgment. In the covering letter that accompanies the submitted manuscript, it must be confirmed that all authors fulfilled both conditions.

Conflict of interest. Authors are expected to inform the Editor, in a letter accompanying the submitted manuscript, of any commercial association that might pose a conflict of interest, such as ownership, stock holdings, equity interests and consultant activities, or patent-licensing situations. Such information is confidential, is not given to the consultants, and does not play a part in the decision of the quality or timeliness of the manuscript. If the manuscript is accepted, the author and the Editor will determine how best to release the information. The usual and customary listing of sources of support and institutional affiliations on the title page is proper and does not imply a conflict of interest; only where there is a possible conflict of interest is the author(s) expected to inform the Editor.

Disclaimer. Statements and opinions expressed in articles and communications herein are those of the author(s) and not necessarily those of the Editor(s) or publisher, and the Editor(s) and publisher disclaim any responsibility or liability for such material. Neither the Editor(s) nor the publisher guarantees, warrants, or endorses any product or service advertised in this publication, nor do they guarantee any claim made by the manufacturer of such product or service.

General requirements for preparation of manuscripts

The original and two good-quality photocopies of the manuscript and three sets of glossy prints of illustrations are required.

Manuscripts must be typed double-spaced on one side only of 22 × 28 cm (8½ × 11 inch) white bond paper with 1-inch margins at top, bottom, and sides. Number pages consecutively in the upper right-hand corner in the following order: title page, condensation, abstract, body of text, acknowledgments, references, legends, and tables.

Title page. The title page (page 1) should contain in sequence the title (concise and suitable for indexing purposes); author line with first name, middle initial, and last name of each author and each author's highest academic degree (both MD and PhD are acceptable); city(ies), state(s) in which the study was conducted; divisional, or departmental, and institutional affiliations at the time the study was performed; source(s) of financial support; presented line, if applicable; disclaimers, if any; name, address, and business and home telephone numbers of author to whom requests for reprints should be addressed (if reprints will not be available, it should be so stated); and name, address, and business and home telephone numbers of author responsible for correspondence concerning the manuscript if different from author to whom reprint requests are addressed. At the bottom of the title page supply a short title for the running head not exceeding 52 characters (including word spaces).

Condensation. On page 2 of the manuscript provide a brief, concise condensation, typed double-spaced, that will appear with the title in publication of the Contents pages of the JOURNAL. It should be a single sentence, limited to a maximum of 25 words, delineating the essential point(s) made in the manuscript.

Abstract page and key words/phrases. On manuscript page 3 type the abstract, double-spaced, with the required margins and headed by the title of the article and name(s) of author(s). Abstracts for regular articles, Current Investigation, Clinical Opinion, and Current Development may not exceed 150 words. Abstracts for case reports and brief communications may not exceed 50 words. Below the abstract list 3 to 5 *key words* or short phrases for indexing purposes.

Text. Do not hesitate to write your manuscript in the first-person, active voice if it is more appropriate to the information you wish to convey. The passive voice is generally more effective for describing techniques or observations, since the emphasis is on the "action" rather than on the person performing the action.

Only standard abbreviations are to be used. Consult the *Council of Biology Editors Style Manual* or the AMA's *Manual for Authors and Editors*. Abbreviations in the title are not acceptable. They should be avoided, if possible, in the abstract. In the text they should be kept to a practical minimum. The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement.

Either the generic, chemical, or proprietary names of drugs may be used. If the generic or chemical name is used, authors may, if they desire, insert the proprietary name in parentheses after the first mention in the text, with the name of the manufacturer and city and state.

Regular articles are customarily organized into the following sections: an introduction and headings that identify Material and Methods, Results, and Comment. Authors may wish to summarize their findings in a short paragraph at the end of the Comment section. This format may not be appropriate for some types of articles.

In the introduction, state concisely the purpose and rationale for the study and cite only the most pertinent references as background.

In the *Material and Methods* section describe briefly (but in sufficient detail to permit other workers to evaluate and reproduce the results) the plan, patients and/or experimental animals and controls, methods and procedures utilized, and statistical method(s) employed.

In the *Results* section present the detailed findings. Include mentions of all tables and/or figures. Avoid duplication of text and supporting material. Emphasize only your important observations; do not compare your

Estimating length of manuscripts

The length of text material (introduction through Comment section) in regular manuscripts accepted for publication normally ranges from 750 to 4200 words (an average of 2000 words). A 4200-word text can seldom be accepted, especially if tables and figures are included. The average manuscript of 2000 words of text with abstract, 3 tables with captions, 2 figures with legends, and references makes a 5.7-page article in the JOURNAL. The 2000 words of text alone make approximately 8 pages of manuscript typed double-spaced with the required 1-inch margins (approximately 250 words per page). A table or figure that occupies both columns of half a JOURNAL page is equivalent to approximately 500 words in manuscript. Thus, if a greater number of illustrations and tables are used, the length of the text should be adjusted accordingly.

observations with those of others. Such comparisons and comments are reserved for the Comment section.

In the *Comment* section state the importance and significance of your findings but do not repeat the details given in the Results section. Limit your opinions to those strictly indicated by the facts in your report. Compare your findings with those of others. No new data should be presented in this section.

Acknowledgments. Acknowledge only persons who have made substantive contributions to the study.

References. A reasonable number are allowed, except in case reports and brief communications (limited to 2) and in manuscripts for the Current Development section (for which there is no limit). Number references consecutively in the order in which they are mentioned in the text. Use the format of the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" (Vancouver style) (Ann Intern Med 1988;108:258-65). Journal titles should conform to abbreviations used in *Cumulated Index Medicus*.

Examples (if six or fewer authors, list all; if seven or more authors, list three then et al.):

JOURNAL: Flamm BL, Fischermann E, Quilligan EJ, et al. Vaginal delivery following cesarean section—use of oxytocin augmentation and epidural anesthesia. AM J OBSTET GYNECOL 1984;148:759-63.

BOOKS: James VHT, Folkerd EJ, Bonney RC, Beranek PA, Reed MJ. Factors influencing estrogen production and metabolism in postmenopausal women with endocrine cancer. In: van Herendael HB, Riphagen FE, Goessens L, van der Pas H, eds. The climacteric, an update. Lancaster, England: MTP Press, 1983:29.

Personal communications and unpublished data, if essential, may be used but not as numbered references. If they are used, they are to be referred to, within parentheses, at the appropriate location in the text. If used, the author(s) must obtain written and signed permission for their use from the individual being quoted. This signed permission must accompany the manuscript when it is submitted to the Editor. Abstracts are not acceptable as numbered references.

Illustrations and tables. Illustrations and tables should supplement, not duplicate, the text; presentation of data in either one or the other will suffice.

A reasonable number of halftone and line illustrations will be reproduced without charge, but special arrangements must be made with the Editors for *color illustrations* at a cost of $525 per page (one side).

For *color photographs* submit original transparencies and two sets of unmounted prints on glossy (smooth-surface) paper. Polaroid prints are not acceptable. Color transparencies must have a color balance (consistency in lighting and film speed) that is acceptable to the author and Editors before acceptance for publication. Please note that 35 mm transparencies are enlarged to twice their original size. If it is important to deviate from this standard, please so indicate when the material is submitted. The *top, first author's last name,* and *figure number* must be indicated on the front of each transparency and the back of each print. Consistency in size of illustrations within the article is strongly preferred.

For *black-and-white illustrations* submit three sets of 3×4 inch (minimum) to 5×7 inch (maximum) unmounted, glossy photographic prints. All lettering must be done with commercially available paste-on letters (or numbers) or by a professional; typed or freehand lettering is not acceptable. All lettering must be in proportion to the drawing, graph, or photograph. Original drawings, appropriately done in black India ink, roentgenograms, and other material must be submitted as glossy photographic prints with good black-and-white contrast. Consistency in size within the article is strongly preferred. Any special instructions regarding sizing should be clearly noted.

Do not use paper clips or mar the surface of prints in any way.

Figures must be cited consecutively in the text in Arabic numerals and identified thusly on the back of the print (gummed label with): author(s) name(s), title of article, number, and top marked clearly.

Figures will be returned only on request by the author.

Tables should be typed on separate sheets of paper, one table to a page, and included at the end of the text. They should be numbered in Roman numerals. Each table must be cited in sequence at an appropriate point in the text. Captions should be brief yet indicate clearly the purpose or content of each table, and each column should be precisely defined by headings. Abbreviations and special designations should be explained in a footnote to the table. *If a table or any part thereof has been taken from copyrighted material, a legend to the table must give full credit to the original source.* Special arrangements must be made with the Editors for elaborate tables because of space limitations.

Legends to illustrations. Legends for all figures must be typed double-spaced on paper separate from the text of the manuscript, and these pages must be numbered in sequence after the references. Titles should be included in

the legend, *not* on the print. Original magnifications should be provided. *If an illustration has been taken from copyrighted material, the legend must give full credit to the original source.*

Computer-generated illustrations. *Black-and-white illustrations* submitted must be legible and clearly printed in jet-black ink on heavy coated paper with either a glossy or dull finish. Any patterns or shadings must be dark enough for reproduction and must be distinguishable from each other. Lines, symbols, and letters should be both smooth and complete. The legend for the illustration should not appear on the print. On the back of each print the name of the first author and the figure number should be given and the top indicated. Original individual laser or plotter prints are to be submitted *unmounted* with the manuscript. Laser prints should be full size at 300 dots per inch (DPI) or greater full-page resolution; multiple illustrations on a page cannot be accepted. Dot matrix prints and photographic halftones are not acceptable. *Color illustrations* are acceptable, but special arrangements must be made with the Editors. The colors used must be dark enough and of sufficient contrast for reproduction. With the exception of fluorescent colors, all colors can be reproduced in four-color illustrations. The preparation and submitting of color prints should follow the preceding guidelines for black-and-white computer-generated illustrations.

Permissions. Direct quotations, tables, or illustrations that have appeared in copyrighted material must be accompanied by *written permission* for their use from the copyright owner and original author along with complete information as to source. Photographs of identifiable persons must be accompanied by signed releases or else all recognizable features masked.

Requirements for special sections

Case reports and brief clinical and basic science communications. Limit of 700 words, 2 references. Include abstract of 50 words maximum, 3 to 5 key words/phrases for indexing purposes, and short title. If tables and/or figures are used, an equivalent number of words must be deducted from the total (see "Estimating Length of Manuscript").

Current Investigation. Same requirements as for regular article.

Clinical Opinion. Limit of 3000 words. Include abstract of 50 to 150 words, 3 to 5 key words/phrases, and short title. Submit to Dr. Zuspan.

Current Development. Limit of 6000 words. Include abstract of 50 to 150 words, 3 to 5 key words/phrases, and short title. Submit to Dr. Zuspan.

Correspondence. Two types of correspondence will be considered for publication. (1) A Letter to the Editors commenting on an article that has appeared in the JOURNAL should be brief and directly related to the published article. The editorial staff reserves the right to shorten letters if necessary and to make minor editorial alterations without reference to the writer. Letters may be published together with a reply from the original author. If the original author does not respond, a notation indicating "Response declined" will be published. As space for letters is limited, only a selection of letters submitted may be published. (2) A brief case presentation or a short report of a pertinent observation in the form of a Letter to the Editors will be considered for publication. All letters should be typed double-spaced. The original and a good photocopy must be submitted. Letters should be sent to Dr. Zuspan.

Announcements. Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60 U.S., and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, The C.V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, MO 63146-3318.

Books. Books received will be listed in the JOURNAL. They should be sent to Dr. Gerbie. No books will be returned.

Reprints

Reprints of articles must be obtained from the author. The corresponding author will receive a price schedule and order form at the time of publication. Reprints in quantities must be ordered from the publisher with the author's consent.

Business communications

Communications of a business nature and all advertising communications should be addressed to: Journal Publisher, The C.V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, MO 63146-3318, or call Journal Advertising Production Manager (314) 872-8370.

Checklist

—Letter of submission
—Copyright transfer letter
—Copies of other manuscripts containing duplicated data (see paragraph "Previous Publication")
—Original and two photocopies of manuscript
—Title page
—Title of article
—Full name(s), highest academic degree(s), and affiliations of author(s)
—Line citing financial support
—Author to whom correspondence is to be sent, including address and business and home telephone numbers
—Reprint requests line or line stating reprints not available
—Short title
—Condensation (double-spaced)
—Abstract (double-spaced), 3 to 5 key words/phrases
—Article proper (double-spaced)
—References (double-spaced), on a separate sheet
—Legends (double-spaced), on a separate sheet
—Tables (double-spaced), on a separate sheet
—Illustrations, properly labeled (three sets of glossy prints)
—Permission to reproduce published material
—Informed consent for patient photographs

The only journal pr[illegible] review of the entire field

mplete Coverage
ch issue provides a com-
te and systematic review
the past year's devel-
ments in one or two
tions of obstetrics and
necology. Together the six
monthly issues cover the
ire field of obstetrics and
necology.

notated References
th each review, the author
ects and annotates the year's most
nificant papers on that topic, high-
hting the key findings.

EDWARD E. WALLACH, Editor

Editorial Board

WC Andrews	CB Hammond	RD Kempers	M Mizuno	K Semm
S Campbell	AL Herbst	EA Lenton	T Mori	M Seppala
J Cohen	R Iizuka	A Ludmir	JT Queenan	N-O Sjoberg
JL Currie	V Insler	B Lunenfeld	JA Rock	JC Warren
AH DeCherney	TRB Johnson	GD Malkasian, Jr.	RH Schwarz	JD Woodruff
Sir J Dewhurst				

Section Editors

W Beck	A DeCherney	A Ludmir	A Rosenfield	K Semm
F Chervenak	D Foster	J Niebyl	J Sanfillipo	EE Wallach
J Currie	MGR Hull	J Rock	R Schwarz	JD Woodruff

Authoritative Reviews
Each section consists of a series of short, highly-illustrated reviews. Using a comprehensive bibliography, leading experts evaluate all new findings in their fields.

World Bibliography
In each issue, the bibliography of current world literature lists all relevant papers published in the past year. It is specially compiled by obstetricians and gynecologists from over 200 journals.

Current Opinion in OBSTETRICS & GYNECOLOGY

Review articles · Recommended reading
Bibliography of the world literature

Vol 1 · 1989 · No 1

Vol 1/No 1	THIS ISSUE	October
	In vitro fertilization · A DeCherney Infectious diseases · R Schwarz	
Vol 1/No 2	General obstetrics, Office gynecology, Adolescent and pediatric gynecology	December
Vol 2/No 1	Maternal/fetal medicine, Gynecologic oncology	February
Vol 2/No 2	Prenatal diagnosis, Fertility and infertility, Gynecologic pathology	April
Vol 2/No 3	Reproductive endocrinology, Gynecologic surgery	June
Vol 2/No 4	Gynecologic urology, Family planning, Endoscopy	August

CS CURRENT SCIENCE

Volume 1 Number 1 October 1989

In vitro fertilization
Section Editor: A. DeCherney

S. Boyers	Fertilization and implantation
J. Overstreet	The male factor in IVF
R. Marrs	Clinical aspects and techniques of IVF
S. Corson	GIFT and similar procedures
B. Younger	Ethical and moral issues and government legislation
R. Winston	Ovarian stimulation and the luteal phase
A. Luciano	Oocyte and embryo donation
S. Muashir	Freezing techniques

Infectious diseases
Section Editor: R. Schwarz

L. Westrom	PID and other STDs
S. Gall	HIV infection and AIDS
S. Faro	Surgical and postoperative infection
. Eschenbach	Intrauterine infection, premature rupture of membranes, premature labor, and neonatal infection
J. Sever	Perinatally acquired infections and screening
L. Gilstrap	Urinary tract infection
J. Thomason	Vaginitis

Current world literature

In vitro fertilization
Infectious diseases
Index to subjects

an papillomavirus infection

J.D. Oriel

Harley Street, London, UK

urrent Opinion in Infectious Diseases 1989, 2:2–6

ons by human papil-
equences, have been
esearch. Much of this
p between HPV and
liscoveries of the last
ely slow, and many
nswers. The effective
HPV-induced lesions

viruses

e been detected in
16 and 18 are asso-
gnant disease of the
al types; their DNAs
in invasive cancers.
designated HPV 45,
cervical lesion show-
tosis [1]. Subsequent
of genital tract infec-
; it is associated with
ectrum, from invasive
lium.
histological sections
ed tissue has been
tudied, firstly to assess the sensitivity of this technique in
comparison with other hybridization procedures such as

a biotin–streptavidin polyalkaline phosphatase comp
detection system; they demonstrated the presence
HPV 6 and 11 in benign condylomas and also in
case of cervical carcinoma HPV 16 was present in
cases of vulval intra-epithelial neoplasia and in four ca
of squamous cancer of the anal canal. Shibata *et al.*
used an enzymatic *in vitro* gene amplification techni
— the polymerase chain reaction; the assay detected H
16 or 18, without cross-reactivity to HPV 6 or 11, in
biopsies from women with severe cervical dysplasia
invasive cancer.

Wilbur *et al.* [5] examined 180 exophytic genital lesi
for HPV by haematoxylin and eosin light microscopy,
munocytochemistry for HPV capsid antigen and *in*
hybridization for HPV messenger RNA. Of 96 histolo
cally confirmed condylomas, *in situ* hybridization
positive in 80 (83%), the predominant types being H
6 and 11, but immunocytochemical assay was posi
in only 53 (55%) of the cases. Jenkins *et al.* [6]
cluded that routine HPV antigen staining of colposc
biopsy specimens is of very limited diagnostic value,
that the technique is best reserved for cases which
colposcopically abnormal but in which no definite hi
logical or cytological abnormality can be found. Mors
al. [7] compared the results of DNA hybridization
cytological findings on 164 Papanicolaou stained cerv
scrapes from 143 women, 124 scrapes were regarde
'normal', with no cytological features suggestive of
vical intra-epithelial neoplasia (CIN), and 40 were f
women with colposcopically and histologically confir
CIN. HPV DNA, predominantly HPV 6 or 16, was pre
in 64 (39%) of the 164 specimens. Cytological char
keratin
multinu
be sam
nges al

Human papillomavirus infection Oriel

araffin-embedded

ction of human
using the poly-
67:225–230.
araffin-embedded

tion of infection
omata: — a com-
and *in situ* nu-
1988, 89:505–510.
hybridization was
y was positive in

tion and human papillomavirus type 16 DNA sequences in cervical intra-epithelial neoplasia and normal tissue in the west of Scotland: evaluation of treatment policy. *Br Med J* 1988, 296:381–385.

HPV 16 or 18 sequences were detected in 20 out of 27 colposcopically abnormal biopsy samples, and in 13 out of 27 paired normal samples from the same patients. A cofactor other than HPV is necessary for pre-neoplastic disease to develop.

15. KREBS H-B, SCHNEIDER V: Human papillomavirus-associated
• lesions of the penis — colposcopy, cytology, and histology. *Obstet Gynecol* 1987, 70:299–304.

83 (65%) male partners of 127 women with CIN had small HPV-associated lesions: 80 were condylomas and 3 penile dysplasia.

16. SCHNEIDER A, DE VILLIERS E-M, SCHNEIDER V: Multifocal squa-
•• mous neoplasia of the female genital tract — significance

Human papillomavirus infection

BANKS L, MATLASHEWSKI G, PIM D, CHURCHER M, ROBERTS C, CRAWFORD L: **Expression of human papillomavirus type-6 and type-16 capsid proteins in bacteria and their antigenic characterization.** *J Gen Virol* 1987, **68**:3081–3090.

BARRASSO R, DE BRUX J, CROISSANT O, ORTH G: **High prevalence of papillomavirus-associated penile intraepithelial neoplasia in sexual partners of women with cervical intraepithelial neoplasia.** *N Engl J Med* 1987, **317**:916–922.

BARTON SE, DAVIES JM, MOSS VW, TYMS AS, MUNDAY PE: **Asymptomatic shedding and subsequent transmission of genital herpes simplex virus.** *Genitourin Med* 1987, **63**:102–105.

BEKASSY Z, WESTROM L: **Infrared coagulation in**

CAUSSY D, ORR W, DAYA AD, ROTH P, REEVES W, RAWLS W: **Evaluation of methods for detecting human papillomavirus deoxyribonucleotide sequences in clinical specimens.** *J Clin Microbiol* 1988, **26**:236–243.

CHOO KB, PAN CC, HAN SH: **Integration of human papillomavirus type 16 into cellular DNA of cervical carcinoma: preferential deletion of the E2 gene and invariable retention of the long control region and the E6/E7 open reading frames.** *Virology* 1987, **161**:259–261.

COLLINS JE, JENKINS D, MCCANCE DJ: **Detection of**
• **human papillomavirus DNA sequences b in situ DNA-DNA hybridisation in cervic intraepithelial neoplasia and invasive carcinoma — a retrospective study.** *J Clin Pathol* 1988, **41**:289–295 [2].

CONFERENCE: **Human papillomavirus and**

Call **TOLL FREE:** (800) 552-5866 or in PA call (215) 790-2279

Become a CHARTER SUBSCRIBER and receive one issue FREE!

VA89 Current Opinion in OBSTETRICS & GYNECOLOGY, Volume 1, 1989/90 (8 issues) V603

☐ Yes! Please enter my **Personal Subscription** ~~$88~~. **$87.50**
☐ **Institutional Subscription $170.**

Payment Method: ☐ Check enclosed, payable to Current Science
☐ Bill me ☐ VISA ☐ Mastercard ☐ American Express

Card Number _____ Exp. _____

Signature _____ Date _____

Money-Back Guarantee: If not completely satisfied with this journal, you may cancel your subscription by writing to Current Science within 30 days of receiving the first issue. Your payment will be fully refunded.

Name _____

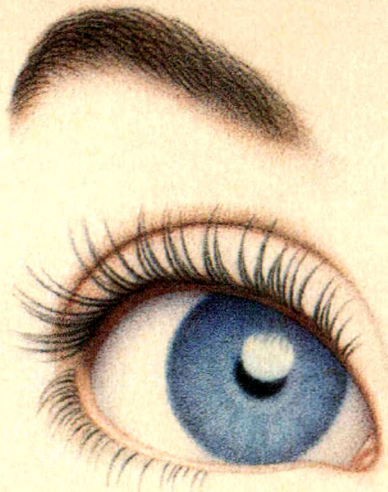
Address _____

_____

City/State/Zip _____

MAIL TO:

CS CURRENT SCIENCE
CURRENT SCIENCE
DEPARTMENT VA
1201 LOCUST STREET
PHILADELPHIA, PA 19107-9824

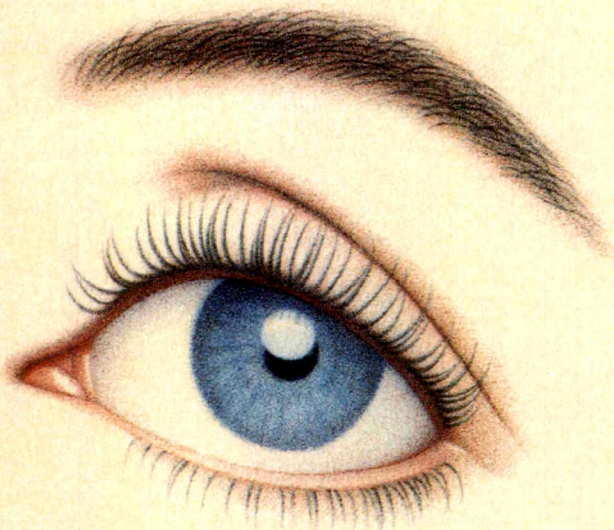


"When breakthrough bleeding happened to me I was scared, and that's why I called my doctor."

She deserves a







...etter start

TRI·LEVLEN®

Levonorgestrel and ethinyl estradiol tablets—Triphasic regimen

54%* less breakthrough bleeding* than Ortho-Novum® 7/7/7

Demonstrated in the largest parallel comparative trial of triphasic OCs to date†[1]

*Serious as well as minor side effects have been reported with the use of all oral contraceptives. The physician should remain alert to the earliest symptoms of serious disease and discontinue oral contraceptive therapy when appropriate. Please see full prescribing information, a brief summary of which follows.

†100 women randomly assigned to Tri-Levlen® tablets or Ortho-Novum® 7/7/7; 50 women placed on nonhormonal contraception as a control. Evaluations were made at baseline and once a month for 6 months.

One cycle of Tri-Levlen® tablets therapy provides: ethinyl estradiol–30 mcg/day for 6 days, 40 mcg/day for 5 days and 30 mcg/day for 10 days; levonorgestrel–50 mcg/day for 6 days, 75 mcg/day for 5 days and 125 mcg/day for 10 days. One cycle of Ortho-Novum® 7/7/7 therapy provides: ethinyl estradiol–35 mcg/day for 21 days; norethindrone–500 mcg/day for 7 days, 750 mcg/day for 7 days and 1000 mcg/day for 7 days.

Ortho-Novum® is a registered trademark of Ortho Pharmaceutical Corp.

BERLEX Dedicated to innovation in OB/GYN medicine

TRI-LEVLEN® 28 Levonorgestrel and ethinyl estradiol
TRI-LEVLEN® 21 tablets—Triphasic regimen

BRIEF SUMMARY

Tri-Levlen®—6 brown tablets, each containing 0.050 mg of levonorgestrel (d(-)-13 beta-ethyl-17-alpha-ethinyl-17-beta-hydroxygon-4-en-3-one), a totally synthetic progestogen, and 0.030 mg of ethinyl estradiol (19-nor-17α-pregna-1,3,5(10)-trien-20-yne-3,17-diol); 5 white tablets, each containing 0.075 mg levonorgestrel and 0.040 mg ethinyl estradiol; 10 light-yellow tablets, each containing 0.125 mg levonorgestrel and 0.030 mg ethinyl estradiol (7 light-green tablets containing inert ingredients are included in the 28-day triphasic regimen).

Indications and Usage—Tri-Levlen Tablets are indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OCs) as a method of contraception.

Contraindications—OCs should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5). 8. Benign or malignant liver tumor which developed during the use of OCs or other estrogen-containing products.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with the use of OCs is well established. Three principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OCs are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION—An increased risk of myocardial infarction associated with the use of OCs has been reported, confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing myocardial infarction, regardless of whether the patient was an OC user or not. OCs, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE—In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism, including coronary thrombosis, is directly related to the dose of estrogen used in OCs. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the U.K. estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OCs according to age, smoking habits and duration of use. The overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000). the risk being concentrated in older women, in those with a long duration of use and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking and duration of use, nor to compare the effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. The available data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. The estimates of risk of death for each method include the combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OCs) plus the risk attributable to pregnancy or abortion in the event of method failure. This latter risk varies with the effectiveness of the contraceptive method. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OCs in women over 40 who smoke. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with OCs increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes or history of preeclamptic toxemia and, especially, by cigarette smoking. The physician and the patient should be alert to the earliest manifestations of thrombophlebitis and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four-to-six-fold increased risk of postsurgery thromboembolic complications has been reported in OC users. If feasible, OCs should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Great Britain involving cerebrovascular disease and another study in the United States concerning myocardial infarction suggest that an increased risk of these conditions in users of OCs persists after discontinuation of the OC. In the British study, the risk of cerebrovascular disease remained elevated in former OC users for at least six years after discontinuation. In the U.S. study, an increased risk of myocardial infarction persisted for at least 9 years in women 40- to 49-years-old who had used OCs for five or more years. The findings in both these studies require confirmation since they are inconsistent with other published information.

2. *Ocular Lesions:* There have been reports of neuro-ocular lesions, such as optic neuritis or retinal thrombosis, associated with the use of OCs. Discontinue OC medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. *Carcinoma:* Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina and liver. Certain synthetic progestogens, none currently contained in OCs, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case-control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time OCs were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs. Several studies have found no increase in breast cancer in women taking OCs or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with OCs, found an excess risk in the subgroups of OC users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of OCs has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with OCs. Close clinical surveillance of all women taking OCs is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use OCs.

4. *Hepatic Tumors:* Benign hepatic adenomas have been found to be associated with the use of OCs. One study showed that OC formulations with high hormonal potency were associated with a higher risk than lower potency formulations. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of OCs. Two studies relate risk with duration of use of OCs, the risk being much greater after 4 or more years of OC use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking OCs. The relationship of these drugs to this type of malignancy is not known at this time.

5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring and Malignancy in Female Offspring.* The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence at the present time that OCs further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OCs. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including OCs, in pregnancy. One case-control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. It is recommended that, for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period and further use of OCs should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue OCs with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease:* Studies report an increased risk of surgically confirmed gallbladder disease in users of OCs and estrogens. In one study, an increased risk appeared after 2 years of use and doubled after 4 or 5 years of use. In one of the other studies, an increased risk was apparent between 6 and 12 months of use.

7. *Carbohydrate and Lipid Metabolic Effects:* A decrease in glucose tolerance has been observed in a significant percentage of patients on OCs. For this reason, prediabetic and diabetic patients should be carefully observed while receiving OCs. An increase in triglycerides and total phospholipids has been observed in patients receiving OCs. Three studies have been performed with the Tri-Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets Triphasic Regimen) formulation and no significant alterations in lipid metabolism were noted, with the exception of a slight increase in triglyceride levels in one study. The clinical significance of these findings remains to be defined.

8. *Elevated Blood Pressure:* An increase in blood pressure has been reported in patients receiving OCs. In some women, hypertension may occur within a few months of beginning OC use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure and in the fifth year of use is two-and-a-half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given OCs. Hypertension that develops as a result of taking OCs usually returns to normal after discontinuing the drug.

9. *Headache:* The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent or severe, requires discontinuation of OCs and evaluation of the cause.

10. *Bleeding Irregularities:* Breakthrough bleeding, spotting and amenorrhea are frequent reasons for patients discontinuing OCs. Irregular breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of OCs. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. *Breast-feeding:* OCs given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in OCs has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of OCs should be deferred until the infant has been weaned.

Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OCs. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical examination and PAP smear being performed. 2. Under the influence of estrogen-progestogen preparations preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. OCs may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving OC therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by OC therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of OC therapy when relevant specimens are submitted. 10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OCs: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test.

Information for the Patient—See Patient Package Labeling

Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Carcinogenesis—See "Warnings" section for information on the carcinogenic potential of OCs.

Pregnancy—Pregnancy Category X. See "Contraindications" and "Warnings."

Nursing Mothers—See "Warnings."

Adverse Reactions—An increased risk of the following serious adverse reactions has been associated with the use of OCs (see "Warnings"): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies.

There is evidence of an association between the following conditions and the use of OCs, although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients receiving OCs and are believed to be drug-related. Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally: gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; dysmenorrhea; amenorrhea during and after treatment; temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement and secretion; change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses.

The following adverse reactions have been reported in users of OCs, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, hemolytic uremic syndrome.

Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OCs by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Dosage and Administration—To achieve maximum contraceptive effectiveness, Tri-Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets—Triphasic Regimen) must be taken exactly as directed and at intervals not exceeding 24 hours. (If Tri-Levlen is first taken later than the first day of the first menstrual cycle of medication or postpartum, contraceptive reliance should not be placed on it until after the first 7 consecutive days of administration. The possibility of ovulation and conception prior to initiation of medication should be considered.) Any time the patient misses 1 or 2 brown, white or light-yellow tablets, she should also use another method of contraception until she has taken a tablet daily for 7 consecutive days.

For full details on dosage and administration see prescribing information in package insert. 60611-1 5/86

Reference: 1. Data on file, Berlex Laboratories, Inc.

TRI-LEVLEN is a registered trademark of Berlex Laboratories, Inc.

TRI·LEVLEN®

Levonorgestrel and ethinyl estradiol tablets—
Triphasic regimen

BERLEX
Dedicated to innovation in OB/GYN medicine

BERLEX Laboratories, Inc., Wayne, New Jersey 07470

© 1989, Berlex Laboratories, Inc. All rights reserved.

430-118

American Journal of Obstetrics and Gynecology

Founded in 1920

volume 161 *number* 2 AUGUST 1989

CLINICAL SECTION

Clinical Opinion

Missed abortion: Still appropriate terminology?

Gabriella Pridjian, MD, and Atef H. Moawad, MD
Chicago, Illinois

The term *missed abortion* has been in the obstetric literature more than 100 years. With ultrasonographic evaluation of early pregnancy, its textbook definition (death of a fetus without its expulsion for ≥8 weeks) is rarely fulfilled. Yet the term *missed abortion* is still extensively used today with regard to early pregnancy losses. We recommend its abandonment and in its place the use of the following terms: *first trimester intrauterine fetal death, second trimester intrauterine fetal death,* and *anembryonic gestation.* (AM J OBSTET GYNECOL 1989;161:261-2.)

Key words: Missed abortion, anembryonic gestation, nomenclature, ultrasonography

In 1847 Henry Oldham coined the term *missed labor* to describe the situation in which there was death of the ovum without its expulsion in a pregnancy of >28 weeks' gestation. In 1878 J. Mathews Duncun introduced the term *missed abortion* in the *Medical Times and Gazette* to describe a similar situation in which the gestation was <28 weeks.[1] The term *missed labor* has been appropriately lost as obstetrics has developed into a more exact science. However, the term *missed abortion* is still widely used today. *TeLindes' Operative Gynecology* defines missed abortion as a dead fetus retained for ≥8 weeks, or a "prolonged period."[2] *Comprehensive Gynecology* defines missed abortion as fetal death before 20 weeks' gestation without expulsion of any fetal or maternal tissue for ≥8 weeks thereafter.[3] The term was coined in an effort to describe the process of expulsive forces exerted on the conceptus. A missed abortion is a clinical diagnosis made when there are no expulsive forces exerted for a prolonged period of time after the death of the conceptus. The remainder of the spectrum of expulsion are threatened, inevitable, incomplete, and complete abortion.

Before the advent of ultrasonographic techniques, the clinician used lack of growth of the uterus as one of the major clinical signs of death of the fetus, and waited a "prolonged period" to ascertain the diagnosis. The use of the term *missed abortion* today is confusing because ultrasonographic evaluation can assess the status of the conceptus in a much shorter period of time. "Missed" in modern obstetrics implies that the physician could have made the diagnosis earlier, but did not. There should no longer be instances in which missed abortion is diagnosed or pregnancy evaluation is delayed to make the diagnosis. Nor should the term be misused. For example, a patient at 10 weeks' gestation with an ultrasonographic diagnosis of a nonviable fetus cannot be considered to have a missed abortion, by definition, because 8 weeks or a prolonged period has not passed since death of the fetus.

Early pregnancy loss can be a devastating emotional event for a woman and her partner. When possible signs and symptoms of an early miscarriage are evident, evaluaton of the integrity of a first trimester pregnancy should be done expeditiously. Ultrasonography should be performed at the first prenatal visit if the size of the uterus is smaller than dates, if pregnancy has been associated with significant spotting, bleeding, or cramping, or if at ≥8 weeks' gestation no fetal heart tones are heard with Doppler.

It is no longer appropriate to delay diagnosis of a nonviable conceptus until the next visit to determine whether there is growth of the uterus. In our institution, as in others, transvaginal ultrasonography is performed for first trimester pregnancy evaluation if the transabdominal scan is unsatisfactory. Advantages to the transvaginal approach include higher resolution,

From the Department of Obstetrics and Gynecology, The University of Chicago, Chicago Lying-In Hospital.
Reprint requests: Gabriella Pridjian, MD, Department of Obstetrics and Gynecology, The University of Chicago, 5841 S. Maryland Ave., Chicago, IL 60637.

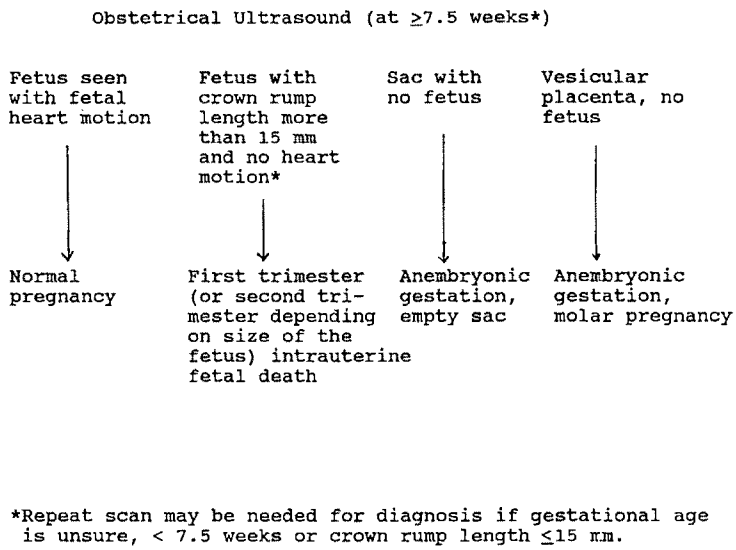


Fig. 1. Nomenclature of early pregnancy status based on transabdominal and, if needed, transvaginal pelvic ultrasonography.

especially in obese patients, and minimal preparation or patient discomfort, as the maternal bladder need not be full. If by ultrasonography, there is no fetal heart activity yet the fetus has a clearly visible body >15 mm, or if dating is excellent (infertility patients) and there is no fetal heart motion at ≥7.5 weeks' gestation, a "first (or second) trimester intrauterine fetal death" is diagnosed.[4] The diagnosis of an "anembryonic gestation, empty sac" or "anembryonic gestation, molar pregnancy" is made when at ≥7.5 weeks there is no fetus[5] (Fig. 1).

When nonviability of a pregnancy is ascertained, a dilatation and curettage procedure should be promptly scheduled. Delay of evacuation of the intrauterine contents may lead not only to hemorrhage and infection during spontaneous abortion, but also to undue anguish by the parents.

Even in instances in which the patient is seen late in pregnancy, the term *missed abortion* is not informative. For example, if a patient at 16 weeks' gestation by accurate last menstrual period is found to have a much smaller uterus on examination and a fetus without heart motion by ultrasonography, biometric measurements should be done to date the fetus in an effort to evaluate when the death occurred. A diagnosis of first trimester or second trimester fetal death may be made on the basis of biometric fetal measurements. If no fetus is found, a diagnosis of anembryonic gestation is much simpler and more descriptive. (Although the conceptus is, by definition, considered an embryo until 8 weeks after fertilization, or 10 weeks after the last menstrual period, for simplicity we have elected to use the term *first-trimester fetal death* to include death of both embryo and fetus in the first trimester.)

The study of the causes of early pregnancy loss may benefit if in the medical record the diagnosis is more precise. It may be that certain chromosomal complements are associated with anembryonic gestations (polyploidy),[6] whereas others are more associated with intrauterine fetal deaths. Maternal factors such as uterine anomalies or fibroids may be more associated with first trimester fetal death, whereas external factors such as infection may be more associated with anembryonic gestations. This information may help us to better counsel patients after they experience a pregnancy loss, especially when the chromosome studies are not available.

In summary, the term *missed abortion* should be abandoned for more accurate descriptions of early fetal losses. We recommend the use of the terms *anembryonic gestation* (empty sac or molar pregnancy), *first-trimester intrauterine fetal death*, or *second trimester intrauterine fetal death*, diagnosed by ultrasonography to be used in its place.

REFERENCES

1. Ricci JV. One hundred years of gynecology (1800-1900). Philadelphia: The Blankston Company, 1945:489.
2. Grimes DA. Surgical management of abortion. In: Mattingly RF, Thompson JD, eds. TeLinde's Operative Gynecology. 6th ed. Philadelphia: JB Lippincott, 1985:516.
3. Mishell DR. Abortion. In: Dreegemueller W, Herbst AL, Mishell DR, Stenchever MA, eds. Comprehensive gynecology. St. Louis: CV Mosby, 1987:375.
4. Jeanty P, Romero R. Obstetrical ultrasound. New York: McGraw Hill, 1984:45-7.
5. Sabbagha R. Diagnostic ultrasound applied to obstetrics and gynecology. Hagerstown: Harper & Row, 1980:66.
6. Simpson JL, Golbus MS, Martin AO, Sarto GE. Genetics in obstetrics and gynecology. New York: Grune and Straton, 1982:124-7.

Multidisciplinary team counseling for fetal anomalies

Robert P. Lorenz, MD,[a,b] and Margueritte H. Kuhn, MD[a]
Royal Oak and Detroit, Michigan

Prenatal management of fetal anomalies requires a cooperative effort of the family, obstetrician, ultrasonographer, neonatologist, pediatric subspecialists, nursing staff, geneticists, and social workers. Our approach to these patients is to convene all team members and the family shortly after an anomaly is identified to review the diagnosis, prognosis, plans, and alternatives. Team counseling offers a clear definition of the problem, avoids unnecessary confusion and anxiety for the family, and provides a supportive environment during this family crisis. Our experience with 28 families has been favorable and is described herein. (Am J Obstet Gynecol 1989;161:263-6.)

Key words: Fetal anomalies, prenatal counseling, genetics, perinatal grief

In 1961 Solnit and Stark[1] described the birth of a defective newborn as "the sudden loss of the baby that was expected, and sudden birth of a feared, threatening, anger-evoking child." Davidson[2] described the parental reaction to the live birth of a child with an anomaly as "death of the wished for child." Irvin et al.[3] described the stages of response as shock, denial, sadness and anger, equilibrium, and reorganization.

With recent advances in ultrasonography and prenatal genetic testing, a previous pediatric problem is increasingly becoming the responsibility of the obstetric team. The identification of a fetal anomaly is a devastating family crisis that shatters the usual expectation of a normal pregnancy, birth, and subsequent care of a healthy newborn. The psychologic response to the ultrasonographic finding of a fetal anomaly is similar in some to the birth of a newborn with an anomaly, with elements of shock, denial, anger, and disorientation. When a fetal diagnosis is established remote from delivery, the additional burden of prolonged anticipation and uncertainty combined with the lack of opportunity to directly bond with a visible child requires the full support of the medical team and the extended family.

There have been significant improvements in the ability to identify, intervene, and maximize outcome for the fetus with an anomaly. Management of pregnancies complicated by fetal anomalies is often best treated at tertiary perinatal centers with the resources and the subspecialist teams to assist in these rare problems. In the past such problems were managed by an obstetrician who would request consultation and then transfer care of the fetus to the pediatric team after birth. The family would meet the consultants and pediatric team when the neonatal care began. In cases with complex fetal problems, the family would meet several physicians, nurses, and support personnel, often for the first time during an emergency when urgent decision making was necessary. This "linear" or "sequential" model of one-on-one interactions (Fig. 1) typically may involve the following at different and sequential times: ultrasonography, a visit with the obstetrician, repeat ultrasonography, another visit with the obstetrician (or his/her associate), a consultation with the maternal-fetal medicine specialist, another visit or discussion with the obstetrician, a consultation with the relevant pediatric subspecialist, antepartum fetal testing (e.g., nonstress tests), admission for labor and delivery, care by a neonatologist and nursing team, further consultation and possible surgery in the newborn period, and meetings with social workers, clergy, and others. Meanwhile, the professionals who are involved are reviewing information and discussing differential diagnoses, prognosis, recommendations for testing and management (e.g., timing and mode of delivery). These professional discussions are usually also one-on-one, involving one member (e.g., one obstetrician in a group of four associates) of each group involved. In addition, the patient relays the information from her visits to her partner and often to her other children, her close friends, relatives, and clergy. These family support members have usually not accompanied the patient to most of her visits in the above sequence. Schematically, it is not unusual to have a total of 72 or more one-on-one conversations among interested parties before resolution of the clinical problem (Fig. 1).

As the number of individual conversations increases over the extended time of the patient's workup and consultation process, the probability of unnecessary confusion and anxiety (in addition to the inevitable confusion and anxiety that such a diagnosis carries) rises

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, William Beaumont Hospital, Royal Oak,[a] and Wayne State University, Detroit.[b]
Reprint requests: Robert P. Lorenz, MD, Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, William Beaumont Hospital, 3601 W. 13 Mile Road, Royal Oak, MI 48072.

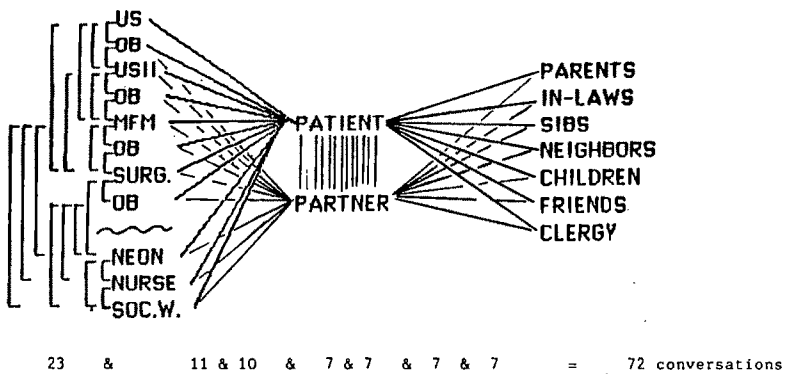


Fig. 1. One-on-one conversations in sequential management model. *MFM,* Maternal-fetal medicine; *NEON,* neonatologist; *NURSE,* neonatal nurse; *OB,* obstetrician; *Soc.W,* social worker; *SURG,* pediatric surgeon consultation; *US,* ultrasonography; *USII,* repeat or level II ultrasonography.

Table I. Multidisciplinary team members

Team members	*No.**
Physicians	
Primary obstetrician	10
Maternal-fetal medicine consultant	28
Ultrasonographer	20
Neonatologist	20
Pediatric cardiologist	3
Pediatric geneticist	8
Pediatric infectious disease consultant	1
Pediatric neurosurgeon	6
Pediatric surgeon	3
Pediatric urologist	3
Resident	28
Nursing staff	
Neonatal intensive care unit nurse	1
Perinatal nurse specialist/coordinator	6
Other	
Cytogenetics specialist	3
Reproductive genetics counselor	17
Social worker	26

*Number of times each member participated in 28 conferences.

dramatically. In addition, the likelihood of full understanding and agreement among all the professionals involved is reduced when one-on-one conversations are the primary mode of planning. Usually someone not involved in these discussions (e.g., nurses, obstetrician, or pediatric surgeon's partner on call) becomes involved in care without full prospective understanding.

Thus the sequential method of management is not the best method to provide a coordinated, supportive, and clear plan to a family facing the crisis of a diagnosis of a fetal anomaly.

Model of multidisciplinary team counseling

Multidisciplinary team counseling for fetal anomalies is a coordinated meeting of caregivers and the family to discuss differential diagnoses, prognosis, and recommendations for care. Principal elements include a conference among the professionals, a conference with the family, and a written report. When a fetal anomaly is first identified (usually by ultrasonography, occasionally by cytogenetic studies), the attending obstetrician is immediately informed and offered the assistance of the team. Within 24 to 48 hours appropriate members are assembled for team counseling. If subsequent diagnostic studies later in pregnancy or patient concern warrants a second counseling session, the same members are reassembled with the family.

Professional conference. Before the conference, a complete review of the prenatal record, genetic history, history, and physical examination by the maternal fetal medicine consultant occurs. Then the family waits in a separate area while the case is presented to the assembled team (see Table I). The ultrasonographic images and reports are presented and discussed. After discussion, a consensus is reached with regard to differential diagnoses, range of prognosis, and recommendations for further testing and therapy (e.g., timing and mode of delivery, possible neonatal surgery). Then the members delegate the topics that each will discuss when the family joins them.

Team meeting with family. Before the meeting, the family is informed of the names and responsibilities of the team members and the purpose of the meeting. Questions are encouraged.

The family joins the conference and sits together near the obstetrician. The obstetrician introduces the other team members and may act as the conference leader. Ultrasonographic results are presented, and each team member reviews the assigned topic and describes his or her role in subsequent care. The patient and her family then can ask questions of anyone present. The family is offered a visit to the neonatal intensive care unit after the conference. The family is given a list of the team members, their departments, and phone numbers for future reference.

A written summary of the complete history, physical

Table II. Types of fetal anomalies*

System	*n*
Amniotic fluid	9
Cardiac	7
Central nervous system	9
Cleft palate	1
Cytomegalovirus	1
Cystic hygroma	1
Cytogenetic	4
Growth abnormality	5
Hydrops	3
Renal	5
Ventral wall defect	7
TOTAL	52

*Many fetuses had more than one finding.

Table III. Sequential versus multidisciplinary team counseling

Difficulties with sequential visits
Many individual visits with different professionals
Delays between each visit
Partner may not always be present
Benefits of team visits
Fewer visits
Rapid resolution
Patient, partner, and family hear all input together
All professionals hear each other's input simultaneously
All professionals hear everything patient hears
One written plan

examination, ultrasonographic findings, conference participants, discussion, and conclusions related to diagnosis, prognosis, plan, and counseling session is sent to the obstetrician, all members of the team, and all inpatient units (e.g., delivery room, neonatal intensive care unit) where the family will receive care.

Results

During the development of this program (1985 to 1987) there were 104 (0.6%) newborns delivered at William Beaumont Hospital who were admitted to the neonatal intensive care unit with congenital anomalies. Of these newborns, 30 (29%) died in the newborn period. Many patients did not have prenatal diagnosis of the congenital anomaly.

This report describes the approach used for 28 families who had multidisciplinary team counseling for fetal anomalies (Table II). Family participants varied in number from one (one case when the patient came alone) to five. Grandparents of the fetus were the most frequent participants in addition to the partner. Professional participants varied in number from four to nine. The primary obstetrician was on staff at our institution in 23 cases; in 10 cases (43%) he or she was a participant in the conference. Of the five cases referred from other institutions, three had care transferred to our center subsequent to the conference. The combined conferences last an average of 1 hour.

Of the 14 cases diagnosed before 24 weeks' gestation, twelve chose pregnancy termination. Three of the 16 ongoing pregnancies ended in fetal demise, and two in death in the first month of life.

Follow-up included postpartum visits by members of the team, ongoing counseling by the Perinatal Loss Service after a death, and subsequent genetic counseling in many cases. Further follow-up information was received from the primary obstetrician in some cases.

Reactions to this approach from the patients and their families has been overwhelmingly positive. Patients and their families are impressed with the assembled team who are prepared to help their baby. Instead of being intimidated by a large group of strangers, the patients and their families perceived the team conference as reassuring and helpful. In our experience, they seem more prepared for the subsequent birth than patients who underwent sequential management. When the fetal prognosis is poor, the grief process seems to proceed more smoothly when this thorough initial team conference is the beginning. Fewer patients have sought a second opinion since this program began, which we believe reflects a better understanding and acceptance of the problem and plan. When second opinions are requested, the provision of the written record of the multidisciplinary team counseling helps to introduce the second opinion team to what has actually occurred, further reducing the potential for confusion.

Professionals find the conference format educational, and a direct and simple way to analyze a complex problem and decide on a plan. They can all hear what the family hears, and observe their reactions and questions. It saves time for everyone because patients are less likely to initiate extra telephone calls and visits to review information. The written report is a tremendous aid in subsequent management and counseling.

Comment

Sequential counseling versus multidisciplinary team counseling for fetal anomalies is compared in Table III. The latter offers a more rapid response and formulation of a consensus plan, which is most helpful to the family. It removes the family perception of differences of opinion (or openly clarifies what the differences are) among the professionals who will be involved in perinatal care. It provides an opportunity for the family to meet in advance the people who will be caring for their child, before critical decision making is necessary. It is a useful eductional tool for all members of the team and provides updates on all aspects of an approach to fetal anomalies. It provides a supportive group of

knowledgeable family members and professionals to help the patient and her partner in a time of crisis. Sixty-one percent of the cases described ended in pregnancy termination, fetal death, or neonatal death. In these cases, the team management conference becomes a central element in management of perinatal grief. The grief process is supported by the presence of well-informed family members and the professional team.

Principles of counseling families with a newborn with an anomaly[3, 4] and with a perinatal loss[5-7] have been previously addressed. Families should be openly counseled together soon after the diagnosis, in a setting that is private and unhurried. professionals involved include a perinatologist and a chaplain or social worker[8] for perinatal loss and the pediatrician and a physician in training[3] for the newborn with a congenital anomaly. For the family of a fetus with a fetal anomaly, we believe a larger group for the initial management conference is more beneficial. Subsequent grief counseling after a death is assigned to one member of the initial counseling team.

Ideally, the primary obstetrician is involved in the counseling session. As the person chosen by the patient to assist her with her pregnancy and birth, he or she represents a trusted and familiar figure in a time of uncertainty, fear, and axiety. In the first few years of this program, the obstetrician was able to and chose to participate in 43% of the conferences. Participation increased in the later years as obstetricians understood the team conference and its benefits. In many cases, schedules did not permit participation of all members of the team, or the obstetrician, in these hastily assembled conferences. It is understandable that some obstetricians chose not to participate in a setting of an unfamiliar fetal problem and in a format of a group session. Such a format may be uncomfortable for some physicians who have been trained and are more comfortable in a one-on-one patient interaction in an office setting. On the positive side, obstetricians who have participated are enthusiastic about the format, and many of them now utilize it and participate when clinical circumstances warrant this approach.

Multidisciplinary team counseling requires effort on the part of the members, flexibility in making schedule changes to participate, and time. In our experience, the effort is worthwhile and results in better coordination of care and more effective support of the family. We believe this is truly a "better mousetrap," and encourage others to adapt it to their management of fetal anomalies, as well as pursue formal studies of its effectiveness compared with other approaches to fetal anomaly counseling and management.

REFERENCES

1. Solnit AJ, Stark MH. Mourning and the birth of a defective child. Psychoanal Stud Child 1961;16:523.
2. Davidson GW. Understanding death of the wished for child. Springfield, Illinois: OGR Corp, 1979.
3. Irvin NA, Kennell JH, Klaus MH. Caring for the parents of an infant with a congenital malformation. In: Klaus MH Kennell JH, Parent-infant bonding. 2nd ed. St. Louis: CV Mosby, 1982.
4. Fost N. Counselling families who have a child with a severe congenital anomaly. Pediatrics 1981;67:321-4.
5. Peppers LG, Knapp RJ. Motherhood and mourning. New York: Praeger, 1980.
6. Woods JR Jr., Esposito JL, eds. Pregnancy loss. Baltimore: Williams & Wilkins, 1987.
7. Borg S, Lasker J. When pregnancies fail. Boston: Beacon Press, 1981.
8. Woods JR Jr, Klein BA. Perinatal support services at the University of Cincinnati, In: Woods JR, Esposito JL, eds. Pregnancy loss. Baltimore: Williams & Wilkins, 1987.

Breast disease programs in obstetrics and gynecology: A plea for training in mammography

Norbert Gleicher, MD
Chicago, Illinois

Obstetrics and gynecology residency programs have started to integrate structured breast disease teaching programs into the curricula. I suggest that mammography training should be the cornerstone of programs on the diagnosis of breast diseases. The implementation of such a program into a teaching department is described in an attempt to facilitate similar efforts elsewhere. (AM J OBSTET GYNECOL 1989;161:267-70.)

Key words: Mammography, breast disease, medical education

Recommendations by a variety of professional bodies have mandated the establishment of teaching programs for the diagnosis of breast diseases within departments of obstetrics and gynecology. Although this mandate was primarily directed at residents within approved obstetrics and gynecology residency programs and could have been fulfilled through elective periods and other forms of cooperation outside the departments of obstetrics and gynecology, our department decided to develop its own intramural program. The following is a description of the program development process through its inception, planning, and implementation phases. From the subsequent comments it will be apparent that my strong preference lies with programs within obstetrics and gynecology departments and not in efforts outside the department. It is hoped that the subsequent pages will convince others of this viewpoint and will help to facilitate the necessary implementation process elsewhere.

Historical perspective

Little doubt can exist that the female breast is a reproductive organ. In that function it represents the only reproductive organ outside the treatment perview of our specialty. Except for some minor geographic exceptions, diagnostic as well as surgical management of breast disease has in this country become exclusively the territorial property of radiologists and surgeons. This is despite the fact that gynecologists more than any other medical specialty serve as primary care providers to women, perform breast examinations, and diagnose breast disease, inclusive of carcinoma.

Although it is most frequently the gynecologist who first faces breast pathology, it is usually the radiologist who performs diagnostic procedures of mammography and breast ultrasonography and the surgeon who obtains biopsy specimens and operates when required. The patient by that time has seen three physicians, has made three appointments, and has waited in three waiting rooms. She also, in a way, has been abandoned by the primary physician responsible for her reproductive health, her gynecologist.

The concept of preventive screening was invented in our specialty when Papanicolaou developed the Papanicolaou smear in an attempt to decrease the incidence of invasive carcinoma of the cervix.[1] Large scale Papanicolaou smear screening has reduced the annual incidence of newly diagnosed invasive carcinomas of the cervix by 50%.[2] Quite correctly, the American College of Obstetricians and Gynecologists objected to earlier recommendations by the American Cancer Society, which had suggested that low-risk patients did not require an annual Papanicolaou smear.[3] A very strong argument for annual Papanicolaou smears was the concomitant physical examination that, it is hoped, is inclusive of a breast examination.

At present approximately 110,000 new cases of carcinoma of the breast occur annually in the United States. Approximately 37,000 women die annually from the disease.[4] European studies have conclusively demonstrated that routine mammography screening can reduce mortality from breast cancer ≤60%.[5,6] To the gynecologist this should not come as a surprise. After all, we invented the concept of precancerous lesions.[1] As with most other tumors, survival after breast cancer correlates closely with tumor size at time of diagnosis.[7] Because mammography can detect

From the Department of Obstetrics and Gynecology, Mount Sinai Hospital Medical Center, and Rush Medical College.
Supported in part by the Foundation for Reproductive Medicine, Inc., Chicago, Illinois.
Reprint requests: Norbert Gleicher, MD, Mount Sinai Hospital Medical Center, California Avenue at 15th St., Chicago, IL 60608.

breast cancer of nonpalpable size, mammography can and should be seen as the "Papanicolaou smear" of the breast. This concept has been recognized and has been the basis for broadly based screening recommendations formulated by various medical organizations and endorsed by the American College of Obstetricians and Gynecologists as early as 1979.[8]

Why the gynecologist?

If one accepts the notion of wide-scale mammography screening, then one has to address practicality, efficiency, and costs of such an effort. All three of these issues point toward the gynecologist. Because the gynecologist is usually the only health care provider women see on a regular (i.e., annual) basis, mammography screening should take place when and where her other screening concerns (Papanicolaou smear, pelvic exam, breast exam) are addressed. Such an approach is not only logical, but also convenient for the patient and is the most cost-effective approach. If all regularly required screening processes can be combined into one visit, costs have to compare favorably with all alternatives. Such an approach will return the primary care responsibility for breast care to the gynecologist and thus reestablish our specialty as the exclusive providers of female reproductive health care. An argument can be made that the quality of breast care in this country often lags behind European countries, because no specialty has assumed primary responsibility for the female breast. By the assumption of this responsibility, our specialty can potentially save more lives than with any other presently recommended screening program.

If we accept responsibility for the diagnosis and proper care of female breast disease, then one has to conclude that we have to learn mammography. An analogy to the performance of pelvic ultrasonography immediately comes to mind. Initially in the perview of radiologists, the logic of clinical practice has shifted pelvic ultrasonography increasingly toward the gynecologist. As the pelvic mass on bimanual exam requires ultrasonographic follow-up, so does the breast mass radiographically (and at times by ultrasonography).

Residency programs are now required to provide curricula in ultrasonography. Both general and subspecialty boards expect certain levels of ultrasonography expertise from candidates. If the diagnosis of breast disease is to be taught to our house staff, then mammography interpretation needs to be taught immediately after instruction of proper breast examination and long before breast biopsy and other surgical issues become relevant.

Since the diagnosis of breast disease has entered our residency program curricula, faculty have to teach the topic. In our department this was seen as an opportunity to integrate mammography into a newly established breast disease program that included a dedicated mammography unit.

Program development

Establishment of a departmental breast program was clearly initiated by the newly established requirements to teach the pathophysiology and diagnosis of breast disease to our house staff. In an attempt to develop a rational and a feasable program approach for our institution, we concluded that we had to proceed through the following developmental steps:

1. Didactic education of faculty and house staff. Because none of our faculty had the expertise to provide the required information, a lecture series with outside speakers was scheduled over an approximately 6-month period.

2. Program emphasis on breast examination in the departmental outpatient clinic. Under faculty supervision, the house staff was closely inspected in the performance of breast examinations. Each house officer received a "breast examination card" on which attending physicians had to certify that they witnessed 20 correctly performed breast examinations.

3. Establishment of a dedicated mammography unit for breast cancer screening. Because the institutional political situation did not allow the placement of this unit within the hospital confines, the unit (General Electric Mammoview Mammography System, Naperville, Illinois) was placed in the private practice facility of the geographic full-time faculty group. The already available ultrasonography unit at that site (General Electric RT 3600 with 7.5 linear transducer, Naperville, Illinois) was upgraded to allow the performance of breast ultrasonography when needed in support of mammography.

4. Faculty training in mammography and breast ultrasonography. Faculty were sent outside the department for training in mammography and breast ultrasonography. All faculty members who wished to receive "certification" to read mammograms had to attend both a basic and advanced mammography course offered by the widely respected mammographer L. Tabar. This requirement was not only established because of the noted excellence of these courses, but also to assure uniformity of the reading process.

The introduction of mammography into the department came 6 years after the establishment of processes that introduced obstetric and gynecologic ultrasonography to faculty and house staff. Then and today, selected faculty members underwent training first. Once their level of competence reached satisfactory levels, senior residents were integrated into the scan-

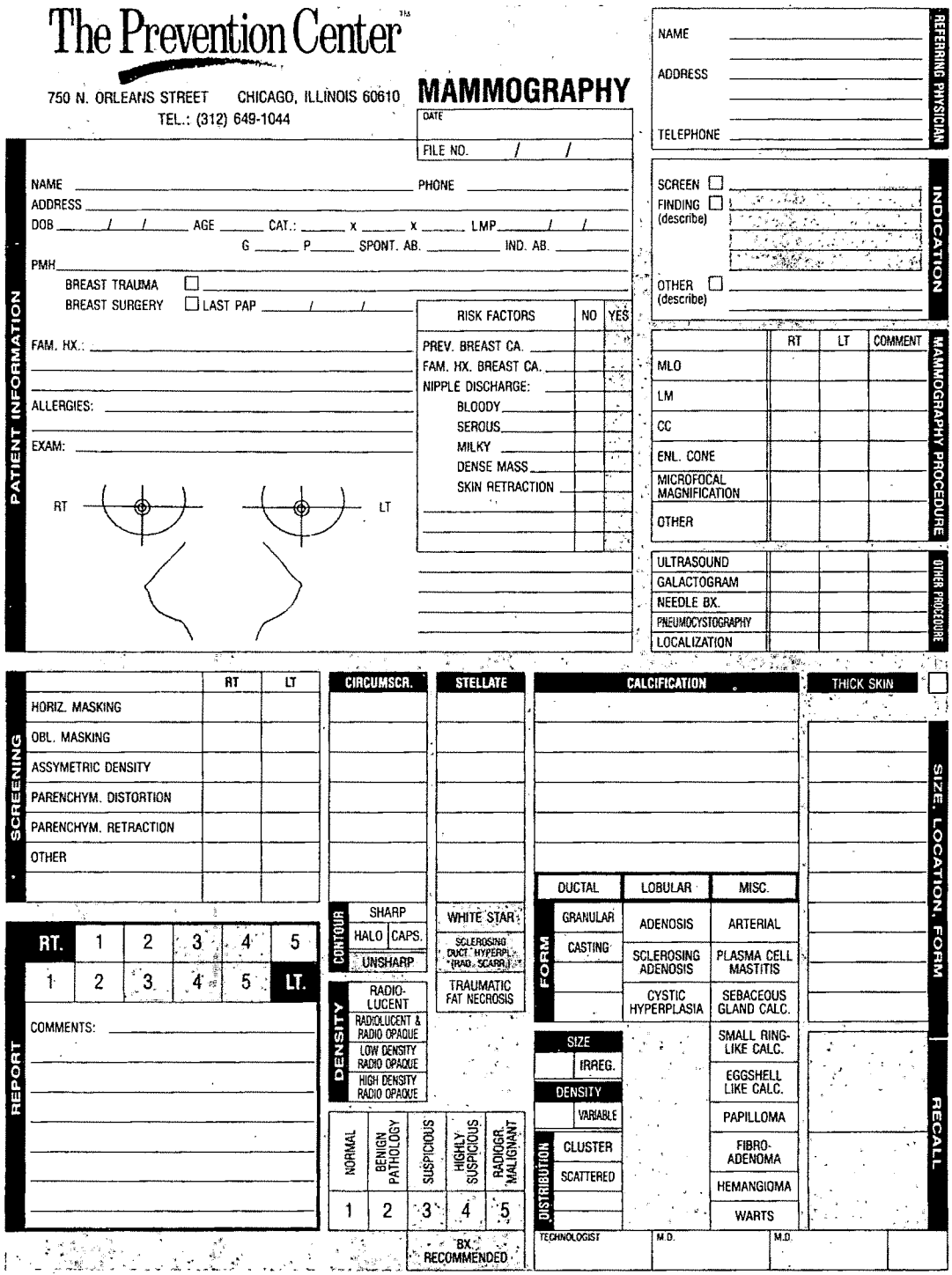


Fig. 1. Mammography reading and reporting form was developed to apply standard mammography reading techniques to mammography reading process in department. Stepwise approach to mammography is forced on reading physician because each reading step has to be indicated separately. Final result of reading process is detailed description of each finding. Note that all findings indicative of potential malignancy and all high-risk factors in patient's history are highlighted in red *(shaded area)*. Each breast is classified in classes I to V as indicated. Classes III to V indicate biopsy recommendation.

ning process. Over a 4-year period practical hands-on education reached the first-year residents. Over the past 2 years all house staff have been actively involved in the routine performance of obstetric as well as gynecologic ultrasonography in the department. In 1987 approximately 90% of all departmental scans were performed by residents with very satisfactory sensitivity and specificity.

Although faculty training in mammography was not completed until July 1988, the education process for selected senior residents began in March 1988 when the mammography unit opened. It was expected the unit would perform 20 to 25 breast examinations per day by July 1988. Mammography reading skills are projected to reach first-year residents by 1992.

To emphasize mammography as the "Papanicolaou smear" of the breast we developed a mammography reading report sheet that follows standard reading recommendations by L. Tabar but classifies each breast in accordance with Papanicolaou smear classifications into five classes (Fig. 1). This results in a reading process with a clear recommendation of whether to obtain a biopsy specimen.

Also a recommendation of L. Tabar,[9] we decided to double-read each mammography study. This provided an opportunity to collaborate with our radiology colleagues who double-read each film. Although we believed strongly in the placement of the breast diagnosis program within the obstetrics and gynecology department, we also believed that professional teamwork between medical disciplines was essential if the best possible care was to be provided to patients. Double-reading gave the opportunity of collaboration with the radiology department. The performance of breast surgery established this opportunity for collaboration with the surgery department. By the association of one general surgeon with special interest in breast surgery with the program, we provided the necessary surgical expertise to the patient, and to the teaching program at the same time. Although three departments are thus involved in the breast program, its structure is clearly different from any other program of which we are aware. Not only is a clear primary responsibility established, but it is established within our specialty. We believe this not only improves our teaching process for breast disease but, more importantly, has greatly improved medical care for our patient population.

REFERENCES

1. Papanicolaou GN, Traut HF. Diagnosis of uterine cancer by the vaginal smear. New York: Commonwealth Fund, 1943.
2. American College of Obstetricians and Gynecologists. Periodic cancer screening for women (Statement of Policy). Washington DC: American College of Obstetricians and Gynecologists, 1980.
3. American College of Obstetricians and Gynecologists Tech Bull No. 81, October 1984.
4. Leis HP Jr. Epidemiology in breast cancer. In: Strombeck JO, Rosato FE, eds. Surgery of the breast: diagnosis and treatment of breast diseases. Stuttgart: Georg Thieme Verlag, 1986:100-9.
5. Tabar L, Gad A, Holmberg LH, et al. Reduction in mortality from breast cancer after mass screening with mammography. Lancet 1985;1:829-32.
6. Verbeek ALM, Holland R, Sturmans F, Hendriks JHCL, Mravunac M, Day NE. Reduction of breast cancer mortality through mass screening with modern mammography. Lancet 1984;1:1222-6.
7. Leis HP Jr. Prognosis in breast cancer. In: Strombeck JO, Rosato FE, eds. Surgery of the breast: diagnosis and treatment of breast diseases. Stuttgart: Georg Thieme Verlag, 1986:110-9.
8. American College of Obstetricians and Gynecologists. Statement of policy. Washington, DC: American College of Obstetricians and Gynecologists, 1979.
9. Tabar L, Dean PB. Teaching atlas of mammography. 2nd ed. Stuttgart: Georg Thieme Verlag, 1985.

Make researching easier with the Ten-Year Cumulative Index (1978-1987) to the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY

The Ten-Year Cumulative Index is a complete guide to more than 30,000 pages of original articles, case reports, letters, and editorials published in the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY from 1978 through 1987 (volumes 130 to 157). Numbering more than 800 pages, the Cumulative Index includes both a subject and an author index. This hard-cover volume is $64.50 ($69.00 international). Prices include shipping. *Payment must accompany all orders and must be in U.S. funds, drawn on a U.S. bank.*

Contact The C.V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146 USA; phone (314) 872-8370, ext. 394. In the U.S., call toll-free: 1-800-325-4177, ext. 394. Please allow six weeks for delivery.

Intrauterine growth retardation: Standards for diagnosis

Robert L. Goldenberg, MD, Gary R. Cutter, PhD, Howard J. Hoffman, MA, Judith M. Foster, MPH, Kathleen G. Nelson, MD, and John C. Hauth, MD
Birmingham, Alabama

An intrauterine growth-retarded infant is commonly defined as one weighing less than the 10th percentile in birth weight for its gestational age. However, because there is no standard population from which to derive these percentiles, the birth weights that serve as the cutoff point in various published studies may differ by several hundred grams at any gestational age. For this reason, we examined the studies from which the currently used 10th-percentile standards are derived to determine which factors may be responsible for the variation. In addition to obvious differences in the populations and geographic areas on which they were based, studies differed in how gestational age was determined, whether the gestational age was "rounded" or given in completed weeks, which types of infants were excluded, whether the studies were hospital or population based, and whether they were controlled for sex of the infant and race and parity of the mother. These differences in study methodology may be as or more important than the population differences in defining the 10th percentile cutoffs. A single national standard for intrauterine growth retardation would allow comparison between studies of risk factors, diagnostic tests, management, and long-term follow-up status of fetuses and infants with intrauterine growth retardation. (Am J Obstet Gynecol 1989;161:271-7.)

Key words: Intrauterine growth retardation, small for gestational age, low birth weight

Fetal growth insufficient for length of gestation is associated with an increased risk of perinatal mortality and morbidity.[1] Common to each investigation of infants with intrauterine growth retardation (IUGR) is a decision about the criteria that will define the condition, usually a birth weight below an arbitrarily chosen point at each gestational age. These cutoff points have included the 3rd[2] and 5th percentile of birth weight,[3] 2 SD below the mean,[4,5] and the more commonly used 10th percentile.[6] Since race and parity of the mother and the sex of the infant influence birth weight, standards specific for these factors also have been published.[7-9] It is obvious that use of different birth weight percentiles, or correction of the standard for race, parity, and sex of the infant have a major effect on the birth weights that define IUGR. However, there are substantial differences in the various published standards that are defined by the 10th percentile alone.

Because infants of certain weights will be diagnosed as having IUGR in some studies but not others, many of the IUGR-related risk factor and infant outcome studies are not comparable, which often leads to conflicting results. Our review, comparing the 10th percentile birth weights in various reports, was undertaken to better understand the populations from which the standards were developed and the methods used to determine the 10th percentile values ultimately published.

From the Perinatal Epidemiology Unit, the Departments of Obstetrics and Gynecology and Pediatrics, and the School of Public Health, The University of Alabama at Birmingham, and the Biometry Branch, Prevention Research Program, National Institute of Child Health and Human Development, National Institutes of Health.
Supported in part by National Institutes of Health Contract No. N01-HD-4-2811.
Reprint requests: Robert L. Goldenberg, MD, Department of Obstetrics and Gynecology, The University of Alabama at Birmingham, University Station, Birmingham, AL 35294.

Methods

The studies reviewed were selected from those published in the English literature since 1963 that presented in tabular form values for the 10th percentile of birth weight for gestational age. These studies were characterized by location, source of data, the method used to define gestational age, exclusions, sample size, patient characteristics, and the methods used to determine the 10th percentile values. In addition, to illustrate the differences for selected gestational ages, the actual 10th percentile values from each of the studies are presented. When available, the 10th percentiles for boys and girls, black and white infants, and primiparous and multiparous women also are presented.

Results

The 10th percentile birth weights at several gestational ages from the various studies are presented in Table I. When available, the 10th percentiles for male and female infants, black and white infants, and infants of parous and nonparous mothers are presented separately. In addition, for each group of studies defined by the same sex, or inclusion of both sexes, the maximum difference in 10th percentile birth weight be-

Table I. Tenth percentile birth weight values at various gestational ages

Author	Sex	Population description		Birth weight (gm) at gestational age (wk)					
		Race	Parity	28	32	36	38	40	42
Babson et al.[14]	M,F	W	P,M	695	1351	2173	2602	2880	3039
Brenner et al.[10]	M,F	W,B	P,M	770	1310	2190	2510	2750	2830
Goldenberg et al.*	M,F	W,B	P,M	891	1381	2232	2597	2849	2959
Lubchenco et al.[6]	M,F	W	P,M	860	1290	2050	2430	2630	2720
Naeye and Dixon et al.[12]	M,F	W,B	P,M	967	1534	2121	2570	2776	2778
Williams et al.[17]	M,F	W,B	P,M	727	1301	2229	2629	2848	2965
Difference				**272**	**244**	**182**	**199**	**250**	**319**
Carr-Hill and Pritchard[15]	M	W	P	—	1430	2147	2661	2888	2891
Carr-Hill and Pritchard[15]	M	W	M	—	1519	2277	2768	3001	2978
Hardy et al.[13]	M	W	P,M	—	1510	2250	2526	2840	2966
Hardy et al.[13]	M	B	P,M	—	1629	2237	2471	2692	2743
Hoffman et al.[9]	M	W	P,M	843	1548	2259	2621	2890	3010
Hoffman et al.[9]	M	B	P,M	907	1577	2227	2544	2676	2660
Kloosterman[7]	M	W	P	930	1280	2090	2600	2900	3030
Kloosterman[7]	M	W	M	930	1380	2210	2700	3050	3190
Lubchenco et al.[6]	M	W	P,M	915	1320	2105	2505	2700	2730
Miller and Merritt[8]	M	W	P	—	—	—	2840	3080	3280
Miller and Merritt[8]	M	W	M	—	—	—	2860	3190	3450
Miller and Merritt[8]	M	B	P,M	—	—	—	2730	2900	3080
Milner and Richards[11]	M	W	P,M	860	1410	2230	2600	2890	2910
Thomson et al.[16]	M	W	P	—	1370	2310	2690	2910	2930
Thomson et al.[16]	M	W	M	—	1680	2310	2750	2980	2980
Williams et al.[17]	M	W,B	P,M	762	1348	2278	2696	2944	3086
Difference				**168**	**400**	**220**	**355**	**514**	**790**
Carr-Hill and Pritchard[15]	F	W	P	—	1153	2164	2491	2774	2437
Carr-Hill and Pritchard[15]	F	W	M	—	1530	2102	2631	2864	2907
Hardy et al.[13]	F	W	P,M	—	1424	2180	2448	2710	2861
Hardy et al.[13]	F	B	P,M	—	1533	2164	2422	2604	2646
Hoffman et al.[9]	F	W	P,M	758	1527	2170	2446	2713	2802
Hoffman et al.[9]	F	B	P,M	788	1564	2181	2545	2592	2603
Kloosterman[7]	F	W	P	810	1180	1950	2500	2800	2950
Kloosterman[7]	F	W	M	820	1260	2010	2680	2930	3050
Lubchenco et al.[6]	F	W	P,M	870	1250	1960	2405	2630	2630
Miller and Merritt[8]	F	W	P	—	—	—	2720	2970	3170
Miller and Merritt[8]	F	W	M	—	—	—	2800	3050	3260
Miller and Merritt[8]	F	B	P,M	—	—	—	2650	2890	3100
Milner and Richards[11]	F	W	P,M	830	1330	2150	2510	2770	2810
Thomson et al.[16]	F	W	P	—	1310	2280	2530	2770	2780
Thomson et al.[16]	F	W	M	—	1330	2350	2600	2830	2820
Williams et al.[17]	F	W,B	P,M	678	1219	2169	2587	2817	2936
Difference				**192**	**411**	**400**	**395**	**458**	**823**

P, Primiparous; *M*, multiparous.

The differences in birth weight between the highest and lowest values in studies in which the sex of the infant was similar (M and F, M or F) were calculated.

* Unpublished observations.

tween the standards at each gestational age studied is presented. There are large differences in 10th percentile cutoff points between the various standards, with the differences >500 gm at some gestational ages.

We hope to explain some of the reasons for this variation (Table II) by discussing the methods used to construct each set of standards.

The most widely used birth weight standards are

those of Lubchenco et al.[6] from Denver. This study population consisted of 5635 live-born white infants. Exclusions consisted of infants with anencephaly, hydrocephalus, hydrops fetalis, and maternal diabetes as well as all nonwhite infants. In addition, 55 infants thought to be too large for gestational age at delivery also were excluded. Gestational age was calculated from the first day of the last menstrual period (LMP) and was presented in completed weeks. As shown in Table I, these standards tend to be lower than the others, especially at later gestational ages. This difference has been thought, at least partially, to be a result of the high altitude of the Denver area. It should be noted that the very small number of infants studied at many of the gestational ages make the confidence limits of the cutoff points at many gestational ages very large.

Brenner et al.[10] analyzed data from 30,772 deliveries at 21 to 44 weeks' gestational age in Cleveland. The population was 53% white and 47% nonwhite (98% black). Data were analyzed from singleton births from pregnancies not complicated by preeclampsia, breech presentation, erythroblastosis, diabetes mellitus, or congenital abnormalities. Gestational age was calculated and rounded to the nearest week from the first day of the last normal menstrual period. Correction factors for parity, race, and infant sex were calculated and presented, but 10th percentile measurements for these subgroups were not presented.

Miller and Merritt[8] analyzed extensive anthropometric data on a mixed population of 6000 black and white infants born at The Univesity of Kansas Medical Center from 1973 to 1978. Their intention was not to do a population-based study but instead to develop normal standards for fetal growth in a population of surviving infants devoid of risk factors for IUGR. From their original population of 6000, they presented data by parity and sex for 1200 term white infants and by sex for 473 term black infants. For an infant to be included in the study, the gestational age as determined by LMP, ultrasonography, maternal clinical measurements, and a newborn examination had to be in agreement. The 10th percentile standards in this study were considerably higher than those in other published studies (Table I).

The fetal growth standards developed by Kloosterman[7] were also reviewed. These standards were constructed by investigation of 80,000 mostly white pregnancies in Amsterdam between 1931 and 1965. Eight percent of those cases in which the gestational age was unknown or uncertain, stillbirths with maceration, and multiple births were excluded. The LMP was used to determine gestational age in completed weeks. The mean and various percentile weights for each gestational age were determined by use of the birth weight. The authors considered the differential effect of sex and parity (primiparous and multiparous women) on birth weight and constructed curves for each of the four combinations of sex and parity.

Milner and Richards[11] used data from England and Wales to construct fetal growth curves. They chose a random sample of 9.7% of all births at the National Health Service Hospitals from 1967 to 1971. After exclusions, this sample represented 8.5% of all births at these hospitals and consisted of 271,519 births. Birth weights were analyzed at each gestational age by sex of the infant and maternal parity.

Data from the Collaborative Perinatal Project of the National Institute of Neurological and Communicative Disorders and Stroke were analyzed by Naeye and Dixon.[12] These data were collected prospectively from 12 hospitals in the United States between 1959 and 1966. The study population consisted of 48,239 single-born neonates who were alive and well at 1 year of age. The racial distribution consisted of 46% black, 46% white, and 8% other. Gestational age was based only on the LMP; the reported gestational age was rounded to the nearest week. Birth weights of survivors only were plotted by gestational age. Cases were found to cluster at birth weight intervals consistent with a bimodal distribution similar to that described by Milner and Richards.[11] The lowest weight cluster was chosen at each gestational age.

Birth weight for gestational age data from The Collaborative Perinatal Project were also reported by Hardy et al.[13] In this report, in contrast to that of Naeye and Dixon,[12] 10th percentile values were reported by race and sex. The population consisted of 18,481 white and 19,504 black singleton live-born infants with known birth weight. The data presented were reported as collected. There were no adjustments or exclusions. For this study, gestational age was based on the LMP only and was rounded to the nearest week.

Babson et al.[14] reviewed 39,895 deliveries at two maternity hospitals in Portland, Oregon, from 1959 through 1966. The mothers were primarily of Northern European descent, lived near sea level, and received private prenatal care. The gestational age, rounded to the nearest week, was generally calculated from the first day of the LMP. However, in determining the gestational age at delivery, clinical data were at times taken into consideration. Also all fetal deaths, multiple births, and nonwhite infants were excluded from analysis.

In a recently published monograph, Carr-Hill and Pritchard[15] present an extensive discussion of the development of birth weight standards. The population for their analysis consisted of 17,528 singleton births in Aberdeen from 1975 to 1980. All stillbirths and births from multiple pregnancies were excluded. Gestational age was recorded in completed weeks since the first day of the LMP, and only women with certain LMPs were included. They presented their 10th percentile data divided by sex of the infant and parity of the

Table II. Characteristics of studies that used 10th percentile to determine IUGR

Author	*Location*	*Source of data*	*Gestational age method*	*Exclusions*	*Sample size*	*Characteristics of population*	*Methods*
Lubchenco et al.[6]	Denver, Colo.	Hospital births, 1948-1961	LMP only No clinical correction Completed wk	Nonwhites Anencephaly Hydrocephalus Hydrops Diabetics "Too large" infants	5,635	White High altitude Spanish 30% Low socioeconomic status	Percentiles read from midpoint of week and smoothed arithmetically
Brenner et al.[10]	Cleveland, Ohio	Hospital births, 1962-1969	LMP only No clinical correction Rounded to nearest wk	Many clinical conditions	30,772	53% white 47% black Mixed socioeconomic status	Percentiles smoothed by 2-point weighted means
Miller and Merritt[8]	Kansas	Hospital births, 1973-1978	LMP, ultrasonography, clinical dating and pediatric dating all agree Completed wk	All known risk factors for IUGR Congenital anomalies Perinatal deaths	1,200 White 473 Black	Mixed black and white	Data presented by race, sex, and parity
Kloosterman[7]	Amsterdam, Netherlands	Hospital births, 1931-1965	Not stated	Stillbirths Multiple births Gestational age unknown or uncertain	80,000	White Lower socioeconomic status	"Smooth line drawn through original crooked one"
Milner and Richards[11]	England and Wales	Births at National Health Service hospitals, 1967-1971	LMP only No clinical correction Completed wk	Stillbirths Multiple births Births before admission No gestational age or birth weight on chart	271,519	White Mixed socioeconomic status	Data available by sex and corrected for nonnormal distribution
Naeye and Dixon[12]	U.S. hospitals	Births at hospitals participating in CPP, 1959-1966	LMP only No clinical correction Rounded to nearest wk	Multiple births Deaths before age 1	48,239	46% Black 46% White 8% Other	Cluster probability plot method controlling for nonnormal distribution

CPP, Collaborative Perinatal Project; *NCHS*, National Center for Health Statistics.
* Unpublished observations.

mother (a total four standards) from 32 to 42 weeks' gestational age.

The 10th percentiles presented from The University of Alabama are calculated from a hospital-based data set of 4497 live births from women with a "sure" LMP (unpublished observations). Approximately 70% of the mothers were black. No exclusions were made for congenital anomalies. Gestational age at delivery was calculated from a prenatally determined estimated date of confinement by not only the "sure" last menstrual period but also other clinical indicators of gestational age. The authors found that use of the obstetrician's best prenatal estimate of gestational age resulted in a greater percentage of the births at 39 and 40 weeks and less variability of birth weight at each gestational age than in the "sure" LMP data derived from the same population. These corrections also resulted in relatively higher 10th percentiles at later gestational ages and lower 10th percentiles at the earlier gestational ages.

All of the previously described studies utilized hospital data. It has been suggested that 10th percentiles of birth weight at each gestational age may differ significantly between hospital data and population-based data. Therefore it was also of interest to examine population-based studies for comparative purposes.

One such study was by Thomson et al.[16] Data were collected from 52,004 legitimate, singleton births in Aberdeen from 1948 to 1964. Infants with congenital anomalies and macerated stillbirths were excluded from the study. Gestational age was determined from the first day of the last normal menstrual period and was classified as certain, uncertain, or unknown. In the uncertain group, clinical information combined with the LMP was used to define the gestational age. Those with unknown gestational ages, comprising about 10% of all births, were excluded. Gestational age data were reported in completed weeks. The data were analyzed, and percentiles of fetal weight were constructed by parity and sex.

Hoffman et al.[9] (1968) used a 50% sample of all live

Author	Location	Source of data	Gestational age method	Exclusions	Sample size	Characteristics of population	Methods
Hardy et al.[13]	U.S. hospitals	Births at hospitals participating in CPP, 1959-1966	LMP only No clinical correction Rounded to nearest wk	Multiple births Other than white or black	18,481 White 19,504 Black	Low socioeconomic status	Data presented by race and sex
Babson et al.[14]	Portland, Ore.	Births at two maternity hospitals, 1959-1966	LMP (usually) Clinical corrections Rounded to nearest wk	Stillbirths Multiple births Nonwhites	39,895	Private patients, mostly of Northern European descent	Uncorrected
Carr-Hill and Pritchard[15]	Aberdeen, Scotland	Births at one hospital, 1975-1980	LMP only No clinical corrections Completed wk	Stillbirths Multiple births Uncertain LMPs excluded	17,528	White Mixed socioeconomic status	Data presented by sex and parity
Goldenberg et al.*	Birmingham, Ala.	Births at two hospitals, 1984-1986	LMP sure Clinically corrected Completed wk	Unsure LMP No prenatal care	4,497	70% Black 30% White Low socioeconomic status	3-point weighted mean
Thomson et al.[16]	Aberdeen, Scotland	Geographically based, 1948-1964	LMP certain and uncertain Clinically corrected Completed wk	Congenital anomalies Stillbirths Unknown gestational age	52,004	White Mixes socioeconomic status	Data presented by parity and sex Not smoothed
Hoffman et al.[9]	United States	NCHS 50% sample of all U.S. live births, 1968	LMP only No clinical corrections Completed wk	Multiple births Not white or black No LMP available	1,164,871	Mixed white and black socioeconomic status mixed	Data presented by race and sex
Williams et al.[17]	Calif.	State vital statistics data, 1970-1976	LMP only No clinical corrections Completed wk	Inappropriate weight for gestational age	2,265,478 live births 3,738 stillbirths	59% White 26% Spanish 10% Black 5% Other	Cluster probability method used for correcting nonnormal distributions

CPP, Collaborative Perinatal Project; *NCHS,* National Center for Health Statistics.
* Unpublished observations.

U.S. resident births in 36 states and the District of Columbia in which the first day of the LMP was reported on the birth certificate. Data from 1,164,871 births were analyzed. Exclusions included births to women not reporting an LMP, births to women in states that did not record LMP on the birth certificate, multiple births, births to women not recorded as white or black, and births less than 20 weeks' gestational age. In this paper, birth weight for gestational age percentiles, including the 10th percentiles, were presented for four groups separately by race and sex of the infant.

Williams et al.[17] conducted a large population-based study in California with matched birth and death certificate data. As in the article by Hoffman et al.,[9] the large sample size (2,288,806 births) allowed for evaluation of confounding variables such as sex, race, and plurality. Infants with congenital anomalies or antenatal complications were not excluded from this study. Gestational age was calculated from date of birth and date of last normal menstrual period. The 10th percentiles of birth weight were calculated in weekly intervals. This study attempted to address the problem of inaccuracies in gestational age that result in skewed distributions of birth weight. The greatest effect was thought to be on the 90th percentile because of "heavy" infants who were mistakenly given low gestational ages, whereas the 10th percentile was believed to be only slightly affected.

Comment

One of the major difficulties in understanding the literature devoted to IUGR is determining which infants have been included in any particular study. Since IUGR is most commonly defined as a birth weight < the 10th percentile at each gestational age, the population from which the standards are derived to define the 10th percentile is of major importance. However, in study after study the investigators appear to choose standards with little concerns for the relationship of that "standard population" to their own. In addition,

infants that are classified as having IUGR by one standard are not by other standards. Therefore studies that evaluate risk factors for IUGR, the use of various tests for IUGR, and follow-up studies of infants with IUGR are often not easily compared, at least in part, because the birth weights used to define the condition at any gestational age are not similar.

This study clearly shows that the 10th percentile birth weights at each gestational age from various published standards differ substantially, even occasionally by >500 gm. However, in many cases despite the apparent large differences, especially at the early and late gestational ages, because of very small numbers, the 10th percentiles may not even be statistically different[6,8] (unpublished observations). When real differences do exist, it would appear that the racial composition and socioeconomic status of the population, the height of the geographic area above sea level, and which infants were excluded may influence the birth weight distribution. Equally as important, however, appears to be the method used to determine the gestational age. For example, a number of studies examined infants' birth weights retrospectively and discarded some cases because the gestational ages were not compatible with their birth weights, or because mathematic modeling made it unlikely that certain groups of infants were of a gestational age in agreement with the LMP.[6,8,14] Furethermore, some studies rounded the gestational age,[10,12-14] whereas other authors presented the data in completed weeks.[6,11] Most used the LMP alone to determine the gestational age at delivery, but clinical data were used to influence the gestational age in at least three studies[8,14] (unpublished observations). In summary, there are so many methodologic differences between studies that differences that do appear to exist in the 10th percentiles between populations may possibly be attributed solely to methodologic differences. Certainly there has been no standard method for determining the birth weight for gestational age "standards."

The following discussion addresses some of the issues that must be settled in selecting a population and determining the methodology if a uniform set of standards for the diagnosis of IUGR is to be developed.

First, the issue of whether to use separate 10th percentile standards established for race, sex, and parity for diagnosing IUGR is a difficult one to resolve. For example, although it is apparent that black infants generally weigh less than white infants, and their 10th percentile cutoff points are less, it is not clear that this difference is solely a result of less genetic potential for growth. Instead, black infants have more risk factors for low birth weight,[18] including lower maternal socioeconomic status and more instances of maternal hypertension. Therefore the issue regarding race and potential for growth does not appear resolvable. However, since most U.S. studies of IUGR involve racially mixed populations, there are advantages to having a single standard that is based on a racially mixed U.S. population. However, in other cases the use of race-specific standards may be appropriate. These standards should be developed from the same population.

Male newborns weigh more than females, and the 10th percentile cutoff is higher for males than females. Since there do not appear to be more risk factors in mothers who are delivered of male infants[18] and since female infants do not appear to be at a disadvantage because of their lower weight, it seems reasonable to use sex-specific standards. Otherwise, infants classified as having IUGR will include a disproportionate number of apparently healthy female infants.

Infants born to parous women are generally heavier than those born to nulliparous women; their 10th percentile cutoffs are heavier as well. Currently it is not clear whether primiparous women have more or less risk factors for IUGR (i.e., more toxemia but possibly less chronic hypertension, a lower prepregnancy weight, etc.) or if their infants fare less well. However, it would appear that the genetic potential for fetal growth is equivalent in multiparous and primiparous pregnancies, but that there are factors related to the primiparous state itself that tend to constrict growth. In view of the likelihood that fetuses born to primiparous mothers have more growth potential than they generally attain, and since most studies of IUGR are performed on populations of mixed parity, in most studies we would recommend the use of a single standard based on a population of mixed parity for practical purposes.

There is reasonable evidence that infants born to mothers residing at high altitudes have smaller babies,[6,19] and some have recommended using standards based on geographic location. Because there is no reason to believe that these "high altitude" babies have less genetic potential to grow than other babies, it seems reasonable to conclude that the high altitude is a growth retardant and should be considered a risk factor for IUGR. Since it is not reasonable to base standards for diagnosing IUGR on a population at high risk for this condition, we would recommend that the standards be derived from a population whose fetal growth is not influenced by geographic constraints.

Despite the fact that nearly all standards are based on LMP data alone, virtually all studies involving infants with IUGR are now reported with gestational ages calculated from the LMP but modified by clinical data and ultrasonography. It makes little sense to use gestational age standards for diagnosing IUGR based on LMP alone when the infant's gestational age will be

determined differently for most studies. Therefore we recommend that for the derivation of standard 10th percentiles, the gestational ages should be LMP-based but should be confirmed by early clincal and ultrasonographic data.

When establishing standards, the issue of which infants to exclude for medical or risk factor indications is also difficult to resolve. Some authors, especially those using vital statistics data, exclude no cases.[9, 17] Others exclude some infants with congenital anomalies but include others.[6] Most, but not all, exclude multiple births. Miller and Merritt[8] excluded not only abnormal infants, perinatal deaths, and multiple births, but also those with any known risk factor for IUGR such as smoking. Although there is clearly no one "right" choice as to which infants should be excluded in developing a percentile standard for IUGR, the use of a consistent method is important if studies on IUGR in various populations are to be compared. For consistency and to eliminate the idiosyncratic nature of exclusions or inclusions in so many studies, one obvious solution in developing standards would be to include all live-born infants born to the population evaluated.

In conclusion, we strongly believe that there should be a single, clearly defined U.S. population from which the percentiles for defining IUGR are derived, and that unless there are specific reasons to the contrary, studies on IUGR performed in the U.S. should use these standards. Although there is general consensus that the 10th percentile should be used for most studies, those who want to evaluate greater or lesser degrees of growth retardation may want to use the 3rd, 5th, 15th, or 25th percentile. These percentiles should be derived from the same standard population for consistency. Similarly, for researchers interested in using race- or parity-specific values, the race- or parity-specific percentile standards should be derived from this standard population.

The characteristics of the specific population on which the standards should be based are subject for debate. For example, we believe that the standards should be derived from a hypothetical population that is representative of U.S. pregnancies on the basis of demography, risk factors, and perinatal outcome. Obviously, there is room for discussion (and disagreement among the authors) about other specific decisions regarding sex, race, parity, multiple birth, certain congenital anomalies, etc. In addition, there are also important decisions to be made, with regard to method, such as how the gestational age should be determined and/or confirmed. Nevertheless, we believe that after appropriate discussion and compromise, representatives from appropriate decision-making groups should be able to reach agreement on a standard population.

Much of the confusion that presently surrounds IUGR would be eliminated if The American College of Obstetricians and Gynecologists, The American Academy of Pediatrics, The Society of Perinatal Obstetricians, and the appropriate committees of the National Institutes of Health, The Centers for Disease Control, and The National Center for Health Statistics could together endorse the same national standard, and U.S. researchers would use this single common standard in their studies.

REFERENCES

1. Starfield B, Shapiro S, McCormick M, et al. Morality and morbidity in infants with intrauterine growth retardation. Pediatrics 1982;101:978-83.
2. Fitzhardinge PM, Steven EM. The small-for-date infant. II. Neurological and intellectual sequelae. Pediatrics 1972;50:50-7.
3. Michaleis R, Schulte F, Nolte R. Motor behavior of small for gestational age newborn infants. Pediatrics 1970; 76:208-15.
4. Gruenwald P. Growth of the human fetus. AM J OBSTET GYNECOL 1966;94:1112-9.
5. Usher R, McLean F. Intrauterine growth of live-born Caucasian infants at sea level: standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. Pediatrics 1969;74:901-10.
6. Lubchenco LO, Hansman C, Dressler, et al. Intrauterine growth as estimated from liveborn birth-weight data at 24 to 42 weeks of gestation. Pediatrics 1963;32:793-800.
7. Kloosterman GJ. On intrauterine growth. The significance of prenatal care. Int J Gynaecol Obstet 1970;8:895-912.
8. Miller HC, Merritt TA. Fetal growth in humans. Chicago: Year Book, 1979:31-57,127-41.
9. Hoffman HJ, Stark CR, Lundin FE, et al. Analysis of birth weight, gestational age, and fetal viability, U.S. births, 1968. Obstet Gynecol Surv 1974;29:651-81.
10. Brenner WE, Edelman DA, Hendricks CH. A standard of fetal growth for the United States of America. AM J OBSTET GYNECOL 1976;126:555-64.
11. Milner RDG, Richards B. An analysis of birth weight by gestational age of infants born in England and Wales, 1967 to 1971. J Obstet Gynaecol Br Commonw 1974;81: 956-7.
12. Naeye RL, Dixon JB. Distortions in fetal growth standards. Pediatr Res 1978;12:987-91.
13. Hardy BJ, Drage JS, Jackson EC. The first year of life. Baltimore: Johns Hopkins University Press, 1979:38-70.
14. Babson SG, Behrman RE, Lessel R. Fetal growth. Liveborn birth weights for gestational age of whtie middle class infants. Pediatrics 1970;45:937-44.
15. Carr-Hill R, Pritchard C. The development and exploitation of empirical birthweight standards. New York: Stockton Press, 1985:123-32.
16. Thomson AM, Billewicz WZ, Hytten FE. The assessment of fetal growth. J Obstet Gynaecol Br Commonw 1968; 75:903-16.
17. Williams RL, Creasy RK, Cunningham GC. Fetal growth and perinatal viability in California. Obstet Gynecol 1982;59:624-32.
18. Kramer MS. Determinants of low birth weight: methodological assessment and meta-analysis. Bull WHO 1987; 65:663-737.
19. Unger C, Weiser JK, McCollough RE, et al. Altitude, low birthweight and infant mortality in Colorado. JAMA 1988;259:3427-32.

Anal and perianal abnormalities in prepubertal victims of sexual abuse

David Muram, MD
Memphis, Tennessee

We describe anal and perianal findings in 310 prepubertal children who were determined to be victims of sexual abuse; 206 (66%) had perinea that appeared normal. Abnormal findings were present in 104 children (34%): anal gapping in 61 children; skin tags in 44; rectal tears in 33; sphincter tears in 15; human papillomavirus lesions in 4; perineal scarring in 2; and bite marks in 1. Normal-appearing perianal and anal regions were noted in 150 of 175 children (85%) who denied anal assault, and in 11 of 70 (16%) who described such assault. In comparison, anal and perianal abnormalities were observed in 59 of the 70 children (84%) who gave a clear history of anal assault, but in only 25 of 175 (15%) who denied such abuse. Failure to document perianal abnormalities in almost two thirds of the patients demonstrates the limitations of the medical evaluation in validating allegations of sexual abuse. (Am J Obstet Gynecol 1989;161:278-81.)

Key words: Children, sexual abuse, anus, perianal abnormalities

Child sexual abuse is a common and serious problem that affects children regardless of their age, sex, socioeconomic class, or geographic location.[1, 2] Anal assaults comprise a significant proportion of these attacks.[2, 3] Although genital injuries are often recognized as possible signs of abuse,[4, 5] anal and perianal injuries are sometimes dismissed by physicians as being associated with common bowel disorders (e.g., constipation or diarrhea).[6-8] This article describes the anal and perianal findings in a large group of prepubertal children who were determined to be victims of sexual abuse by their history and by the local investigative teams of Child Protective Services, a branch of the Tennessee Department of Human Services.

Material and methods

The demographics of Shelby County, Tennessee, and the referral patterns to the Pediatric Gynecology Clinic have been described in previous articles.[2, 4] Between Jan. 1, 1986, and April 30, 1988, the Tennessee Department of Human Services referred 310 prepubertal children between the ages of 1 and 10 (163 white and 147 black; 41 boys and 269 girls) who were determined by their history and the ensuing investigation by the Tennessee Department of Human Services to be victims of sexual abuse. All patients in this study group were examined in an office setting by the same examiner; this allowed consistent and uniform data collection. All children in the study group were prepubertal, as confirmed by the medical examination. For the evaluation of the perianal region, the children were placed in one of three positions: frog-legged supine, "knee-chest" position, or across the parent's lap, simulating the "knee-chest" position. The glutei were separated so that the anus and perianal area could be fully inspected. In addition to the unaided examination, the perianal area was also inspected either through a Zeiss or Codman colposcope under ×16 magnification. The area was inspected under regular illumination and through a green filter that better demonstrates vascular patterns. No solutions or dyes were applied to enhance visualization. The examiner recorded changes in skin color, texture, and all other lesions or abnormalities.

The various perianal abnormalities observed in the study population were defined as follows:

1. Inflammation—redness, swelling, tenderness of the perianal tissues

From the Section of Pediatric Gynecology, Department of Obstetrics and Gynecology, University of Tennessee.
Received for publication July 29, 1988; revised November 22, 1988; accepted March 6, 1989.
Reprint requests: David Muram, MD, Department of Obstetrics and Gynecology, University of Tennessee, 853 Jefferson Ave., Memphis, TN 38103.

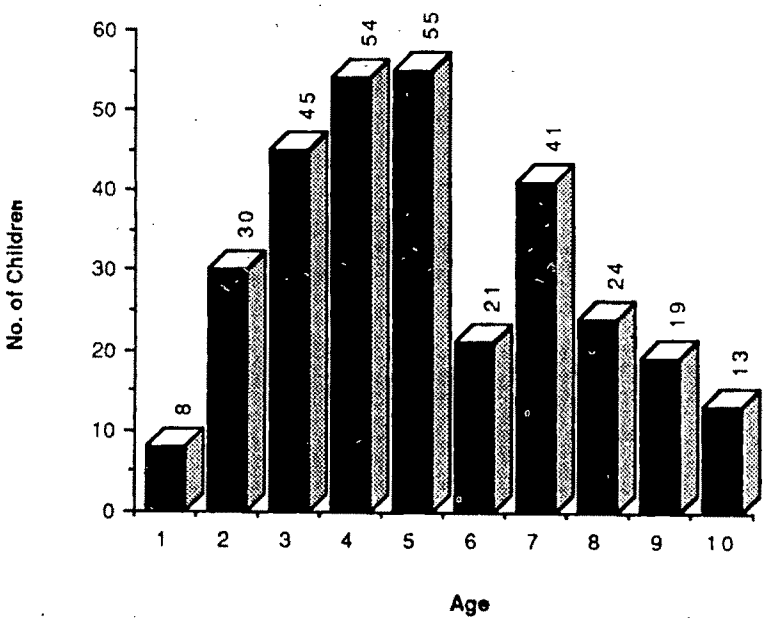


Fig. 1. Age distribution of the study group.

2. Rash—perianal skin eruption
3. Ulceration—loss of the superficial layer of the skin or mucosal surface
4. Skin tag—excess of perianal skin raised near the mucocutaneous junction that could not be eliminated by stretching nearby tissues
5. Fissure—a deep linear cleft or ulcer at the mucocutaneous line of the anus
6. Anal gapping—relaxation of the anal sphincter to a diameter ≥1 cm when the glutei were separated

The genital abnormalities were classified into one of three categories, as have been previously described in detail[4]:

1. *Category 1.* Normal-appearing genitalia
2. *Category 2.* Nonspecific findings—abnormalities of the genitalia that could have been caused by sexual abuse but also are often observed in patients who are not victims of sexual abuse (e.g., inflammation and scratching)
3. *Category 3.* Specific findings—the presence of one or more abnormalities strongly suggesting sexual abuse.

The allegations

Many children described a complex abusive situation in which repetitive sexual acts were performed over a variable time period. In some instances, it was difficult to evaluate the progression of the abusive relationship, the time span over which the abuse occurred, and the number of attacks. In 175 of the 310 children (56%), the abusive relationship affected other areas of the victim's body and did not involve the anal area (e.g., oral–genital contact). In another 65 children (21%), it was impossible to ascertain from the child's description if anal assault occurred. In only 70 instances (23%) did victims clearly describe an assault involving the anal area. Some were combined with genital assault (n = 47), anal coitus alone (n = 18), or digital manipulation of the anal area (n = 5).

Results

Age distribution. The mean age of the children in the study group was 5.2 years (range, 1 to 10 years). A difference in age distribution by racial group was noted; black children were older (mean age, 5.7 years) than white children (mean age, 4.8 years). The age distribution for the entire study group is shown in Fig. 1.

Findings. Two hundred and six children (66%) had normal-appearing perinea. Abnormal findings were present in 104 children (34%): anal gapping was found in 61 children, either as an isolated finding (n = 12), or in association with other findings. Skin tags were found in 44, either as an isolated finding (n = 21), or in association with other findings. Rectal tears were seen either as an isolated finding (n = 12), or in combination with other findings (n = 21). Sphincter tears were observed as an isolated finding (n = 1), or with anal gapping, rectal tears, or skin tags (n = 14). In four patients, perineal human papillomavirus lesions were seen; in two patients, perineal scarring was noted in association with perianal skin tags. One girl had bite marks on the perianal area. The findings are listed in Table I.

Table I. Perianal and anal findings in prepubertal children ($N = 310$) who were victims of sexual abuse

	No. of patients
No abnormalities	206 (66%)
Skin tags	21
Anal gapping, rectal tears	18
Anal gapping	12
Rectal tears	12
Anal gapping, rectal tears, skin tags	7
Anal gapping, skin tags	6
Perianal warts	4
Skin tags, rectal tears	3
Rectal tears, sphincter tears	3
Anal gapping, sphincter tears, rectal tears, skin tags	3
Anal gapping, sphincter tears	3
Skin tags, perineal scarring	2
Anal gapping, sphincter tears, rectal tears	2
Redness, irritation	1
Redness, irritation, rectal tears	1
Sphincter tears	1
Sphincter tears, skin tags	1
Skin tags, redness, irritation	1
Bite marks	1
Anal gapping, sphincter tears, rectal tears, perineal scarring	1
Anal gapping, rectal tears, proctoepisiotomy	1
TOTAL	104 (34%)

Normal-appearing anal and perianal regions were noted in 150 of 175 children (86%) who denied anal assault and in 11 of 70 (16%) who described anal assault. In comparison, anal and perianal abnormalities were observed in 57 of the 70 children (81%) who gave a clear history of anal assault but in only 23 of 175 (13%) who denied such abuse.

The anal and genital abnormalities among the children in this study group were correlated. Of the 310 patients, 125 children were found to have normal-appearing genitalia. Seventy-one patients had category 2 findings. Abnormalities suggestive of abuse were found in 114 (38%) patients. When the various genital abnormalities were compared with the presence or absence of perianal findings, children who had anal abnormalities were more likely to have normal genital examination (Table II). However, inclusion of genital findings increased the sensitivity of the medical evaluation. A combination of anal and genital abnormalities was seen in 53 (17%) patients, and either genital or anal abnormalities were found in an additional 182 (59%) patients. Only 75 (24%) patients had no abnormality of the genital or perianal regions. Even when only genital abnormalities highly suggestive of abuse were included (category 3), both anal and genital abnormalities were seen in 28 (9%) patients and either genital or anal abnormalities were found in an additional 159 (51%) patients.

Table II. Correlation between perianal and anal findings and genital abnormalities

	No. of patients		
Genital abnormalities	*Category 1*	*Category 2*	*Category 3*
Anal abnormalities present ($n = 104$)	51	22	27
Anal abnormalities absent ($n = 206$)	74	47	85

$\chi^2 = 6.314$, $p = 0.043$.

Comment

Anal abuse is a common form of sexual assault against children, particularly boys.[4,6] Although the genital abnormalities suggestive of sexual abuse have been described, there is no consensus regarding which perianal or anal abnormalities are the result of sexual abuse and which have other causes (e.g., constipation with passage of large, hard stools). Such determination may not be easy because the anal sphincter and anal canal allow some room for dilatation. A tear of the anal mucosa or sphincter rarely occurs after a digital assault. Penetration by a larger object may result in some degree of injury and may vary from a mere swelling of the anal verge to gross tearing of the sphincter. In the period immediately after penetration, sphincter laxity, swelling, and small mucosal tears of the anal verge may be observed. The swelling subsides and the mucosal tears heal within days; occasionally skin tags form. The anal sphincter regains function if it was not severed. Repeated acts of anal penetration over a prolonged period cause the anal sphincter to become loose, forming an enlarged opening.[9] The diagnosis of venereal disease affecting the anal canal is often delayed or even missed. When a child is infected with *Neisseria gonorrhoeae*, genital infection is associated with vaginal discharge and often the child is brought for medical care. Anal infection is often asymptomatic. Other disorders may cause perianal abnormalities similar to the ones observed in this series. Perianal abnormalities are often observed in children with Crohn's disease or Hirschsprung's disease. The anal canal gapes when the buttocks are gently drawn apart in children with significant constipation. This is a normal anorectal reflex initiated by the distended rectum. Usually stool may be seen in the anal canal. Minor small fissures may be seen after defecation for 2 to 3 weeks. Reddening of the perianal area may be found in children with fecal soiling.[7] Unfortunately, children who were abused often suffer from functional constipation and a damaged anal sphincter often causes fecal soiling.

Although many patients in this study group had normal-appearing perianal regions, the absence of physical findings is easily explained. Many victims of

anal assault do not sustain significant physical injuries because the anal sphincter and anal canal are capable of dilatation. Penetration, even by an adult penis, may occur without significant injury. The pain that follows anal assault may cause spasm of the anal sphincter and result in functional constipation, thus providing an explanation of the child's complaints of pain and rectal bleeding. Most children do not immediately disclose incidents of sexual abuse. Many victims do not have an examination for weeks, months, or even years after the abuse occurred. By then the injuries often have healed completely and the physical examination reveals few, if any, abnormalities.

Although we were able to detect a large number of children with anal and perianal abnormalities, it is difficult to conclude from this study which of these findings are diagnostic of anal abuse. The operating procedures of the clinic identify the patients as probable victims of sexual abuse at the time of registration; this may possibly cause bias by the examiner. More controlled studies are required to identify the specific findings indicative of anal assault. The association of other abnormalities (e.g., signs of neglect, genital injuries, etc.) should raise suspicion by the examiner that the patient is a victim of abuse. However, abnormal findings should not be interpreted in isolation. The findings of the medical examination must be correlated with other methods of validation such as a history from the child. To conclude from the findings of the medical examination alone that a child is a victim of abuse may cause enormous harm to the child and the family.[10]

As expected, children who were able to describe anal assault in detail were more likely to have perianal abnormalities. Memory of anal assault may suggest that the child experienced a more intense pain and thereby was more likely to have been injured. Fifty-nine of the 70 children (84%) who described anal assault had abnormalities on physical examination. In contrast, the examination was reported as normal in most instances when the child denied that anal assault had occurred. Observation of other abnormalities, particularly genital tract injuries, increases the sensitivity of the medical evaluation of child victims of abuse.

In this study group children who had normal results of the genital examination were more likely to have anal abnormalities. It is tempting to speculate that children who were primarily victims of anal assault were less likely to suffer a genital assault and thereby had a normal genital examination. I believe that this erroneous conclusion is caused by the inclusion of a significant number of male victims in this series. Only one male patient had genital abnormalities. All the remaining male patients had normal genital examinations, but a significant number of these had anal abnormalities, accounting for the erroneous impression.

"The etiology of most perianal fissures, irritation, and skin tags is often not evident, but may be trauma secondary to overzealous cleaning, constipation with passage of large hard stools, scratching induced by irritation from *Enterobius vermicularis* or eczema, or other perianal conditions."[11] Although the possibility of sexual abuse is not mentioned in this well-accepted textbook, child sexual abuse should be considered a potential cause of perianal injury. With the increasing number of children who are reported as victims, physicians who treat young patients must become proficient in the evaluation of sexual abuse. When abnormalities suggestive of perianal injury are detected, the physician should gently inquire about the possibility of sexual abuse. If such history can be obtained, further investigation is required. Some states have laws that require that all incidents of child sexual abuse be reported to a designated agency,[2] and many medical centers have developed multidisciplinary units for the evaluation of child victims of abuse.[12] Physicians may choose to refer patients whom they think have been sexually abused to these multidisciplinary units for further evaluation.

In summary, this study has documented that children who complained of anal assault are more likely to have some abnormality of the perianal region. Although these abnormalities are not always the result of sexual abuse, I suggest that when perianal abnormalities are detected, further evaluation may be required to protect a potential child-victim from further abuse. Such abnormalities could be considered as corroborating findings to a child's complaint of sexual assault.

REFERENCES

1. Kemp CE. Sexual abuse, another hidden pediatric problem. Pediatrics 1978;62:382-9.
2. Muram D, Weatherford T. Child sexual abuse in Shelby County, Tennessee: two years of experience. Adolesc Pediatr Gynecol 1988;2:114-8.
3. Hobbs CJ, Wynne JM. Child sexual abuse—an increasing rate of diagnosis. Lancet 1987;2:837-41.
4. Muram D. Child sexual abuse—genital findings in prepubertal girls. I. The unaided medical examination. AM J OBSTET GYNECOL 1989;160:328-33.
5. Emans SJ, Woods E, Flagg N, Freeman A. Genital findings in sexually abused, symptomatic, and asymptomatic girls. Pediatrics 1987;79:778-85.
6. Hobbs CJ, Wynne JM. Buggery in childhood—a common syndrome of child abuse. Lancet 1986;2:792-6.
7. Clayden G. Anal appearances and child sexual abuse. Lancet 1987;1:620-1.
8. Roberts REI. Examination of the anus in suspected child sexual abuse. Lancet 1986;2:1100.
9. Paul DM. The medical examination in sexual offenses against children. Med Sci Law 1977;17:251.
10. Dyer C. First High Court judgment on sex abuse in Cleveland. Br Med J 1987;295:382-3.
11. Shandling B. Diseases of anus, rectum, and colon. *In:* Behrman RE, Vaughan VC, eds. Nelson textbook of pediatrics. 13th ed. Philadelphia: WB Saunders, 1987: 813-5.
12. Muram D, Rivara FP, Buxton BH. Management of sexual abuse in pre-pubertal girls. Pediatr Adolesc Gynecol 1984;2:191-6.

Phosphorus 31 nuclear magnetic resonance examination of female reproductive tissues

Elizabeth A. Noyszewski, MSc, Jyothi Raman, MSc, Suzanne R. Trupin, MD, Barbara L. McFarlin, CNM, MS, and M. Joan Dawson, PhD

Urbana, Illinois

Nuclear magnetic resonance spectroscopy is a powerful method of investigating the relationship between metabolism and function in living tissues. We present evidence that the phosphorus 31 spectra of myometrium and placenta are functions of physiologic state and gestational age. Specific spectroscopic abnormalities are observed in association with disorders of pregnancy and gynecologic diseases. Our results suggest that noninvasive nuclear magnetic resonance spectroscopy examinations may sometimes be a useful addition to magnetic resonance imaging examinations, and that nuclear magnetic resonance spectroscopy of biopsy specimens could become a cost-effective method of evaluating certain biochemical abnormalities. (AM J OBSTET GYNECOL 1989;161:282-8.)

Key words: Magnetic resonance, ^{31}P nuclear magnetic resonance, metabolism, pregnancy disorders, gynecologic diseases

Nuclear magnetic resonance spectroscopy is a powerful method of investigating the relationship between metabolism and function in living tissues and is increasingly being used in studies of the cause of diseases. A related technique, magnetic resonance imaging, has an undisputed role in clinical diagnosis and is increasingly used in diagnosis of diseases of reproductive tissues.[1]

Nuclear magnetic resonance spectroscopy detects biochemical changes that cannot be assessed by other currently available diagnostic procedures. The phosphorus 31 nucleus emits strong signals from a small number of important compounds in living systems and yields estimates of intracellular pH and free intracellular magnesium (magnesium ion) concentration. The energetic status of an organ can be assessed and the concentrations of phosphorus compounds important to membrane synthesis, modulation, and degradation can be determined.

We present evidence that the ^{31}P nuclear magnetic resonance spectra of myometrium and placenta are a function of physiologic state and gestational age. Specific spectroscopic abnormalities are observed in association with disorders of pregnancy and gynecologic diseases. In some instances, these results support current hypotheses concerning the molecular abnormalities responsible for disease and suggest interesting new avenues to explore. Noninvasive nuclear magnetic resonance spectroscopy studies of female reproductive tissues are feasible; our results suggest that they may sometimes be a useful addition to magnetic resonance imaging examinations. However, such examinations are expensive and therefore limited to cases of clear need. Nuclear magnetic resonance spectroscopy could prove useful in quite a different way: as a cost-effective biochemical test on specimens now routinely assessed for histologic abnormalities.

From the Departments of Physiology and Biophysics and Obstetrics and Gynecology, University of Illinois at Urbana-Champaign.

Supported by the Research Board of the University of Illinois Urbana-Champaign and the Servants United Foundation.

Received for publication September 12, 1988; revised March 3, 1989; accepted March 8, 1989.

Reprint requests: M. Joan Dawson, PhD, Department of Physiology and Biophysics, 524 Burrill Hall, 407 S. Goodwin Ave., Urbana, IL 61801.

Material and methods

Sample selection. Placenta from uncomplicated gestations were examined in each trimester. Data on placentas obtained from patients with complications of pregnancy are summarized in Table I and detailed in the Results section. The two hydatidiform moles were studied after evacuation at 7 and 10 weeks.

Normal myometria were examined from eight patients delivered of their infants by cesarean section at 34 to 39 weeks' gestation. Five of these uterine biopsies were performed at the time of elective repeat cesarean section and three were done after unaugmented labors. Myometria were examined from a single diabetic woman and a single hypertensive patient; placental samples were also available from these patients.

Tubal specimens were obtained at the time of postpartum ligation or at the time of hysterectomy. Samples of both normal and cystic ovarian tissues were also obtained.

Fourteen myometrial specimens were examined after hysterectomy in multiparous patients aged 30 to 87

Table I. Comparison of diseased and normal tissue

Tissue	*Disease*	*Alteration*	*p Value*
Myometrium	Hypertension	↑ PN (1.63)	<0.001
Pregnant	(n = 1)		
Nonpregnant	Adenocarcinoma	↑ PME (1.59)	<0.001
	(n = 2)	↑ Pi (1.92)	<0.001
Placenta	Growth retardation		
Third trimester	(n = 4)	↑ GPC (2.02)	<0.001
	Class A diabetes	↓ H^+ (0.16)	<0.003
	(n = 2)		
First trimester	Hydatidiform mole	↑ PME (2.1)	<0.001
	(n = 3)	↓ H^+ (0.24)	<0.001

Number of samples in each category of diseased tissue is given in parentheses. Number of normal samples of myometrium were: pregnant, n = 7; nonpregnant, n = 12. Among normal placentas there were: third-trimester, n = 5; first-trimester, n = 6. Significance levels (p values) were estimated by an unpaired t test. *PN*, Pyridine nucleotide; *PME*, phosphomonoester; *Pi*, inorganic phosphate; *GPC*, glycerophosphorylcholine.

years. Indications for hysterectomy were as follows: two for uterine leiomyomas, one for adenomatous hyperplasia, two for endometrial adenocarcinoma, three for endometriosis, and six for other indications. The Institutional Review Board of the University of Illinois, Urbana-Champaign, approved this study.

Sample handling. After informed consent of the patient was obtained, samples of 100 to 500 mg of pregnant myometrium was trimmed from the edge of low transverse cervical incisions at the time of cesarean section. Samples were placed in oxygen-saturated modified de Jalon's solution (sodium, 170 mmol/L, chlorine, 177 mmol/L; piperazine-*bis*-2-ethane sulfonic acid (PIPES), 15 mmol/L; potassium, 6 mmol/L; glucose, 3 mmol/L, and calcium, 0.3 mmol/L) immediately after examination (within 0 to 2 minutes of delivery). Single lobules of placenta obtained from vaginal deliveries and cesarean section (third trimester) or from pregnancy terminations (first trimester) were also obtained and a small piece (3 to 4 gm) was placed in the ice-cold solution. Samples of uterine tube and ovary were also similarly handled. The pathologist was in the operating room to make his preliminary examination and to release tissues for study. The specimen jars were transported on ice (in a closed, thermally insulated box) to the nuclear magnetic resonance spectrometer. Tissues were superfused with oxygenated de Jalon's solution either at 4° C or 37° C within a 12 or 20 mm diameter nuclear magnetic resonance sample tube during nuclear magnetic resonance spectroscopy examination. Data acquisition was generally completed within 1 to 2 hours.

Nuclear magnetic resonance spectroscopy procedures. ^{31}P nuclear magnetic resonance spectra were obtained at 121.48 MHz on a GN300 wide-bore spectrometer (General Electric, Fremont, Calif.), pulse duration = 20 μsec, flip angle = 35 degrees, pulse interval = 1.5 sec. Resonance positions were measured relative to that of an internal standard in parts per million (i.e., 121.48 Hz = 1 ppm). When possible, the phosphocreatine resonance was used as the internal standard; for the placenta, which often contains no phosphocreatine, the position of the α-nucleotide phosphate peak was used as a reference because in all tissues studied this peak had the most constant chemical shift relative to phosphocreatine (in the uteri reported here, δ(phosphocreatine − α-nucleotide phosphate) = −7.55 ± 0.02, n = 15). Line broadening of 5 Hz was used to reduce noise in the spectra, but no other signal-enhancement procedures were applied. Peaks were assigned on the basis of their resonance positions and the known presence of particular metabolites in the tissue. In some cases unambiguous assignment awaits tissue extraction and further analysis.

The intracellular concentrations of hydrogen and magnesium ions were calculated from the general formula:

$$pX = pK + \log_{10} \frac{\delta - \delta_1}{\delta_2 - \delta}$$

where X = hydrogen or magnesium ions and δ is the observed chemical shift of a titratable species (inorganic phosphate in the pH calculation and β-nucleotide triphosphate in the calculation of free magnesium ions). For the pH calculation at 4° C, pK = 6.71, δ_1 = 3.19, and δ_2 = 5.49, and for magnesium ions, δ_1 = 15.86, δ_2 = 19.02, and pK = 4.11.

Quantitation. Provided that the interval between radiofrequency pulses is more than five times the apparent T_1 relaxation time for the phosphorus nuclei, the peak areas are directly proportional to the concentrations of metabolites present. To determine the effect of pulse interval, spectra were collected at intervals of 1.0, 1.5, 3, 5.5, 10.5, and 15.5 seconds. The sizes of the signals obtained at 1.5-second intervals (the pulse interval used routinely) were then corrected with the ap-

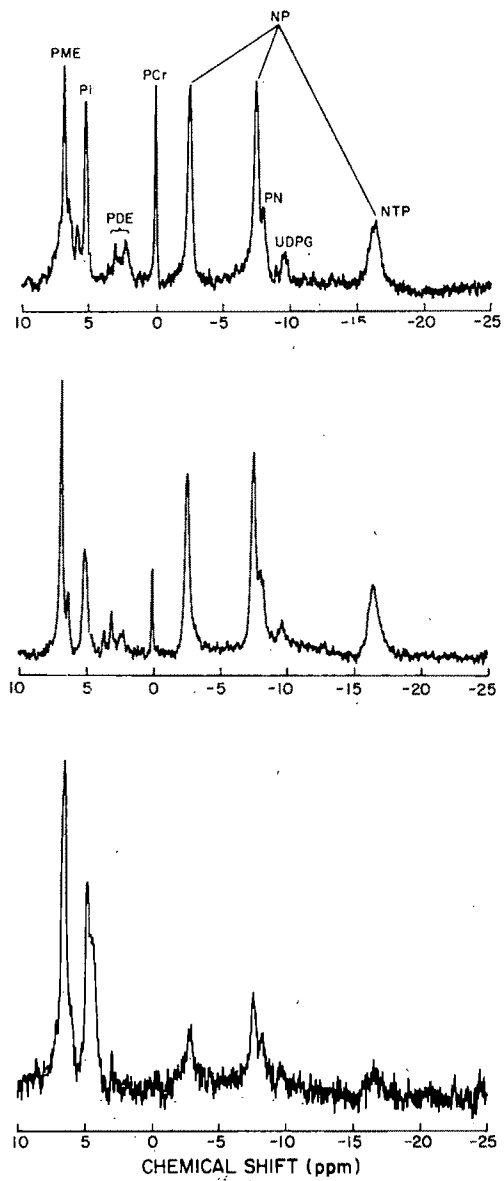


Fig. 1. ^{31}P nuclear magnetic resonance spectra from term pregnant *(top)* and nonpregnant *(middle)* normal myometrium and normal myometrium accompanying endometrial adenocarcinoma *(bottom)*. Isolated myometria (100 to 500 mg) were superfused with oxygenated de Jalon's solution at 4° C. The *y* axes have been adjusted so that, for any given peak, equal signal intensities in the three spectra represent equal concentrations of metabolites. *PME,* Phosphomonoester; *Pi,* inorganic phosphate; *PDE,* phosphodiester; *PCr,* phosphocreatine; *UDPG,* uridine diphosphoglucose; *PN,* pyridine nucleotide (nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate, both oxidized and reduced); *NTP,* nucleotide triphosphate (primarily adenosine triphosphate); *NP,* nucleotide phosphate (primarily adenosine triphosphate plus adenosine diphosphate).

propriate saturation correction factors, which were determined from the T_1 relaxation equation for steady-state conditions.[2] There was no significant difference between T_1 values obtained for pregnant and nonpregnant uterus. The total corrected signal intensity per wet weight of tissue also did not differ. Therefore ratios of signal intensity divided by total intensity are equivalent to concentration ratios.

The concentration of nucleotide triphosphate is estimated from the corrected integral of the β–nucleotide triphosphate peak, which contains no contribution from nucleotide diphosphates. The concentration of nucleotide diphosphate is estimated by subtracting the integral of the β–nucleotide triphosphate peak from that of the γ-nucleotide phosphate peak, which contains contributions from both nucleotide triphosphate and nucleotide diphosphate. The concentration of pyridine nucleotide (nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate, both oxidized and reduced forms) is estimated by subtraction of γ from the α–nucleotide phosphate peak, which contains contributions from all of the nucleotide and pyridine phosphates.

Data analysis and presentation. A tissue evaluation form was attached to the nuclear magnetic resonance spectroscopy records to facilitate correlation of nuclear magnetic resonance spectroscopy data with clinical evaluation; the nuclear magnetic resonance spectroscopy findings were attached to the patient's charts. A spread sheet has been developed to facilitate comparisons of the nuclear magnetic resonance data with the clinical and pathologic findings relevant to the diagnosis of the tissue sample. Data manipulation, graphical representation, and statistical analysis were done with the Asyst software package (Macmillan Software, New York). In comparisons of diseased with normal tissue, or studies of changes in the spectrum as a function of physiologic state of the tissue, data are reported as significantly different if they reach a p value >0.01.

The results and figures shown for concentrations of phosphorus metabolites and free magnesium ions are taken from studies of superfused tissues at 4° C, because this (rather than the more complicated perfusion techniques required to maintain tissues at 37° C) could become a routine procedure in a clinical setting. Under these experimental conditions the myometria, uterine tubes, and ovaries are stable for 12 to 24 hours, as judged by the ^{31}P nuclear magnetic resonance spectra, visual inspection, and tissue weight. Difficulty was experienced in maintaining third-trimester (but not first-trimester) placentas in a metabolically stable state; care was taken to complete the experiments within 1 to 2 hours after delivery. In some cases tissues were perfused or superfused at 37° C and identical results were obtained.

Results

Myometria. Fig. 1 shows typical ^{31}P nuclear magnetic resonance spectra from normal term pregnant (top panel) and nonpregnant (middle panel) myometrium,

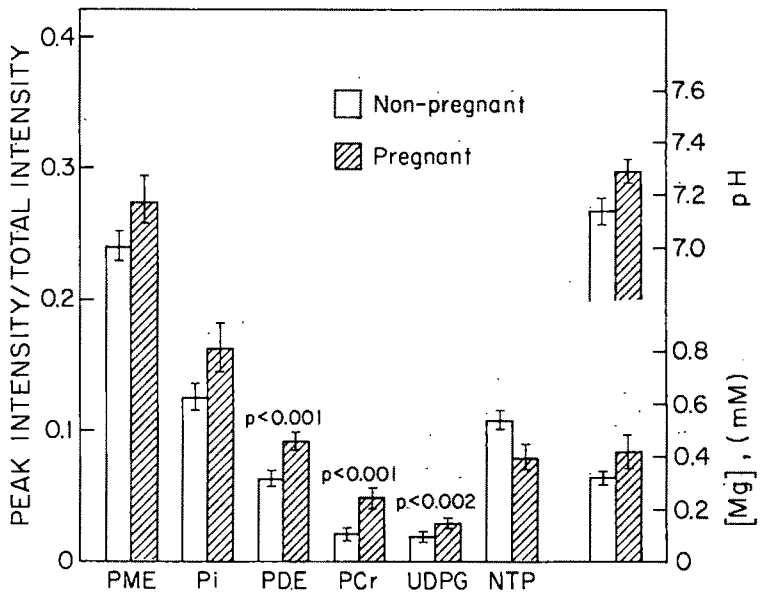


Fig. 2. Summed results on all normal pregnant (n = 7) and nonpregnant (n = 12) myometria, excluding grossly normal specimens from patients with adenocarcinoma. *Error bars* represent ±SEM; p values are for unpaired t test described in Methods. All peak intensities are corrected for saturation so that relative heights of bars are equivalent to relative concentrations. For abbreviations, see Fig. 1 legend.

and grossly normal myometrium from a patient with endometrial adenocarcinoma (bottom panel). The individual nucleotide phosphates resonate so close to each other that they are indistinguishable in the spectra. Adenine is the major (75%) nucleotide in the uterus; its contribution to the total nucleotide phosphate pool does not change with pregnancy.[3] The peak at 6.8 ppm in the phosphomonoester region of the spectrum is in the position of phosphoethanolamine, and the shoulder on the right of this peak is in the position of phosphocholine.[4] Two of the peaks in the phosphodiester region of the spectrum have been identified in mammalian reproductive tissues[4] as glycerophosphorylethanolamine (3.5 ppm) and glycerophosphorylcholine (3.1 ppm). Our spectra of myometria show two peaks, which to our knowledge have not previously been observed: one in the phosphomonoester region at 5.9 ppm and one in the phosphodiester region at 2.3 ppm.

Results of normal myometrium samples are summarized in Fig. 2. There is no significant difference between pregnant and nonpregnant myometrium in concentrations of phosphoethanolamine, inorganic phosphate, and nucleotide triphosphate, whereas phosphocreatine, phosphodiester, and uridine diphosphoglucose are significantly higher in term pregnant than nonpregnant myometrium. Although concentrations are too low for accurate individual quantitation, in pregnant myometrium the peak attributed to glycerophosphorylethanolamine is generally too low for detection and the increase in phosphodiester is largely caused by elevated intensity of the unassigned peak at 2.3 ppm. The unidentified peak at 5.9 ppm is consistently higher in term pregnant than nonpregnant uterus.

Nuclear magnetic resonance spectra of grossly normal myometrium from the two patients with endometrial adenocarcinoma (Fig. 1, bottom panel, and Table I) showed striking differences compared with other myometrial samples. The same spectral characteristics (high phosphoethanolamine and inorganic phosphate with an absence of phosphocreatine) have been observed in cultured human adenocarcinoma cells[5] and in a metastatic adenocarcinoma in the liver.[6] All of the macroscopically normal nonpregnant myometrium obtained from hysterectomies for other reasons, including cervical cancer, showed no ^{31}P spectral biochemical differences from one another. A nondegenerating leiomyoma showed a clearly different biochemical profile from the surrounding normal tissue, including a large acid shift in pH. The myometrial sample obtained from a single patient with hypertension showed significantly increased pyridine nucleotide, as documented in Table I.

Placentas. Fig. 3 shows normal ^{31}P nuclear magnetic resonance spectra of first- and third-trimester placentas (solid lines) and examples of abnormalities (dashed lines). Fig. 4 represents the results on normal first- and third-trimester placentas in graphic form. The relative peak intensities for phosphomonoester, pyridine nucleotide, and nucleotide triphosphate decrease significantly as normal pregnancy progresses, whereas the intensity of the inorganic phosphate peak is substantially increased over the same period.

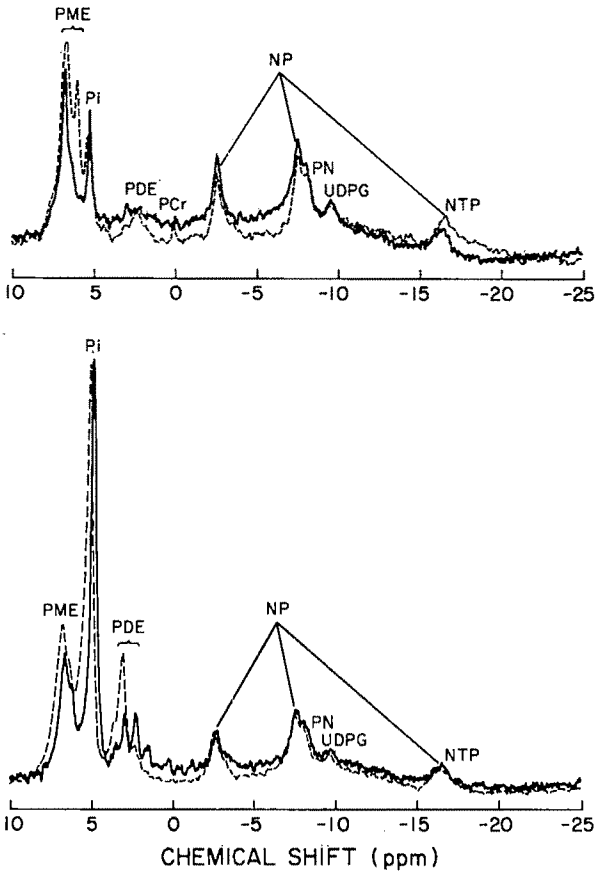


Fig. 3. ^{31}P spectra of normal and abnormal first-trimester (9 to 14 weeks, *top panel*) and third-trimester (35 to 41 weeks, *bottom panel*) placentas. *Solid lines* represent normal placentas and show normal biochemical changes with maturation. Superimposed *dashed lines* represent abnormalities of same gestational age: a 7-week hydatidiform mole and a growth-retarded placenta at 40 weeks. The *y* axes have been adjusted so that for any given peak equal signal intensities represent equal concentrations of metabolites. For abbreviations, see Fig. 1 legend.

The hydatidiform moles (Fig. 3, top, dashed line) show a higher than normal signal intensity in the phosphomonoester region, largely as a result of an increase in the unknown peak at 5.9 ppm, and a decrease in intensity of the inorganic phosphate peak. Placentas from patients with intrauterine fetal growth retardation (Fig. 3, bottom, dashed line) showed a marked increase in the glycerophosphorylcholine peak intensity, together with a significantly alkaline pH in comparison to normal placentas of the same gestational age.

The diabetic patients made up a distinctly abnormal group with a significant intracellular alkalinization that tended to increase with the severity of the disease. Each of the two drug abusers who were studied showed marked abnormalities in their placental ^{31}P spectra; the two spectra did not resemble one another, but neither did their patterns of drug abuse.

Uterine tube and ovary. Spectra from uterine tubes (Fig. 5, top) and ovaries (Fig. 5, bottom) have also been obtained. As might be expected, the spectra from uterine tubes are very similar to those of myometria. The average phosphocreatine/adenosine triphosphate ratio in the three uterine tubes studied was not significantly different from that of nonpregnant myometrium; magnesium binding to adenosine triphosphate was also similar. In the ovarian samples obtained, the phosphocreatine peak is clearly visible and the pH was similar to that of the other reproductive tissues studied. The ovarian cyst showed a higher inorganic phosphate and phosphoethanolamine and a much higher uridine diphosphoglucose than the normal tissue.

Comment

This ^{31}P nuclear magnetic resonance spectroscopy survey of isolated human reproductive tissue has yielded previously unavailable biochemical information and leads to testable hypotheses concerning the relationship between metabolism and function in normal and diseased conditions. Although the number of samples analyzed to date is small, highly significant differences were observed between normal and diseased tissues, suggesting the possibility that accurate diagnostic assessments of the severity of disease could be developed on the basis of these or similar techniques.[7] Continued studies of the biochemical abnormalities we have found could lead to a better understanding of the molecular bases of common reproductive disorders.

When comparison is possible, the results of this study are in good agreement with the results of similar studies of rat tissue (pregnant or nonpregnant uterus,[8] ovary,[4] term placenta[9]), in which dissection was done in the laboratory under controlled conditions and nuclear magnetic resonance analysis was immediately begun. A similar result was also obtained in a single noninvasive study of intact nonpregnant rabbit uterus.[10] For this reason, we believe that our results are reasonable estimates of in vivo tissue metabolite concentrations and how these change with physiologic state.

Tissue specific characteristics and hormonal control. For all of the normal samples, the tissue of origin and its physiologic status can be deduced by inspection of the ^{31}P spectrum. The differences among tissue types relate to the concentrations of phosphorus metabolites involved in generation, storage, and use of metabolic energy (phosphocreatine, nucleotide triphosphate, nucleotide phosphate, pyridine nucleotide, inorganic phosphate), to intracellular pH and free intracellular magnesium concentration, and to the concentrations of phosphomonoesters and phosphodiesters, which are intermediates in membrane synthesis, degradation, and modulation.[11]

The changes observed as a result of pregnancy in

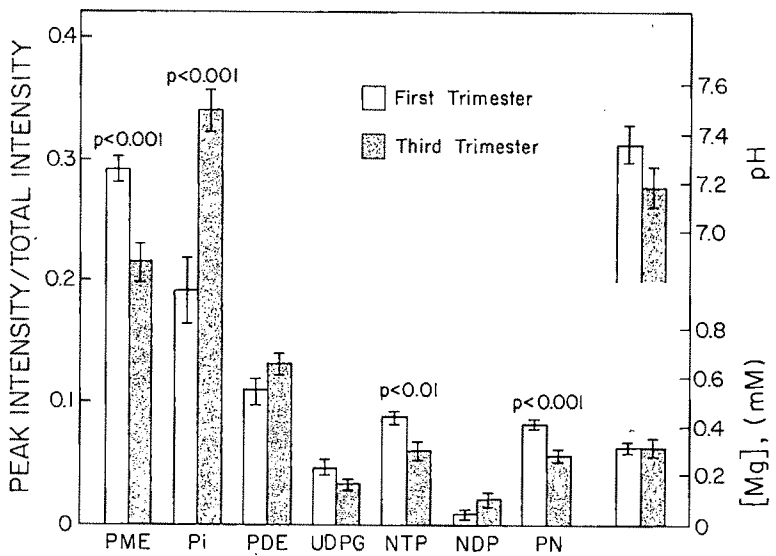


Fig. 4. Summed results of normal first-trimester (n = 6) and third-trimester (n = 5) placentas. Data processing and statistical methods are the same as in Fig. 2, with the exception that no correction for saturation was made. For abbreviations, see Fig. 1 legend.

both rat[8] and human myometrium[7] reflect an increase in energy stores and tend to prepare the uterus for the effort of labor. For example, uridine diphosphoglucose is an intermediate in glycogen synthesis, and phosphocreatine is a buffer for the supply of adenosine triphosphate. Animal studies suggest that phosphocreatine in the myometrium is under hormonal control.[8, 12] There have been suggestions that myometrial intracellular magnesium ions are under hormonal control[12] and we have reported preliminary evidence that free intracellular concentrations of magnesium ions may vary in myometrium and placenta as a result of physiologic status and disease.[7] The results obtained in our study are consistent with these previous observations; both hypertensive and diabetic patients show changes in placental free magnesium ions at the 0.05 level of significance. We have previously suggested that the pronounced increase in inorganic phosphate concentration as a result of placental maturation could be related to phosphorus transport to the fetus and that the decreased phosphomonoester with gestational age could be related to decreased cell turnover.[7]

Proliferative diseases. Currently there is great emphasis in the assessment of the roles of ^{31}P nuclear magnetic resonance spectroscopy in the diagnosis and prognosis of cancer. Our results and those of others who studied breast tumors[13] suggest that the reproductive tract may be particularly ammenable to nuclear magnetic resonance spectroscopy examination. Benign breast tumors have substantially lower phosphoethanolamine content than breast cancers.[14] Our results suggest that grossly normal myometrium from patients with adenocarcinoma (in one case, with no myometrial

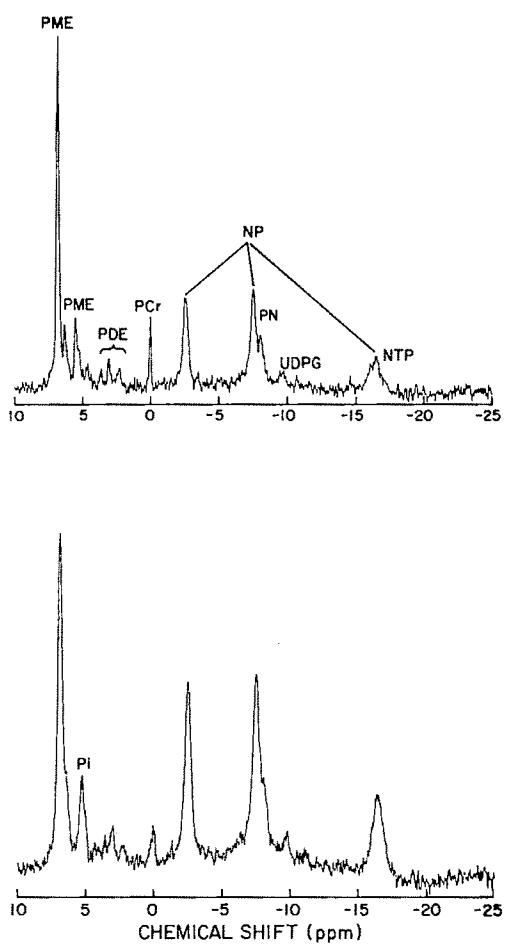


Fig. 5. ^{31}P nuclear magnetic resonance spectra of normal human uterine tube *(above)* and ovary *(below)* superfused at 4° C with oxygenated de Jalon's solution. For abbreviations, see Fig. 1 legend.

invasion) shows characteristics typical of the disease and suggest that ^{31}P nuclear magnetic resonance spectroscopy might provide information of prognostic significance. Conversely, the myometria studied from patients with cervical cancers showed no spectral abnormalities, consistent with a disease that would not normally first spread to the myometrium.

Disorders of pregnancy. The concentrations of high-energy phosphates generally are not altered in the diseased tissues we study, but there are other, more specific spectral changes. For example, the intrauterine growth retardation is associated with an increase in the placental peak attributed to glycerophosphorylcholine; this is consistent with an abnormality in placental membrane composition. A decrease in the intensity of this peak is observed in the skeletal muscle dystrophies,[15] which are known to be associated with abnormal membrane composition. Patients with diabetes show an alkaline placental intracellular pH, consistent with the finding that insulin alters intracellular pH in other tissues.[16]

The future. In an initial study clearly it is possible only to explore the usefulness of nuclear magnetic resonance spectroscopy for diagnosis and for elucidating the cause of disease. The results have been promising and one can imagine routine clinical determinations of placental biochemistry becoming an integral part of patient management. Nuclear magnetic resonance spectroscopy examinations in conjunction with magnetic resonance imaging, although not yet commonplace, yield useful information in selected cases. Our results suggest that endometrial adenocarcinoma might be one of those cases.

Finally, it is now possible to evaluate tissue biopsy samples of 10 to 50 mg blotted weight by ^{31}P nuclear magnetic resonance spectroscopy. We are doing so in preliminary studies of endometrial samples. Therefore nuclear magnetic resonance spectroscopy evaluation of the biochemistry of biopsy specimens on which histologic examinations are now obtained is feasible. Studies of small tissue samples, in contrast to noninvasive nuclear magnetic resonance spectroscopy examinations, involve relatively small capital and running costs and could therefore become routine diagnostic or screening procedures if their utility were to be adequately demonstrated.

REFERENCES

1. Mattison DR, Angtuaco T. Magnetic resonance imaging in prenatal diagnosis. Clin Obstet Gynecol 1988;31:353-89.
2. Fukushima E, Roeder BWS, eds. Experimental pulse NMR: a nuts and bolts approach. Reading, Massachusetts: Addison-Wesley, 1981:164-85.
3. Oliver JM, Kellie AE. The effects of oestradiol on the acid soluble nucleotides of rat uterus. Biochem J 1970; :187-91.
4. Degani H. MR spectroscopy studies of the reproductive organs and associated malignancies. In: McCarthy S, Haseltine F, eds. Magnetic resonance of the reproductive system. Thorofare, New Jersey: Slack, 1987:81-95.
5. Fantini J, Galons JP, Marvaldi J, Cozzone PJ, Canioni P. Growth of a human colonic adenocarcinoma cell line (HT29) on microcarrier beads: metabolic studies by ^{31}phosphorus nuclear magnetic resonance spectroscopy. Int J Cancer 1987;39:255-60.
6. Oberhaensli RD, Hilton-Jones D, Bore PJ, Hands LJ, Rampling RP, Radda GK. Biochemical investigation of human tumours in vivo with phosphorus-31 magnetic resonance spectroscopy. Lancet 1986;2:8-11.
7. Dawson MJ, McFarlane DK, McFarlin BL, Noyszewski EA, Trupin SR. The biochemistry of female reproductive tissues studied by ^{31}phosphorus nuclear magnetic resonance spectroscopy: effects of pregnancy, hormonal manipulation, and disease. Biol Reprod 1988;38:31-8.
8. Dawson MJ, Wray S. The effects of pregnancy and parturition on phosphorus metabolites in rat uterus studied by ^{31}P nuclear magnetic resonance. J Physiol 1985;368: 19-31.
9. Raman J, Trupin SR, Dawson MJ. Magnetic resonance spectroscopy in the evaluation of gynecological diseases and pregnancy disorders. Trophoblast Res 1989 (In press).
10. Haseltine F. Introduction of magnetic resonance of the reproductive system. In: McCarthy S, Haseltine F, eds. Magnetic resonance of the reproductive system. Thorofare, New Jersey: Slack, 1987:9-13.
11. Burt CT. Phosphodiesters and NMR: a tale of rabbits and chickens. TIBS 1985;10:404-6.
12. Degani H, Shaer A, Victor TA, Kaye AM. Estrogen-induced changes in high-energy phosphate metabolism in rat uterus, ^{31}P NMR studies. Biochemistry 1984;23: 2572-7.
13. Victor TA, Lawson CA, Wiebolt RC, Nussbaum S, Shattuck MC, Brodin AG, Degani H. Prediction of hormonal response of human breast carcinoma by ^{31}P MR spectroscopy. In: McCarthy S, Haseltine F, eds. Magnetic resonance of the reproductive system. Thorofare, New Jersey: Slack, 1987:67-79.
14. Degani H, Horowitz A, Itzchak Y. Breast tumors: evaluation with P-31 MR spectroscopy. Radiology 1986;161: 53-5.
15. Chalovich JM, Burt TC, Morris JD, Glonek T, Bárány M. Phosphodiesters in muscular dystrophies. Ann NY Acad Sci 1979;317:649-69.
16. Moore RD, Gupta RK. Effect of insulin on intracellular pH as observed by ^{31}P NMR spectroscopy. Int J Quant Chem Quant Biol Symp 1980;7:83-92.

A new surgical technique to treat refractory labial fusion in the elderly

N. Johnson, MRCOG, FRCS, R. J. Lilford, MRCOG, MRCP, PhD, and D. Sharpe, FRCS, OBE

Leeds and Bradford, England

An elderly woman with refractory labial fusion was treated by labial separation. The subsequent raw area was covered by a rotational skin flap from the thigh. The flap was deroofed of epithelium proximally and tunnelled subcutaneously. The distal portion emerged to cover the raw clitoral area and this successfully prevented contraction and scarring. (Am J Obstet Gynecol 1989;161:289-90.)

Key words: Labial fusion, dyspareunia, clitoris, vulvovaginitis.

A menopausal woman with dyspareunia was first seen with labia minora that could not be separated. Previous treatment attempts failed and the labia minora reunited. A technique suitable for such cases in elderly patients is presented.

Case report

A 53-year-old menopausal female was first seen in good health with a 3-year history of superficial dyspareunia. This was a result of anterior fusion of her labia minora. Three years previously a Fenton procedure combined with surgical separation of the adherent labia was attempted and various hormonal creams were applied to the vulva. These attempts failed and the labia reunited in the midline and obscured the clitoris. In the past, she was delivered of three children vaginally.

Examination of the vulva revealed that both labia minora were united to each other in the midline by a dense fibrous union that could not be separated. The clitoris was completely buried and could not be palpated. The rest of the vulva was atrophic but there were no dystrophic changes.

Under general anesthetic and in the lithotomy position the labia minora were separated along their natural line of cleavage by blunt and sharp dissection. Left alone, the raw edges of the labia came together and it was apparent that even if they were held apart by lateral anchoring sutures the raw area would have strictured and stenosed. It was decided to cover this area by a full-thickness skin flap to prevent contraction and scarring. This was fashioned from the medial aspect of the left thigh (2 inches × 1 inch; Fig. 1). The proximal half of this flap was deroofed of its epithelium by sharp dissection. The flap was rotated around an axis of 90° and was tunnelled subcutaneously until it emerged over the epithelial defect caused by the separation of labia minora. The deroofed epithelium lay under the tunnel and the full-thickness distal portion of the flap covered the raw area above the clitoris. It was secured into place with interrupted monofilament sutures (Fig. 2). The donor site was closed and the patient made an uneventful recovery.



Fig. 1. Labia minora were separated and full-thickness skin flap was fashioned from medial aspect of left thigh.

On the fifth postoperative day the wound was reexamined, and the sutures that secured the healthy flap were removed. By the third postoperative week the flap and donor site had completely healed and the introitus comfortably admitted two fingers. The patient has resumed vaginal intercourse and experiences clitoral stimulation.

Comment

Labial adhesions are generally considered to supervene inflammation in the prepubertal and hypoestrogenized female. However, older women are not immune from the condition[1] and they may have dyspareunia. Most cases can be treated by the application of topical, bland, or estrogen creams and gentle manip-

From the Department of Obstetrics and Gynaecology, St. James's University Hospital, and the Department of Plastic Surgery, Bradford Royal Infirmary.

Received for publication January 19, 1989; accepted March 8, 1989.

Reprint requests: N. Johnson, Department of Obstetrics and Gynaecology, St. James's University Hospital, Leeds, England.



Fig. 2. Proximally, flap is buried subcutaneously and emerges to cover raw area over clitoris. Full-thickness distal portion of flap is secured with interrupted sutures.

ulation. In extreme cases the labia may be separated and held apart with anchoring sutures. However, if the labia are firmly adherent and their separation leaves a large raw area, contraction of the overlying epithelium will occur and cause further stenosis of the introitus, which is likely to be refractory to further treatment. Stricture and scarring can only be prevented by covering the area with a full-thickness skin flap that contains its own blood supply. The only alternative would involve several releasing incisions closed with Z-plasty techniques,[2] but this would still leave the patient with a scarred vulva and wounds over the clitoral area.

The only significant potential complication of the technique we described is flap ischemia. The flap's viability is particularly at risk because it was deepithelialized proximally and because it possesses a random blood supply without a major vascular pedicle. Poor blood flow to the flap can result in wound infection, necrosis, and even sloughing. However, if the flap had failed, the patient would have been left with a homozygous dressing of natural skin that could have been easily regrafted.

This new technique is suitable in elderly patients with cases of labial adhesion that are refractory to all other treatment modalities.

REFERENCES

1. Goldstein AI, Rajcher WJ. Conglutination of the labia minora in the presence of normal endogenous estrogen levels: an exception to the rule. Am J Obstet Gynecol 1972;113:845-6.
2. Dewhurst CJ, Gordon RR. The older patient. In: The intersexual disorders. London: Bailliere Tindall and Cassell, 1969:74-89.

Bound volumes available to subscribers

Bound volumes of the American Journal of Obstetrics and Gynecology are available to subscribers (only) for the 1989 issues from the Publisher, at a cost of $56.00 ($79.00 international) for Vol. 160 (January-June) and Vol. 161 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue in the volume. The binding is durable buckram with the Journal name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact The C.V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318, USA; phone (800) 325-4177, ext. 351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular Journal subscription.

Predictive values, relative risks, and overall benefits of high and low maternal serum α-fetoprotein screening in singleton pregnancies: New epidemiologic data

Aubrey Milunsky, MB, BCh, DSc,[a] Susan S. Jick, MPH,[b] Carol L. Bruell, MS,[a] Dean S. MacLaughlin, PhD,[b] Yean-Kai Tsung, PhD,[a] Hershel Jick, MD,[b] Kenneth J. Rothman, DrPH,[c] and Walter Willett, MD[d]

Boston, Massachusetts

In a prospective study of maternal serum α-fetoprotein screening for both high and low values, we assessed the overall predictive value, sensitivity, specificity and relative risks for congenital defects and complications of pregnancy. Among 13,486 women with singleton pregnancies interviewed at the time of screening (15 to 20 weeks of gestation), 3.9% had high and 3.4% had low values. A high maternal serum α-fetoprotein value was associated with the following adverse outcomes: neural tube defects (relative risk = 224), other major congenital defects (relative risk = 4.7), fetal deaths (relative risk = 8.1), neonatal death (relative risk = 4.7), low birth weight (relative risk = 4.0), newborn complications (relative risk = 3.6), oligohydramnios (relative risk = 3.4), abruptio placentae (relative risk = 3.0) and preeclamptic toxemia (relative risk = 2.3). A low maternal serum α-fetoprotein value was associated with chromosomal defects (relative risk = 11.6) for fetal death (relative risk = 3.3). Either *high or low* maternal serum α-fetoprotein values were associated with 34.2% of all major congenital defects, 19.1% of all stillbirths and fetal-neonatal deaths, 11.0% of major pregnancy complications, and 15.9% of serious newborn complications. Maternal serum α-fetoprotein screening provides an important adjunctive tool for the identification of high-risk pregnancy and adverse neonatal outcome. (AM J OBSTET GYNECOL 1989;161: 291-7.)

Key words: α-Fetoprotein, neural tube defects, Down's syndrome, obstetric complications

Screening for elevated values of maternal serum α-fetoprotein is currently the standard of expected obstetric care in the United States and must be offered in California by law. More than a decade of experience has supported the introduction of routine screening into obstetric practice.[1] Evaluation of low maternal serum α-fetoprotein values for Down syndrome screening currently remains a research area, notwithstanding accumulating reports of benefits.[2-4] Surprisingly few epidemiologic studies have been conducted to evaluate the predictive values and relative risks associated with either elevated or low maternal serum α-fetoprotein values in the same cohort. In this prospective study we assess the sensitivity, specificity, overall predictive values, and relative risks of neural tube and other congenital defects (NTDs), chromosomal abnormalities, and pregnancy complications associated with high and low values in a maternal serum α-fetoprotein screening program.

From the Center for Human Genetics, Boston University School of Medicine,[a] Boston Collaborative Drug Surveillance Program, Boston University Medical Center,[b] the Department of Medicine, Section of Preventive Medicine and Epidemiology,[c] and the Departments of Epidemiology and Nutrition, Harvard School of Public Health, and the Channing Laboratory, Harvard Medical School, and Brigham and Women's Hospital.[d]

Supported by United States Public Health Service grant No. NS 19561 from the National Institute of Neurology and Communicative Diseases and Stroke.

Received for publication September 11, 1988; revised December 8, 1988; accepted February 4, 1989.

Reprint requests: Aubrey Milunsky, MB, BCh, DSc, Center for Human Genetics, Boston University School of Medicine, 80 East Concord St., Boston, MA 02118-2394.

Material and methods

All pregnant women receiving routine prenatal care in the practices of more than 90 participating obstetricians were offered maternal serum α-fetoprotein screening. Only occasional (estimated at <2%) patients declined screening. A serum sample was received by our Center for Human Genetics for 17,405 patients. As part of a study of antecedents to NTDs and other congenital defects, each woman was telephoned by one of our trained nurses for an interview. A total of 16,487 women whose serum samples were received consented to the interview; only 918 (5.3%) declined.

Interviews were conducted between Oct. 1984 and June 1986, and focused specifically on family, medical, and obstetric history with special emphasis on diet, medications, and illness during the first trimester of pregnancy. Questions also covered the environmental

Table I. Selected outcomes after maternal serum α-fetoprotein screening in 13,486 pregnancies

Defect	Total cases	Normal maternal serum α-fetoprotein	High maternal serum α-fetoprotein			Low maternal serum α-fetoprotein		
		No. of cases	No. of cases	Relative risk†	95% Confidence interval	No. of cases	Relative risk	95% Confidence interval
All NTDs	21	2	19	224.0	132.0-380.1	0	—	—
Chromosome defects	21	14	1	1.7	0.2-12.5	6	11.6	5.5-24.7
Other major congenital defects*	82	65	13	4.7	2.8-8.1	4	1.7	0.6-4.5
Fetal death	60	41	14	8.1	4.8-13.4	5	3.3	1.4-7.9
Stillbirth	53	50	1	0.5	0.1-3.2	2	1.1	0.3-4.4
Neonatal death	31	25	5	4.7	2.0-11.2	1	1.1	0.1-8.0
Newborn complications	232	191	29	3.6	2.5-5.1	12	1.7	1.0-3.0
Premature rupture of membranes	372	332	28	2.0	1.4-2.9	12	1.0	0.6-1.7
Polyhydramnios	32	30	2	1.6	0.4-6.5	—	—	—
Oligohydramnios	58	49	7	3.4	1.6-7.1	2	1.1	0.3-4.5
Abruptio placentae	82	71	9	3.0	1.5-5.8	2	0.8	0.2-3.1
Preeclamptic toxemia	223	195	19	2.3	1.5-3.6	9	1.3	0.6-2.4
Other pregnancy complications	563	505	41	1.9	1.4-2.6	17	0.9	0.6-1.5
Maternal diabetes	106	100	4	0.9	0.3-2.6	2	0.5	0.1-2.1
Maternal hypothyroidism	49	44	3	1.6	0.5-5.1	2	1.2	0.3-5.1
Maternal hyperthyroidism	10	9	1	2.6	0.4-19.1	—	—	—
Maternal illness	401	370	15	1.0	0.6-1.6	16	1.2	0.7-1.9
Low birthweight	308	256	43	4.0	3.0-5.3	9	1.0	0.5-1.9
None of the above	11,674	10,897	385			392		
Total†	13,486	12,495	530	—	—	461	—	—

*Minor defects are included with "none of the above" outcomes in calculation of relative risk for major congenital defects. If excluded, relative risk does not change.

†The numbers do not add up to the total because a woman may be in more than one category.

and occupational exposures within the year before conception. The vast majority of the interviews occurred before the maternal serum α-fetoprotein screening results were known to either the patient or the interviewer; 94% of patients in this study were between 15 and 20 weeks' gestation.

Pregnancy outcome was ascertained from a brief questionnaire mailed to the delivering physicians or eventually to the mothers themselves when necessary. Basic information concerning delivery, birth, pregnancy complications, and newborn health was the focus of this questionnaire.

This article reflects the outcome data for 13,486 (82%) of the interviewed women. Approximately 1500 had not yet been delivered at this time. (Subsequently, 159 women with multiple pregnancies were excluded.) Physicians provided 84% of the outcome data; the remaining 16% of the outcome questionnaires were completed by mothers. To date 10% have been lost to follow-up.

Low birth weight was defined as <5.5 pounds. Well-established criteria were used to define major and minor birth defects.[5] Newborn complications included any "special care" of the infant within the first few days of life such as surgery or treatment of jaundice, infection, respiratory difficulty, or complications of prematurity. Pregnancy complications included intrauterine growth retardation, abruptio placentae, placenta previa, precipitate labor, shoulder dystocia, nuchal cord, premature labor, postmaturity (>42 weeks' gestation), bleeding during pregnancy, or fetal loss.

Maternal serum α-fetoprotein assays were done by radioimmunoassay (Clinical Assays, Cambridge, Mass.) as previously reported.[6] Ultrasonographic studies were routinely requested for determination of an accurate gestational age for all mothers whose maternal serum α-fetoprotein values were ≥2.0 multiples of the median or ≤0.4 multiple of the median in the initial phase of the study and later when the odds for Down syndrome were ≥1:270 (see explanation below). Level II ultrasonography was recommended after confirmation of gestational age for maternal serum α-fetoprotein values between 2.0 and 2.4 multiples of the median. Amniocenteses were recommended in all pregnancies after confirmation of gestational age when maternal serum α-fetoprotein values were ≥2.5 multiples of the median or early in the study when the maternal serum α-fetoprotein value was ≤0.4 multiple of the median and subsequently if the odds for Down syndrome were ≥1:270. Later, consistent with practice in other major United States maternal serum α-fetoprotein screening centers, the lower-limit cutoff was changed to reflect

Table II. Sensitivity, specificity, and predictive values for selected pregnancy outcomes after detection of abnormal maternal serum α-fetoprotein values

	Sensitivity (%)	*Specificity (%)*	*Predictive value*	
			Positive (%)	*Negative (%)*
Elevated maternal serum α-fetoprotein values				
Spina bifida	90.9	96.0	1.9	99.99
Anencephaly	100.0	96.0	1.7	100.0
Other NTDs	50.0	95.9	0.4	99.98
Other major congenital defects	16.7	96.0	2.5	99.5
Fetal death	25.5	96.0	2.6	99.7
Neonatal death	16.7	96.0	0.9	99.8
Newborn complications	13.2	96.1	5.5	98.5
Oligohydramnios	12.5	96.0	1.3	99.6
Abruptio placentae	11.3	96.0	1.7	99.4
Preeclamptic toxemia	8.9	96.0	3.6	98.4
Maternal hyperthyroidism	10.0	96.0	0.2	99.9
Low birth weight	14.4	96.2	8.1	98.0
Low maternal serum α-fetoprotein values				
Chromosome defect	39.1	96.5	2.0	99.9
Fetal death	10.9	96.5	1.1	99.7

odds for Down syndrome. These odds were chosen because they were equivalent to known midtrimester risks for women aged 35 years who were routinely informed of such risks and the option of amniocentesis. The actual maternal serum α-fetoprotein level at these odds were in fact very close to the ≤0.4 multiple of the median used initially. Pregnancies that spontaneously aborted (categorized as fetal death) or were terminated for a diagnosed fetal defect were included among known outcomes.

Relative risks of specific outcomes and 95% confidence intervals were calculated for women with high or low maternal serum α-fetoprotein values, and women with normal values were used for comparison.[7] Sensitivity, specificity, and predictive values were computed for high and low maternal serum α-fetoprotein values.

Results

The age range of the 13,486 women in this study extended from 14 to 43; 90% of the women were 20 to 34 years old. Demographic information was available for 97.2% of the subjects. Ninety-four percent of the subjects were white, 1% each were black, Oriental, or of other races, and 2.8% were unknown. Eighty-two percent lived in the Boston area, 11% lived elsewhere in Massachusetts, 3.6% elsewhere in New England, 1% in states distant to New England, and in 2.8% residence information was not available.

Pregnancy outcomes after second-trimester maternal serum α-fetoprotein screening in 13,486 women are summarized in Table I. Of these, 12,495 women (92.7%) had a normal maternal serum α-fetoprotein value, 530 (3.9%) had a high value, and 461 (3.4%) had a low level. Women with a high level were at increased risk for pregnancies resulting in NTDs (relative risk = 224), other major congenital defects (relative risk = 4.7), fetal deaths (relative risk = 8.1), neonatal death (relative risk = 4.7), and newborn complications (relative risk = 3.6). Increased relative risks were also noted for major pregnancy complications including oligohydramnios (relative risk = 3.4), abruptio placentae (relative risk = 3.0), and preeclamptic toxemia (relative risk = 2.3).

Women with low maternal serum α-fetoprotein values were almost 12 times more likely to have offspring with a chromosome defect and were more than three times more likely to suffer a fetal death when compared with women with normal levels.

We next calculated the sensitivities, specificities, and predictive values of the maternal serum α-fetoprotein screen with respect to those outcomes for which the high or low maternal serum α-fetoprotein value indicates an increased risk for an abnormal outcome (see Table II). The sensitivity of the maternal serum α-fetoprotein test is quite low except in the case of spina bifida (sensitivity = 90.9%) and anencephaly (sensitivity = 100%), indicating that for most outcomes the test does not detect a majority of the cases. The positive predictive values, which indicate what percentage of a positive test will result in an abnormal outcome, are also low. This is a reflection of both the low prevalence of the outcomes and the small number of abnormal outcomes that follow a positive test. However, the specificity values are uniformly quite high; all are approximately 96; this indicates that most women who had a

Table III. Pregnancy outcome according to maternal serum α-fetoprotein level

Pregnancy outcome	Total	Maternal serum α-fetoprotein							
		Normal		High		Low*		High or low*	
	No.	n	%	n	%	n	%	n	%
All pregnancies	13,486	12,495		530		461		991	
NTDs	21	2	0.02	19	3.6	0		19	1.9
Chromosome defects	20	14	0.1	0		6	1.3	6	0.6
Other major congenital defects	82	65	0.5	13	2.5†	4	0.9‡	17	1.7
All major defects combined§	123	81	0.6	32	6.0	10	2.2	42	4.2
Fetal death	54	38	0.3	11	2.1	5	1.1	16	1.6
Stillbirth	48	45	0.4	1	0.2	2	0.4	3	0.3
Neonatal death	24	19	0.1	4	0.8	1	0.2	5	0.5
All other losses combined§	126	102	0.8	16	3.0	8	1.7	24	2.4
Premature rupture of membranes	350	315	2.5	25	4.7	10	2.2	35	3.5
Oligohydramnios	39	34	0.3	3	0.6	2	0.4	5	0.5
Abruptio placentae	74	66	0.5	6	1.1	2	0.4	8	0.8
Toxemia	209	182	1.5	18	3.4	9	2.0	27	2.7
Polyhydramnios	19	18	0.1	1	0.2	0		1	0.1
Other major pregnancy complications combined§	691	615	4.9	53	10.0	23	5.0	76	7.7
Newborn complications	157	132	1.1	18	3.4	7	1.5	25	2.5
Other pregnancy complications	384	352	2.8	20	3.8	12	2.6	32	3.2
Maternal illness	310	295	2.4	6	1.1	9	2.0	15	1.5
Other minor congenital defects	29	29	0.2	0		0		0	
None of the above	11,666	10.889	87.2	385	72.6	392	85.0	777	78.4

Data are arranged in hierarchical order so that each case is covered only once.

*Low maternal serum α-fetoprotein value.

†Gastroschisis (3 cases); renal agenesis (2); multiple anomalies (2); urinary tract obstruction (1); imperforate anus, cloaca, and malformed bowel (1); tetralogy of Fallot (1); congenital toxoplasmosis (1); cystic hygroma (1); absent bladder and cystic kidneys (1).

‡Multiple anomalies (1); endocardial fibroelastosis (1); hydrocephalus (1); branchial fistula (1).

§Subtotals, nonhierarchical.

normal pregnancy outcome also had a normal maternal serum α-fetoprotein level. Similarly, the negative predictive values, which indicate what percentage of people with a normal test result will have a normal outcome, are uniformly high. This is a reflection of the low prevalence of the various outcomes, and the large proportion of women with normal test levels who had normal pregnancy outcomes.

From Table II it can be inferred that all fetuses with anencephaly (nine cases) were detected through maternal serum α-fetoprotein screening. For spina bifida, 10 of 11 cases were detected; this yielded the high sensitivity rate of 90.9%. One of two fetuses with encephalocele had an associated elevated maternal serum α-fetoprotein level.

Since multiple adverse outcomes commonly occurred in the same pregnancy, we also constructed a hierarchical table, with each case being counted only once, for the outcome judged to be the most serious (see Table III). Selected pregnancy outcomes are tabulated for normal, high, and low maternal serum α-fetoprotein values. Calculations from the figures in Table III showed that the frequency of all major congenital defects combined was 0.9%. All fetal or neonatal losses combined also occurred in 0.9% of screened women. These major pregnancy complications combined occurred in 5.1%, whereas a further 2.9% had other miscellaneous pregnancy complications. Newborn complications occurred in 1.2% of cases.

Calculations from the data in Table III indicate that 34.2% of all major congenital defects combined were associated with either a high or low maternal serum α-fetoprotein value. Similarly, 19.1% of all fetal-neonatal losses had high or low maternal serum α-fetoprotein values; 11.0% of major pregnancy complications and 15.9% of newborn complications were associated with abnormal levels. High or low maternal serum α-fetoprotein values were associated with a further 8.3% of miscellaneous pregnancy complications. The one fetus listed under chromosome defects and high maternal serum α-fetoprotein values (Table II) had triploidy and spina bifida and therefore does not appear under chromosome defects in the hierarchical Table III.

Of the 530 women with elevated maternal serum α-fetoprotein values, 32 (6.0%) had a fetus with some type of major congenital defect, compared with 81 (0.6%) among women with a normal value. Fetal or neonatal loss occurred in 16 (3.0%) of women with high values, whereas a major pregnancy complication was observed in 53 (10.0%). Among women with normal

maternal serum α-fetoprotein values, these losses occurred in 102 (0.8%) and 615 (4.9%) women, respectively. Newborn complications were noted in 18 (3.4%) among the group with high values and in 132 (1.1%) among those with normal values.

Among the 461 patients with low maternal serum α-fetoprotein values, 6 (1.3%) had chromosome defects (two with trisomy 21, two with trisomy 18, an unbalanced 14/21 translocation Down syndrome, and a mosaic XO/XX/XXX), compared with 14 (0.1%) among those with normal maternal serum α-fetoprotein values. None of these patients were of advanced maternal age. Among the 461 patients, 5 (1.1%) experienced a fetal death and 8 (1.7%) lost their fetuses or newborns, compared with 38 (0.3%) and 102 (0.8%), respectively, among those with normal maternal serum α-fetoprotein values.

The probabilities of specific pregnancy outcomes related to previous normal, high, and low maternal serum α-fetoprotein values are shown in Table IV. Of special note for outcomes associated with high values is the 1:28 probability for NTDs, 1:17 for all major congenital defects combined, 1:10 for the major pregnancy complications, and 1:29 for clinically important newborn complications. For the group of outcomes associated with low maternal serum α-fetoprotein values, of special importance is the 1:77 probability for chromosome defects, 1:46 for all major congenital defects combined, and 1:20 for the specifically named major pregnancy complications.

The other major congenital defects associated with either a high or low maternal serum α-fetoprotein value are included in the footnote in Table III. The five neonatal deaths included four with prematurity complications and one with multiple congenital anomalies.

Table IV. Probabilities of selected pregnancy outcomes for normal, high, and low maternal serum α-fetoprotein values

Pregnancy outcome	*Probabilities for maternal serum α-fetoprotein*		
	Normal	*High*	*Low*
NTDs	1:6247	1:28	0
Chromosome defects	1:892	0	1:77
Other major congenital defect	1:192	1:41	1:115
All major congenital defects combined	1:154	1:17	1:46
Stillbirth, fetal or neonatal death	1:122	1:33	1:58
Premature rupture of membranes	1:40	1:21	1:46
Preeclamptic toxemia	1:69	1:29	1:51
Oligohydramnios, polyhydramnios, abruptio placentae, toxemia, and premature rupture of membranes	1:20	1:10	1:20
Newborn complications	1:95	1:29	1:66

The outcome categories are mutually exclusive, each case was counted only once on the basis of the hierarchy in Table III.

Comment

This prospective study of singleton pregnancies documents the increased relative risks associated with elevated or low maternal serum α-fetoprotein values, extends the number and type of pregnancy complications associated with abnormal values, and demonstrates the overall benefits of screening for both high and low maternal serum α-fetoprotein values. An increased maternal serum α-fetoprotein level in this study yields a striking relative risk of 224 for NTDs and a relative risk of 4.7 for other major congenital defects.

Other authors have previously noted increased relative risks in the range of 4 to 5 for low birth weight among pregnant women with high maternal serum α-fetoprotein levels,[8, 9] as we report here. Relative risks of perinatal death varying from 6 to 11[8, 10, 11] are comparable to our findings of 8.1 for fetal death and 4.7 for neonatal death. We add to the list of pregnancy complications predicted by an elevated maternal serum α-fetoprotein value increased risks for oligohydramnios (relative risk = 3.4), abruptio placentae (relative risk = 3.0), preeclamptic toxemia (relative risk = 2.3), and premature rupture of membranes (relative risk = 2.0).

Fetal Down syndrome in association with low maternal serum α-fetoprotein levels has been noted repeatedly.[2-4, 12] A New England Regional Genetics Group collaborative study[13] observed that 1:73 women with low maternal serum α-fetoprotein values had a fetus with an autosomal trisomy (virtually all trisomies 21, 18, or 13), a Yale study[4] noted 1:112, and we observed 1:115. For all chromosome abnormalities associated with low maternal serum α-fetoprotein levels, we observed a rate of 1:77, very similar to the odds of 1:67 reported in the New England group study. This and other studies[4, 12] have also documented low maternal serum α-fetoprotein levels in association with trisomy 18 and trisomy 13. In this study a low maternal serum α-fetoprotein value predicted a relative risk of 11.6 for a serious chromosome defect. Unlike others who reported that approximately 50% of spontaneous abortions were associated with low maternal serum α-fetoprotein levels,[10, 14] we noted that only 9.3% of fetal deaths had low values. Variable lower limits for maternal serum α-fetoprotein, study sizes, the population studied, and ascertainment bias probably explain the differences in reported rates.

Consonant with our past experience[6] and that of many others reviewed elsewhere,[1] elevated maternal serum α-fetoprotein values predicted the detection of

100% and 90.9% of anencephaly and spina bifida cases, respectively. Low maternal serum α-fetoprotein levels facilitated detection of 30% (6/20) cases of serious chromosome defects. Utilization of both high and low maternal serum α-fetoprotein values for screening, which to our knowledge has not previously been reported in a prospective study, proved valuable. For all major congenital defects combined, a high or low maternal serum α-fetoprotein value was found in 34.2% of cases. In addition, 19.1% of fetal-neonatal deaths, 11.0% of other listed major pregnancy complications, and 15.9% of other newborn complications were all preceded by high or low maternal serum α-fetoprotein values.

In previous reports reviewed by Wald and Cuckle,[15] and us,[6] that mainly consisted of laboratory experiences rather than systematically collected epidemiologic data, 41% to 64% of multiple pregnancies were associated with increased maternal serum α-fetoprotein values (≥2.0 multiple of the median). Those reports in part reflect results from women selected for screening by obstetricians because of some suspicion of twins or congenital defects. In association with our study we obtained maternal serum α-fetoprotein values on 159 women with multiple pregnancies. Among these women 85 (53%) had maternal serum α-fetoprotein values ≥2.0 multiples of the median.

The proportion of high maternal serum α-fetoprotein values reported here (3.9%) agrees with results of recent studies.[1] Low maternal serum α-fetoprotein values (equivalent to Down syndrome odds ≥1:270) in recent reports have ranged between 2.7% and 5.3%[4, 13]; ours was 3.4%. The frequency of low values is likely to decrease with expected improvements in radioimmunoassay performance, which we have already experienced after completion of this study after changing to a kit with greater precision in the low α-fetoprotein range. This change alone will probably bring greater efficacy to low maternal serum α-fetoprotein screening.

Physicians should be aware of the benefits of both high *and* low maternal serum α-fetoprotein screening, not only in the context of prenatal detection of congenital defects, but also in early identification of women at increased risk of obstetric complications. Chronic villitis and placental vascular lesions are the likely cause of elevated maternal serum α-fetoprotein values in certain cases, especially in association with subsequent intrauterine growth retardation.[16] Hence maternal serum α-fetoprotein screening is indicated even in the patient scheduled for amniocentesis. Care should be taken that blood is drawn *before* the amniocentesis needle is introduced to avoid the frequent and rapid α-fetoprotein leak from the fetal to the maternal circulation after aspiration.

Determination of a normal amniotic fluid α-fetoprotein value and acetylcholinesterase after documentation of an elevated maternal serum α-fetoprotein value provides considerable but far from complete reassurance of fetal normality. Our data[1] and those of Burton[17] indicate that approximately 5% of this group have a fetus with a major congenital defect, such as a cardiac malformation, hydrocephalus, renal abnormality, a limb reduction defect, or multiple congenital anomaly syndrome. Level II ultrasonography should therefore be done routinely in these cases. Consideration should be given to *serial* sonographic study (perhaps a total of three before the twenty-fourth week) to detect hydrocephalus and other evolving defects not yet evident. A fetal echocardiogram should also be considered since the likelihood of a cardiac defect exceeds 1%.[1]

Finally, pediatricians also need to be aware of the potential implications of a history of an elevated maternal serum α-fetoprotein value, considering the relative risks of 4.7 and 3.6 for neonatal death and serious newborn complications, respectively.

We thank the interviewing team, especially Marsha Thomas Lanes, Brenda Flomenhaft, Shirley Kasten, Maryanne Megliola, and Nancy Powell. We are also indebted to the many obstetricians and their staffs for allowing us to study their patients and providing outcome data, and to their many patients who participated in this study.

REFERENCES

1. Milunsky A. The prenatal diagnosis of neural tube and other congenital defects. In: Milunsky A, ed. Genetic disorders and the fetus: diagnosis, prevention and treatment. 2nd. ed. New York: Plenum Press, 1986:453-519.
2. Merkatz IR, Nitowsky HM, Macri JN, et al. An association between low maternal serum α-fetoprotein and fetal chromosomal abnormalities. AM J OBSTET GYNECOL 1984; 148:886-94.
3. Cuckle HS, Wald NJ, Lindenbaum RH. Maternal serum alpha-fetoprotein measurement: a screening test for Down syndrome. Lancet 1984;1:926-9.
4. DiMaio MS, Baumgarten A, Greenstein RM, et al. Screening for fetal Down's syndrome in pregnancy by measuring maternal serum alpha-fetoprotein levels. N Engl J Med 1987;317:342-6.
5. Leppig KA, Werler MM, Cann CI, et al. Predictive value of minor anomalies. I. Association with major malformations. J Pediatr 1987;110:531-8.
6. Milunsky A, Alpert E. Results and benefits of a maternal serum α-fetoprotein screening program. JAMA 1984; 252:1438-42.
7. Rothman KJ. Modern epidemiology. Boston: Little, Brown, 1986.
8. Wald N, Cuckle H, Stirrat GM, et al. Maternal serum alpha-fetoprotein and low birthweight. Lancet 1977; 2:268-70.
9. Brock DJH, Barron L, Jelen P, et al. Maternal serum alpha-fetoprotein measurements as an early indicator of low birthweight. Lancet 1977;2:267-8.
10. Doran TA, Valentine GH, Wong PY. Maternal serum α-

fetoprotein screening: report of a Canadian pilot project. Can Med Assoc J 1987;137:235-93.
11. Macri JN, Weiss RR. Prenatal serum alpha-fetoprotein screening for neural tube defects. Obstet Gynecol 1982; 59:633-9.
12. Doran TA, Cadesky K, Wong PY, et al. Maternal serum α-fetoprotein and fetal autosomal trisomies. AM J OBSTET GYNECOL 1986;154:277-81.
13. New England Regional Genetics Group Collaborative Study of Down Syndrome Screening. Combining maternal serum α-fetoprotein measurements and age to screen for Down syndrome in pregnant women under age 35. AM J OBSTET GYNECOL 1989;160:575-81.
14. Davenport DM, Macri JN. The clinical significance of low maternal serum α-fetoprotein. AM J OBSTET GYNECOL 1983;146:657-61.
15. Wald NJ, Cuckle HS. Open neural-tube defects. In: Wald NJ, ed. Antenatal and Neonatal screening. London: Oxford University Press, 1984:25-73.
16. Salafia CM, Silberman L, Herrera N, et al. Placental pathology at term associated with elevated midtrimester maternal serum α-fetoprotein concentration. AM J OBSTET GYNECOL 1988;158:1064-6.
17. Burton BK. Outcome of pregnancy in patients with unexplained elevated or low levels of maternal serum alpha-fetoprotein. Obstet Gynecol 1988;72:709-13.

The efficacy of fetal sonographic biometry in Down syndrome screening

Mark T. Peters, MD, Charles J. Lockwood, MD, and Wayne A. Miller, MD
Lincoln and Boston, Massachusetts

Sonographic biometry has been proposed as a second-trimester Down syndrome screening modality. Approaches have relied on the apparent "shortened" femur length of fetuses with Down syndrome. Unfortunately, significant intercenter variation has been reported in the magnitude of this femur length reduction. In an effort to overcome many of these potential biases and better estimate the magnitude of femur length shortening in fetuses with Down syndrome, a retrospective review of femur length differences between 16 Down syndrome and 194 control fetuses was carried out. All scans were performed by one examiner who used the same equipment and measurement technique. A significant reduction in the observed to expected femur length ratio for a given biparietal diameter was identified in the Down versus control fetuses (0.9574 95% confidence interval 0.9197, 0.9952 versus 0.9999 95% confidence interval 0.9913, 1.0086) ($p < 0.008$). However, the magnitude of this reduction was not sufficient to permit the use of this sonographic approach as an isolated marker for fetal Down syndrome. (AM J OBSTET GYNECOL 1989;161:297-300.)

Key words: Sonographic biometry, shortened femur, Down syndrome screening

Current screening modalities for Down syndrome determine risk on the basis of maternal age and maternal serum α-fetoprotein concentrations. However, this approach fails to identify most fetuses with Down syndrome.[1] Recently several investigators[2,3] have identified differences in sonographically determined femur length measurements between Down syndrome and unaffected fetuses and have proposed its application as a potential screening method. Unfortunately, the discriminatory value of discrepancies in fetal biometry between Down syndrome and unaffected fetuses has not been confirmed by all investigators (Table I).[4,5] Differences in sonographic equipment and measurement criteria may contribute significantly to the observed intercenter variation. Heterogeneity in parental racial-ethnic groups, and therefore in anthropometric indexes, may be present between study and control groups, particularly if the latter are derived from a population at low risk for fetal Down syndrome. These differences are unlikely to be constant across all centers, further exacerbating intercenter variability. When longer ascertainment intervals are used to collect Down syndrome versus control cases, another confounding variable is introduced, since operator experience and equipment resolution will improve over time. Finally,

From the Prenatal Diagnostic Center, and the Department of Obstetrics and Gynecology, Tufts University School of Medicine.
Received for publication August 8, 1988; revised November 12, 1988; accepted February 24, 1989.
Reprint requests: Charles J. Lockwood, MD, Director of Perinatal Research, Maternal-Fetal Medicine Section, St. Margaret's Hospital, 90 Cushing Ave., Boston, MA 02125.

Table I. Reports on accuracy of a shorter than expected femur length for a given gestational age or BPD in prenatal identification of fetal Down syndrome

Author	*Sensitivity (%)*	*False positive (%)*	*No. of cases*	*Reference no.*
Benacerraf et al.	68	2	28	3
Lockwood et al.	51	7	35	2
Perrella et al.	42	13	14	4
Peters et al.	13	7	16	—
Porto et al.	8	7	38	5

Table II. Fetal Down syndrome screening efficacy for observed to expected femur length ratio at a given BPD

					PPV		
O/E fl*	*SP (%)*	*95% CI*	*SN (%)*	*95% CI*	*1/250*	*1/500*	*1/1000*
0.97	66	59-73	50	26-74	0.6	0.3	0.1
0.94	86	80-90	43	20-69	1.2	0.6	0.3
0.92	91	86-95	18	5-44	0.8	0.4	0.2
0.91	93	88-96	13	2-38	0.8	0.4	0.2
0.90	96	92-98	13	2-38	1.3	0.7	0.3
0.89	97	93-99	13	2-38	1.7	0.9	0.4
0.84	99	94-99	13	2-38	5.2	2.6	1.3

OE, Observed to expected; *fl,* femur length; *SP,* specificity; *SN,* sensitivity; *PPV,* positive predictive value.
*Cutoff value equal to or less than stated number.

the subjective tendency to minimize gestational age discrepancies between biparietal diameter (BPD) and femur length measurements also contribute to intercenter variability.

We carried out the following retrospective case-control study in an effort to better define the magnitude of fetal biometric differences between Down syndrome and unaffected fetuses and assess the screening applicability of fetal biometry, free of these biases.

Material and methods

From July 1, 1985, to March 1, 1988, more than 2200 fetuses had an ultrasound scan and amniocentesis performed by a single operator (W. A. M.). The indications for amniocentesis included advanced maternal age, abnormal maternal serum α-fetoprotein concentrations, and a family history of genetic disease. In no case was an amniocentesis carried out because of an abnormal ultrasound finding. All scans included a measurement of BPD and femur length. BPD was measured from the skull's leading edge to leading edge at the level of the thalami. Femur length was determined from the largest single measurement. An ATL 2000 with a 3.5 MHz mechanical sector transducer was used for all ultrasound examinations.

Control group. The control group was obtained by random selection of 194 patients who had undergone genetic amniocentesis and were noted to have normal sonographic anatomy, biometry estimates of gestational age within 1½ weeks of menstrual age, and a normal karyotype.

Study group. Sixteen cases of Down syndrome were identified during the study period. All karyotype diagnoses were made from amniotic fluid cultures, and in no case was the sonographic diagnosis of a fetal congenital anomaly made. In all cases both BPD and femur lengths were recorded and the biometry correlated within 1½ weeks of menstrual age in 13 of 16 cases.

Statistical analysis. Using the method described by Benacerraf et al.,[2] a simple linear regression model of the relationship between femur length and BPD in unaffected fetuses was used to derive expected femur length for a given BPD in both groups. To test the statistical significance of observed differences between case and control populations, unpaired *t* tests and χ_2 were used. Confidence interval estimates for the sensitivity and specificity of observed to expected femur length cutoff points were calculated by the method described by Fleiss.[6]

Results

Comparisons of the mean BPD, femur length, and BPD to femur length ratio at given weeks of gestation were made between the Down syndrome and unaffected populations. Although femur lengths were marginally smaller and the BPD to femur length ratios marginally higher in fetuses with Down syndrome, no statistically significant differences were observed at spe-

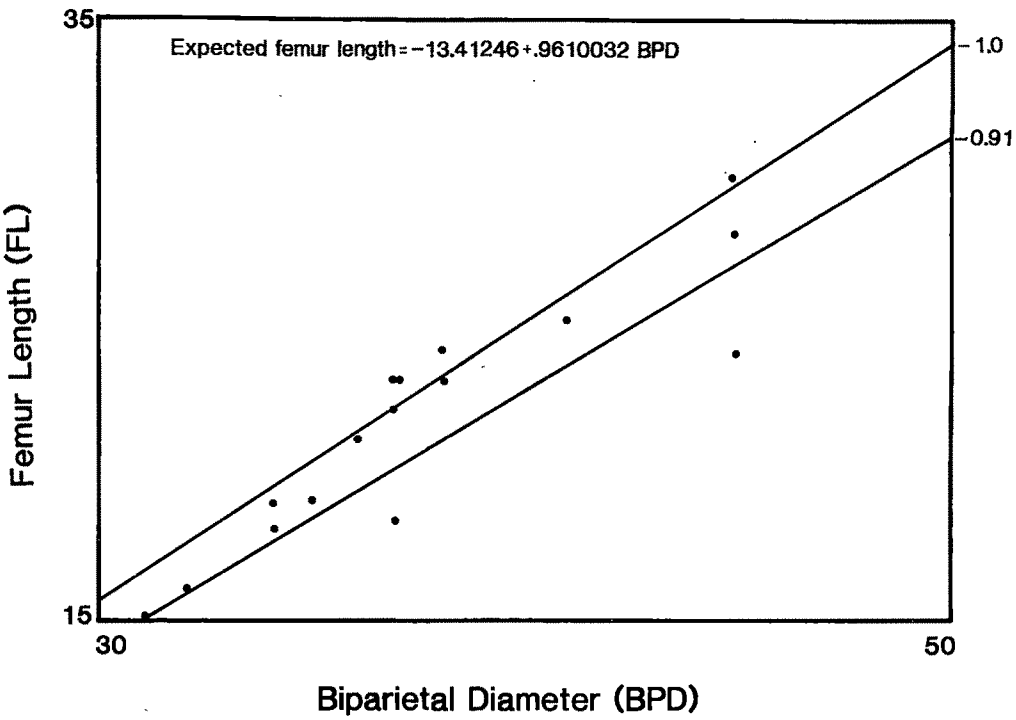


Fig. 1. *Black dots* represent Down syndrome cases. *Top line* is regression line of expected femur length for a given BPD in control group. *Lower line* represents 0.91 of expected femur length for a given BPD.

cific weeks of gestation. The aggregate mean BPD to femur length ratio was significantly higher in the Down syndrome group compared with the control group (1.757 95% confidence interval 1.662, 1.852 versus 1.648 95% confidence interval 1.627, 1.668) ($p <$ 0.004). Only 12.5% (2/16) cases of Down syndrome had a BPD to femur length ratio greater than 1.5 standard deviations above the BPD to femur length mean of the unaffected populations.

The relationship between BPD and femur length in the unaffected population was defined by the following equation: Expected femur length = −13.41246 + 0.9610032 (BPD) (r^2 = 0.883). A first-order regression model proved an optimal description; other regression models did not significantly improve the model.

The expected femur length for a given BPD in the unaffected population is plotted in Fig. 1. The mean ratio of observed to expected femur length for a given BPD was significantly lower in the Down syndrome compared with the control population (0.9574 95% confidence interval 0.9197, 0.9952; n = 16 versus 0.9999 95% confidence interval: 0.9913, 1.0086; n = 194) ($p < 0.008$). Thus for a given BPD, femur lengths of fetuses with Down syndrome were shorter than those of the control group.

Various observed to expected femur length ratio cutoffs were then assessed to determine the efficacy in the identification of fetal Down syndrome cases, which follows the suggestion of Benacerraf et al.[3] The sensitivity, specificity, and positive predictive value of the various cutoff ratios are presented in Table II.

Comment

This retrospective evaluation of sonographically determined biometric differences between second-trimester Down syndrome and unaffected fetus minimizes a number of potential biases present in previous studies. One examiner performed all studies without prior knowledge of the potential for biometric discrepancies in the Down syndrome versus unaffected population. The method of measurement and sonographic equipment used were consistent in both groups throughout the study period. The ascertainment periods were identical in both the study and control populations. The racial and socioeconomic groups were identical in the study and control populations (white, 93% private insurance). Furthermore, single "best" measurements were taken of the BPD and femur length without averaging multiple measurements. These factors should have reduce the potential for subjective reductions in gestational age discrepancies between BPD and femur length measurements.

The lack of statistically significant femur length shortening or elevations in BPD to femur length ratio

at specific gestational age intervals may reflect the small number of Down syndrome cases at given gestational age intervals ($n < 5$). Nonetheless, these observations are in contrast to the findings of Lockwood et al.[2] They first suggested this approach to Down syndrome screening, describing a 50% sensitivity rate and 7% false-positive rate for the detection of Down syndrome fetuses with the use of a BPD to femur length cutoff value of 1.5 standard deviations above the unaffected population's mean BPD to femur length ratio.[2] Implementing an identical approach, only 12.5% (2/16) of fetuses with Down syndrome were identified in this study population with a 7% false-positive rate.

Benacerraf et al.[3] evaluated the Down syndrome screening efficacy of the ratio of observed to expected femur length for a given BPD. They reported optimal screening efficacy with a ratio of 0.91, which produced a 68% sensitivity rate for a 2% false-positive rate.[3] Use of an identical methodology and their optimal cutoff value (0.91) produced a far lower sensitivity rate (13%) and far higher false-positive rate (7%) in this study population. Increasing the false-positive rate to 14% by raising the cutoff ratio to 0.94 afforded an increase in sensitivity to 43%. In contrast, reducing the false-positive rate to 1% by using a cutoff value of 0.86 did not reduce sensitivity (13%).

The finding that there were statistically significant differences between the mean observed to expected femur length value for a given BPD in the Down syndrome versus unaffected population suggests that true biologic differences exist. However, whether these differences are of sufficient magnitude to warrant their use as a screening modality is uncertain. Given that the discriminatory differences in femur lengths (2 to 4 mm) approach the limits of sonographic lateral resolution, far larger numbers of cases and controls must be analyzed to provide a definitive answer. Furthermore, an additional reduction in efficacy can be expected in the prospective application of this modality, since subjective biases will be accentuated. Therefore at this time it does not appear that the detection of a shorter than expected femur length for a given BPD is a sufficient indication for amniocentesis.

One potential value of this sonographic approach is its combination with maternal serum biochemical markers for Down syndrome, such as α-fetoprotein, human chorionic gonadotropin, and unconjugated estriol, as well as maternal age, to assign relative Down syndrome risks to patients as suggested by Wald et al.[7] In this context biometric measurements will either enhance or diminish the risk of Down syndrome without resorting to arbitrary cutoff values, provided they represent an independent measure of Down syndrome risk.[7]

REFERENCES

1. DiMaio MS, Baumgarten A, Breenstein RM, et al. Screening for Down's syndrome in pregnancy by measuring maternal serum alpha-fetoprotein levels. N Engl J Med 1987;317:342-6.
2. Lockwood C, Benacerraf B, Krinsky A, et al. A sonographic screening method for Down syndrome. AM J OBSTET GYNECOL 1987;157:803-8.
3. Benacerraf BR, Gelman R, Frigoletto FD. Sonographic identification of second-trimester fetuses with Down's syndrome. N Engl J Med 1987;317:1371-6.
4. Perrella R, Duerinckx AJ, Grant EG, et al. Sonographic identification of Down's syndrome. N Engl J Med 1988;318:1399.
5. Porto M, McCullock BP, Crade M, et al. Is the BPD/FL ratio a useful screening test for Down's syndrome [Abstract]. Presented at the Eighth Annual Meeting of the Society of Perinatal Obstetricians, Las Vegas, Nevada, February 3-6, 1988.
6. Fleiss JL. Statistical methods for rates and proportions. 2nd ed. New York: John Wiley and Sons, 1981;14.
7. Wald NJ, Cuckle HS, Densem JW, et al. Maternal serum screening for Down's syndrome in early pregnancy. Br Med J 1988;297:883-7.

Pregnancy loss in a small chorionic villus sampling series

Brian A. Clark, PhD, MD, John M. Bissonnette, MD, Susan B. Olson, PhD, and R. Ellen Magenis, MD

Portland, Oregon

Two previous reports have suggested that the pregnancy loss rate with chorionic villus biopsy is high in the initial 100 to 300 cases. In the initial 211 cases of this study, the total pregnancy loss rate (7.1%) and losses not attributed to induced abortion or cytogenetic abnormality (2.8%) do not support this contention. (AM J OBSTET GYNECOL 1989;161:301-2.)

Key words: Chorionic villus sampling, fetal loss rate, spontaneous abortion

Chorionic villus sampling promises to rival amniocentesis as a means of prenatal diagnosis if concerns about its safety and accuracy are resolved. The transcervical aspiration of villi at 9 to 12 weeks' gestation offers several advantages over transabdominal aspiration of amniotic fluid at 14 to 16 weeks' gestation for the clonal growth of amniocytes. An anomalous pregnancy at 12 weeks' gestation can be terminated by aspiration with less morbidity than a pregnancy at 17 to 20 weeks' gestation that requires either a dilation and evacuation procedure or the induction of labor.

In two previous reports it has been suggested that the initial experience with chorionic villus sampling is associated with a higher fetal loss rate in small series compared with those with larger numbers of patients. In response to the suggestion that the fetal loss rate did not appear to decline as larger centers gained experience with chorionic villus sampling, Jackson and Wapner[1] stratified their data in accordance with those centers that had experience with 100 to 200 procedures, those with 200 to 300 procedures, and those with >300 procedures. The fetal loss rates were 4.6%, 3.4%, and 2.7% respectively.[1] The report of a World Health Organization symposium on first-trimester fetal diagnosis and risk that pooled information from three groups, revealed a fetal loss rate of 3.4% for the first 300 procedures that declined to 1.7% after ≥400 procedures had been done.[2]

If chorionic villus sampling is to replace amniocentesis as a universal technique for prenatal diagnosis, it not only must be demonstrated safe in large referral center trials, but it also must be demonstrated safe in centers with moderate caseloads. We report on the experience at University Hospital in Portland, Oregon, where 211 chorionic villus sampling procedures have been done over a period of 3.25 years since initiation of the procedure.

From the Departments of Obstetrics and Gynecology and Medical Genetics, Oregon Health Sciences University.
Received for publication March 3, 1989; accepted March 8, 1989.
Reprint requests: John M. Bissonnette, MD, Mail Stop L-458, Oregon Health Sciences University, 3181 S.W. Sam Jackson Park Road, Portland, OR 97201.

Material and methods

Between April 1, 1984, and Sept. 30, 1988, 211 women with pregnancies between 9 to 12 weeks' gestation underwent chorionic villus sampling. An initial ultrasonography scan ruled out fetal death and pregnancies beyond 12 weeks' gestation; these are not included in subsequent analyses. All patients had cervical cultures before the procedure and those with group B streptococci or *Chlamydia* were treated. During the last 6 months of this period women with severely anteflexed uteri had transabdominal chorionic villus sampling (two cases). Initial experience with chorionic villus sampling was obtained in >80 volunteers with noncontinuing pregnancies between 8 to 12 weeks' gestation. All chorionic villus sampling procedures were performed by one of three physicians.

Chorionic villi were obtained under ultrasonographic guidance with a 3.5 MHz real-time sector scanner with a nylon transcervical catheter (ID 1.06 mm). For the majority (167 cases) the CBC-101 catheter (Vas-Cath of Canada Ltd., Mississauga, Ontario) was used. For the remaining 44 cases a Trophocan catheter (Portex, Inc., Wilmington, Mass) was used. Most samples were obtained with a single pass of the catheter. At no time were there more than three passes. A new sterile catheter was used for each individual catheter insertion. All patients have been followed up to at least 28 weeks' gestation and 180 have completed their pregnancies.

Results and comment

The major indication for chorionic villus sampling was advanced maternal age, with 79% of the women ≥34 years old at the time of delivery. A previous pregnancy with a chromosome disorder (11%) or metabolic defect (8%) accounted for the remainder.

A total of nine chromosomal abnormalities were detected, three of which were balanced translocations or

Table I. Pregnancy outcome of 211 chorionic villus sampling procedures

	n	%
Induced abortion	8	3.8
Loss up to 28 weeks' gestation*	6	2.8
Loss after 28 weeks' gestation	1	0.5
Pregnancies delivered or continued beyond 28 weeks' gestation	196	92.9

*Includes one cytogenetic abnormality (45,X).

inversions. This incidence of cytogenetic abnormality (4.3%) is similar to what has been observed in a large series (4.6%).[3] There were three metabolic disorders detected. Two of the chromosome abnormalities were mosaic: 45,X/46,XY and 45,X/46,XY. The former was confirmed both at amniocentesis and at pregnancy termination, whereas the later showed 46,XY in both amniotic fluid cells and in cord blood at birth. In addition to the two cases of mosaicism nine patients went on to amniocentesis—seven because insufficient tissue was obtained at chorionic villus sampling, one because of culture failure, and one because only maternal cells grew. This rate of subsequent amniocentesis (5.2%) can be compared with 9.9% in a series of >1000 women.[3]

Several approaches have been used to evaluate the pregnancy loss rates after chorionic villus sampling. As discussed in a report of a randomized Canadian collaborative clinical trial group, the most meaningful data will include all losses after a viable pregnancy has been determined at 9 to 12 weeks' gestation. The pregnancy outcome from the present series is given in Table I. The total loss rate of 7.1% is identical to that seen in the chorionic villus sampling (7.6%) and amniocentesis (7.0%) groups of the Canadian collaborative trial, each of which involved >1100 patients. The induced abortion rate in our series was slightly higher (3.8% versus 2.2%) and the losses up to 28 weeks' gestation were somewhat lower (2.8% versus 4.9%) than that seen in the chorionic villus sampling group of the Canadian study. If one uses the conventions of the series,* which do not include induced abortions or spontaneous abortions that occurred in cases of cytogenetic abnormalities, our loss rate of 2.8% does not support the suggestion that the pregnancy loss rate is high in the initial 100 to 300 patients.

We thank Drs. John Buckmaster and Kathleen Kennedy for their assistance. We also acknowledge Ms. Keri Stepper for her assistance.

* Discussed in the beginning of this article.

REFERENCES

1. Jackson LG, Wapner RJ. Authors' reply. Prenat Diagn 1985;5:167.
2. World Health Organization. Risk evaluation in chorionic villus sampling. Prenat Diagn 1986;6:451.
3. Canadian Collaborative Chorionic Villus Sampling–Amniocentesis Clinical Trial Group. Multicenter randomized clinical trial of chorionic villus sampling and amniocentesis. Lancet 1989;1:1.

Maternity shelter care for adolescents: Its effect on incidence of low birth weight

Katherine D. LaGuardia, MD, MPH, Maurice L. Druzin, MD, and Carol Eades, BA
New York, New York

A group of 112 pregnant adolescents residing in a maternity shelter was compared with a group of 113 pregnant adolescents residing in their own homes but receiving identical medical care. The sheltered group was made up of a particularly high-risk group: they were more likely to be black, unmarried, receiving public assistance, and registered later for prenatal care than the group living at home. Despite these differences, the sheltered group delivered significantly fewer infants of low birth weight and preterm gestation than the control group. Variables assessed in this study fail to explain this difference. It is concluded that the services offered in a maternity shelter can have a positive impact on the reduction of low birth weight deliveries in a group of high-risk patients. These results indicate that the social and behavioral correlates of low birth weight remain to be fully defined and understood. (Am J Obstet Gynecol 1989;161:303-6.)

Key words: Adolescent pregnancy, low birth weight, maternity shelter

It has been well documented that pregnant adolescents constitute a population at high risk for delivery of low birth weight infants. According to national figures, in 1978, 17% of all births were to teenagers and yet 24% of low birth weight infants had a teenage mother.[1] The relationship between low birth weight and age is a U-shaped curve, with the highest levels occurring under age 20 years and over age 35 years. This relationship has been known for many years and the subject of much discussión in the literature. The reasons for the higher incidence of low birth weight deliveries among adolescents has not been clearly established.

There appear to be two schools of thought in this debate. One maintains it is because of the physical immaturity of the mother,[2, 3] whereas the other maintains that there is an overrepresentation of risk factors associated with low birth weight among young women.[4-6] It is known that young mothers are more likely black, of low socioeconomic status, to report late for prenatal care, and be unmarried.[1] However, when these factors are rigorously excluded from the analysis, there are no demonstrated differences in prematurity or low birth weight rate among infants born to adolescent mothers and those born to women in the 20- to 34-year-old age bracket.[4, 5]

From the Department of Obstetrics and Gynecology, The New York Hospital–Cornell Medical Center.
Received for publication October 2, 1988; revised February 1, 1989; accepted February 17, 1989.
Reprint requests: Katherine D. LaGuardia, MD, MPH, Department of Obstetrics and Gynecology, The New York Hospital–Cornell Medical Center, 525 E. 68th St., New York, NY 10021.

What continues to remain unclear is whether intervention programs for the prevention of low birth weight are effective in populations with these demographic risk factors. Main et al.[7] failed to demonstrate any difference in the incidence of preterm delivery in a controlled clinical trial whose population consisted of indigent black women with a prematurity rate twice the national average. However, others have found intervention programs to be highly effective.[8, 9] It may be that the etiologic factors, which play an important role in producing preterm births in one population and interventions designed to counteract these factors, may not be universally applicable.

We have examined the potential benefits of intensive social services, behavior modification, and heightened awareness of the problems of pregnancy provided in a sheltered environment (a maternity shelter) on the incidence of low birth weight deliveries among a group of indigent urban teenagers. The outcomes of these pregnancies were compared with those of a comparable group of teenagers residing at home but receiving identical medical care. The study was conducted to examine the hypothesis that a model of social and behavioral intervention can reduce low birth weight rates among a group of high-risk patients.

Material and methods

Data for this study were obtained from a retrospective review of 225 consecutive singleton adolescent deliveries that occurred between 1984 and 1986 at The New York Hospital–Cornell Medical Center in New York City. The study group, totaling 112, consisted of all patients 19 years and under who resided at In-

Table I. Characteristics of patients according to residence

Characteristic	*(1) Study group (sheltered) (N = 112)*	*(2) Control group (nonsheltered) (N = 113)*
Mean age (yr) (range 13-19)	16.5	17.5
Race		
% Black	83*	55
% Hispanic	11*	29
Insurance (% Medicaid)	100*	72
Marital status (% single)	100*	80
% living with		
Parents	—	62
Other family	—	2
Spouse/boyfriend	—	22
Alone	—	4
Unknown	—	10
Mean maternal weight gain (pounds)	30	32
% cigarette smoking	23	35*
Mean week of first prenatal visit	26.4†	21.2
% primary C/S	12	12
Mean birth weight (gm)	3122	3082
% primigravidas	66	51
% preeclampsia	11	9
% anemia (Hct < 34)	52	33

CS, Cesarean section; *Hct,* hematocrit.
*$p < 0.05$ for comparison between columns 1 and 2.
†$p < 0.03$ for comparison between columns 1 and 2.

wood House, a maternity shelter for pregnant adolescents, during their pregnancy and at the time of delivery. The control group, totaling 113, consisted of all patients 19 years and under who lived in their communities during their pregnancy and delivery. If a patient did not live at Inwood House, she was placed in the control group. There was no other process of selection. All patients received similar medical care within the prenatal clinic of the Department of Obstetrics and Gynecology at The New York Hospital. Obstetric care was provided by the house staff with faculty supervision. No specific intervention model was used in the clinic.

Inwood House is one of four maternity shelters in New York City. It receives both private and public funding. All patients must be either referred or approved by the Office of Special Services for Children of the Health Resources Administration of New York City. Criteria for admission include age under 21 years and homelessness. Patients are placed in the shelter on a voluntary basis. However, court-ordered placement exists. All residents receive their prenatal care at The New York Hospital, which is located less than one mile from the home. All are given their own room and meals and are required to attend school. Incentives for compliance with clinic appointments, attendance at school, meals, other activities, and meeting curfew are based on a point system developed by the Inwood House staff. Monetary rewards are administered based on an accumulation of points. Each patient is assigned a social worker who is on-site daily at the shelter. Areas of emphasis by the Inwood House staff during the course of a patient's residence include: (1) contraception, (2) reproductive anatomy, (3) preparation for labor and delivery, including signs and symptoms of preterm labor, (4) plans for the infant after delivery, and (5) plans for the mother after delivery.

Gestational age, which is used as a critical variable in testing the hypothesis of the efficacy of this intervention program, was determined by last menstrual period and serial ultrasound studies and then was correlated with a modified Dubowitz assessment in the neonatal period. Infants with more than a 2-week error between the antenatal assessment and the neonatal assessment would have been excluded from the analysis. However, it was unnecessary to exclude any infants on this basis.

Results

A comparison of characteristics of patients in the control and study groups is presented in Table I. The study group was slightly younger, registered significantly later for prenatal care, was more likely to be black, unmarried, and on public assistance than those in the control group. Despite these differences, the study group delivered significantly fewer infants of low birth weight and premature gestation (defined as <37 completed weeks) than the control group. There was no difference in weight gain, incidence of anemia or preeclampsia, or occurrence of preterm labor between the two groups. However, the control group had significantly more smokers than did the study group (35% versus 23%) ($p < 0.05$). Mean birth weight for the study group was 3122 gm and 3082 gm for the control groups. The primary cesarean section rate for both groups was roughly half that of ward patients over age 20 years (12% versus 24.5%).

As presented in Table II, low birth weight rates were 6.2% and 12.4% for the sheltered and nonsheltered groups, respectively (p = NS). When standardized for both birth weight and gestational age, however, the preterm low birth weight drops to 2.6% and 9.7%, respectively ($p < 0.05$). There was no difference in admissions to the neonatal intensive care unit between the two groups.

Incidence of antepartum hospitalization fails to explain the difference in the preterm low birth weight rate between the two groups. As shown in Table III, although the sheltered group spent more days per person in the hospital for preterm labor, the incidence of hospitalization was statistically similar for both groups for all diagnoses.

Table II. Neonatal outcome by residence

Outcome	*(1) Study group (sheltered) (N = 112)*		*(2) Control group (nonsheltered) (N = 113)*	
% LBW (>500 gm/<2500 gm)	6.2	(*n* = 7)	12.4	(*n* = 14)
% LBW and <37 wk	2.6*	(*n* = 3)	9.7	(*n* = 11)
% LBW and >37 wk	–	(*n* = 4)	2.7	(*n* = 3)
% admitted to ICU	9.0	(*n* = 10)	11.0	(*n* = 12)

LBW, Low birth weight; *ICU,* intensive care unit.
*$p < 0.05$ for comparison between columns 1 and 2.

Table III. Antepartum hospitalization according to residence

Antepartum hospital	*(1) Study group (sheltered)*		*(2) Control group (nonsheltered)*	
	n	%	*n*	%
Diagnosis				
Preterm labor	19	68	10	43
PROM	0	0	2	9
Preeclampsia	2	7	1	4
Medical indication	3	11	6	26
Third trimester bleeding	1	4	1	4
Other	3	10	3	14
Total	28	100	23	100
Average No. of days in hospital/person hospitalized		7.5		6.1
Average No. of days in hospital/person with preterm labor		11.4*		5.8

PROM, Premature rupture of membranes.
*$p < 0.025$ for comparison between columns 1 and 2.

Comment

The assessment of low birth weight in this study was based on the model of Kaltreider and Kohl,[10] in which low birth weight was defined according to both gestational age and birth weight. Premature low birth weight includes those infants delivered at a gestational age of >20 weeks but less than 37 completed weeks and weighing >500 gm but <2500 gm. The term low birth weight population is defined as those infants born after 37 completed weeks and weighing >500 gm but <2500 gm. Although not entirely accurate, this definition allows separation of the truly preterm infant and the growth retarded infant weighing less than 2500 gm but delivered after 37 weeks.

Although a longer-term, prospective study is necessary to analyze more fully the paradoxic outcome revealed in this study, it appears that the services provided in this particular maternity shelter have a positive impact on reduction of low birth weight in a group of high-risk patients. In particular, the heightened awareness of the staff to the problems of pregnancy and the education provided on the signs and symptoms of preterm labor may have contributed to earlier hospitalization and improved outcome. It is also interesting that significantly fewer study subjects smoked. This finding confirms the beneficial effects of behavior modification in this population.

It may also be that an important component contributing to this improved outcome is a reduction of external stress. Those patients residing in the maternity shelter had external stress largely reduced. They had shelter, food, money, and easy access to health care staff. Those in the control group lived in communities further from the hospital, in variable living conditions, and with financial concerns more complex than those at Inwood House. It appears that the social and behavioral correlates of low birth weight remain to be fully defined and understood. Studies that examine the causal factors of low birth weight in the adolescent population need to assess these correlates.

REFERENCES

1. Vital statistics of the United States, 1978. Vol I. Washington, DC: U.S. Government Printing Office, 1982; Department of Health and Human Services publication no. 82-1100.
2. Naeye RL. Teenaged and pre-teenaged pregnancies: con-

sequences of the fetal-maternal competition for nutrients. Pediatrics 1981;67:146-50.
3. Spellacy WN, Mahan CS, Cruz AC. The adolescent's first pregnancy: a controlled study. South Med J 1978;71:768-71.
4. Horon IL, Strobino DM, McDonald HM. Birth weights among infants born to adolescents and young adult women. AM J OBSTET GYNECOL 1983;146:444-9.
5. Merritt TA, Lawrence RA, Naeye RL. The infants of adolescent mothers. Pediatr Ann 1980;9:32-51.
6. Elster AB. The effect of maternal age, parity, and prenatal care on perinatal outcome in adolescent mothers. AM J OBSTET GYNECOL 1984;149:845-7.
7. Main D, Gabbe SG, Richardson D, et al. Can preterm deliveries be prevented? AM J OBSTET GYNECOL 1985; 151:892-8.
8. Papiernik E, Boyer J, Dreyfus J, et al. Prevention of preterm births: a perinatal study in Hagenau, France. Pediatrics 1985;76:154-8.
9. Herron MA, Katz M, Creasy RK. Evaluation of a preterm prevention program: a preliminary report. Obstet Gynecol 1982;59:452-6.
10. Kaltreider FD, Kohl S. Epidemiology of preterm delivery. Clin Obstet Gynecol 1980;23:17-31.

Fetal hyperinsulinism in anencephaly

C. Hubinont, MD, PhD,* N. M. Fisk, MD, U. Nicolini, MD, and C. H. Rodeck, MD**
London, England

Insulin was measured in the blood collected in utero from three midtrimester anencephalic fetuses. The hyperinsulinism found could be due to an underutilization of glucose in the absence of most of the brain and could be responsible for the relatively normal growth in anencephaly despite the absence of the hypothalamohypophysial axis. (AM J OBSTET GYNECOL 1989;161:306-7.)

Key words: Fetal blood, insulin, anencephalic fetuses

The role of the hypothalamohypophysial system in insulin secretion has been studied previously in humans, as a comparison of normal and anencephalic neonates. Van Assche et al.[1] found normal insulin levels, with hyperinsulinemia being confined to a small subgroup of those with both an intact hypophysis and an abnormal maternal carbohydrate tolerance.

Growth in anencephalic fetuses and newborns has been reported to be normal or slightly decreased despite the absence of growth hormone secretion.[2]

Since fetal insulin is a major determinant of fetal growth and since the insulin status of human anencephalic fetuses in utero has not been reported, we measured plasma insulin levels in fetal blood from midtrimester anencephalic fetuses.

From the Institute of Obstetrics and Gynaecology, Royal Postgraduate Medical School, Queen Charlotte's and Chelsea Hospital.
Received for publication February 23, 1989; accepted March 20, 1989.
Reprint requests: C. Hubinont, MD, PhD, Royal Postgraduate Medical School, Institute of Obstetrics and Gynaecology, Queen Charlotte's and Chelsea Hospital, Goldhawk Road, London, England W6 OXG.
**Recipient of a Royal Society and a Council of Europe Fellowship.*
***Supported by a Joseph Foreman Fellowship from Royal Prince Alfred Hospital, Sydney, Australia, and by a Gladys Dodds Fellowship from the Royal College of Obstetricians and Gynaecologists.*

Material and methods

Three fetuses were diagnosed as having anencephaly by ultrasonography at 16, 20, and 24 weeks' gestation (two singleton pregnancies and one twin pregnancy at 24 weeks). Fetal blood was obtained from the affected twin at selective feticide. In the two singleton gestations, fetal blood was aspirated by an ultrasonographically guided needle technique in consenting patients undergoing termination of pregnancy by intraamniotic instillation of prostaglandin E_2.

All three mothers had fasted for 6 to 12 hours. There was no history of maternal glucose intolerance or Rh alloimmunization, both capable of inducing fetal hyperinsulinism.

Insulin assay was performed in duplicate (insulin radioimmunoassay kit, Immunodiagnostics LTD, Tyne & Wear, London). The results were then compared with our normal range, previously established in 33 singleton fetuses (18 to 40 weeks) having blood sampling for prenatal diagnosis of various genetic conditions and later found to be unaffected.

Results

Insulin levels of the three anencephalic fetuses plotted against those of 33 normal fetuses were all higher than the mean + 2 SD for gestational age (Fig. 1).

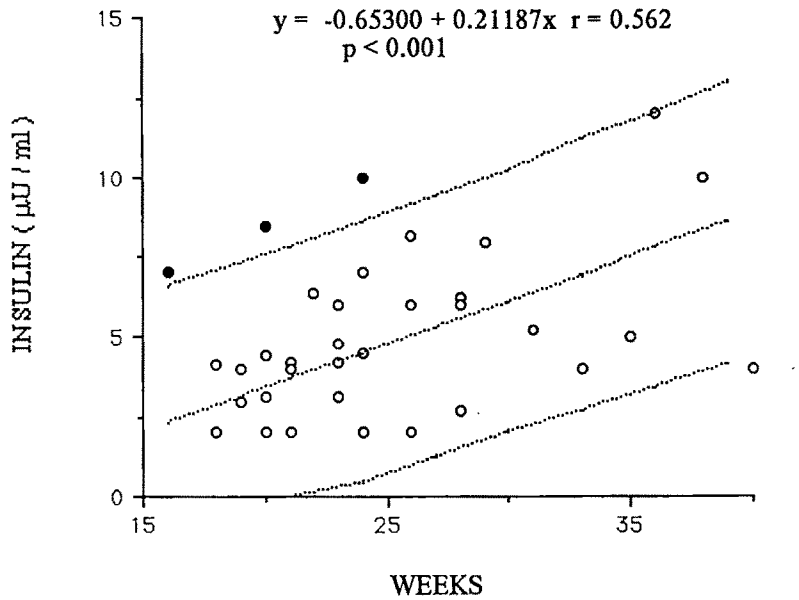


Fig. 1. Plasma insulin concentrations of fetuses with anencephaly(●) plotted against normal values for gestation (○).

Comment

Our finding of hyperinsulinism in anencephalic fetuses differs from the results of Van Assche et al.,[2] but this study was done on newborns whereas our observations were made on fetuses in utero. In fact, the mechanisms of the regulation of insulin secretion in the fetus seem to be different from those in newborns (presence of transplacental transfer of glucose and amino acids), and this may explain the discrepancy in the results.

Hyperinsulinism in anencephalic fetuses might be secondary to high glucose levels, which may be mainly due to underutilization in the absence of most of the brain. Long-term glucose stimulation of the fetal pancreatic β-cells would thus produce hyperinsulinism, as it does in maternal diabetes. Although the features of β-cell stimulation in fetuses of diabetic mothers are usually found only during the third trimester, they have been observed as early as the nineteenth week. This may explain why fetal hyperinsulinism is more marked in the two anencephalic fetuses >20 weeks' gestation.

The presence of hyperinsulinism in anencephalic fetuses suggests that the development and function of their fetal pancreatic β-cells is appropriate, given the increased glucose concentration to which they are probably exposed. Insulin plays an important role in fetal growth; therefore its hypersecretion may compensate for the absence of other growth factors controlled by the hypothalamohypophysial system.

REFERENCES

1. Van Assche FA, Gepts W, De Gasparo M. The endocrine pancreas in anencephalics. Biol Neonate 1970;14:374-88.
2. Vorherr H. Factors influencing fetal growth. Am J Obstet Gynecol 1982;142:577-88.

Antenatal ultrasonographic diagnosis of fetal seizure activity

Helain J. Landy, MD, Alfred N. Khoury, MD, and Peter S. Heyl, MD
Washington, D.C., and Fairfax, Virginia

A case is reported in which fetal seizure activity was observed ultrasonographically but the growth-retarded fetus was chromosomally normal. (AM J OBSTET GYNECOL 1989;161:308.)

Two cases of antenatally diagnosed fetal seizures were reported by Conover et al.[1] In both cases, the seizures were associated with anomalous fetuses. We report a case of fetal seizure activity that was observed ultrasonographically in a chromosomally normal, growth-retarded fetus.

Case report

A 35-year-old woman, gravida 2, para 1-0-0-1, was transferred to Fairfax Hospital at 31½ weeks' gestation with intrauterine growth retardation and decreased amniotic fluid volume. A 46,XX karyotype was known from genetic amniocentesis. Administration of α-methyldopa was begun 3 weeks earlier to treat chronic hypertension. On transfer to Fairfax Hospital, an ultrasonogram showed a structurally normal fetus, although the biparietal diameter measured 1½ to 2 weeks less than long-bone and abdominal circumference parameters. The amniotic fluid volume was subjectively decreased. Results of Doppler flow studies of the umbilical artery were normal. Because blood pressure and creatinine clearance values were normal, antihypertensive medication was discontinued. Results of nonstress tests were reactive, and biophysical profiles were normal. Serial ultrasonograms showed good fetal growth, although the discrepancy in biparietal diameter measurement persisted.

During biophysical profile examination at 35 weeks, oligohydramnios was seen. Rapid, repetitive, and episodic jerking fetal movements were noted. These were thought to represent seizure activity. The patient admitted to having felt similar episodes at least once during the preceding week. A reactive nonstress test and negative contraction stress test were obtained.

A live-born female infant was delivered by cesarean section. Seizure activity was evident immediately. Apgar scores were 4 and 8 at 1 and 5 minutes, respectively; the cord pH was 7.32. The newborn's measurements were as follows: weight, 2060 gm; head circumference, 31 cm (10th to 25th percentile); length, 46 cm (25th to 50th percentile for gestational age, Colorado growth curve). A small midline soft palate cleft was seen and the distal clavicles were poorly ossified.

A thorough search for an underlying cause of the seizures was inclusive. Evaluation ruled out infectious causes (adenovirus, coxsackieviruses A and B, herpes simplex virus, echovirus, enteroviruses types 68 through 71, poliovirus, toxoplasmosis, cytomegalovirus, and group B streptococci) and metabolic causes (including lipid storage diseases, endocrinologic disorders, and other imbalances). Results of head and renal ultrasonograms and echocardiogram were normal. The repeat karyotype was 46,XX. The electroencephalogram initially showed a diffuse burst pattern without suppression with pyridoxine, but the abnormalities resolved a few weeks after birth. Phenobarbital was used only for a short time after birth and was then discontinued.

The infant has remained seizure-free without medication. At 10 months of age, she has shown significant growth delay and weighs only 5100 gm. Microcephaly and motor retardation are obvious, and hearing impairment has been diagnosed. A definite underlying cause remains to be confirmed. Serious neurologic handicaps are anticipated for the future.

Comment

Diagnosing fetal seizure activity is quite rare. In the report of two such cases by Conover et al.[1] both fetuses were anomalous (one had multiple subtle anomalies not detectable with ultrasonography and the other had hydramnios and hydranencephaly). In our case no structural anomalies could be identified ultrasonographically, although intrauterine growth retardation, oligohydramnios, and the possiblity of mild microcephaly existed. Knowing that the fetus had a normal karyotype aided in the decision to manage this pregnancy to optimize fetal salvage.

Fetal seizure activity may be associated with metabolic or structural abnormalities, even in the presence of normal ultrasonographic and genetic evaluations. Since this ultrasonographic finding may be associated with a poor outcome, proper counseling is advised.

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, The George Washington University Medical Center, and the Department of Obstetrics and Gynecology, Fairfax Hospital.
Received for publication February 8, 1989; revised March 8, 1989; accepted March 17, 1989.
Reprint requests: Helain J. Landy, MD, Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, The George Washington University Medical Center, 2150 Pennsylvania Ave., NW, Washington, DC 20037.

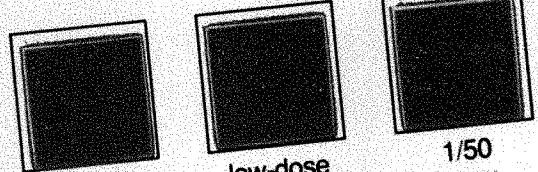
REFERENCE

1. Conover WB, Yarwood RL, Peacock MD, Thomas BA. Antenatal diagnosis of fetal seizure activity with use of real-time ultrasound. AM J OBSTET GYNECOL 1986;155:846-7.

Take the OC preference test.

1.

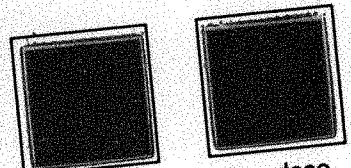
In the United States last year, the OC group with the largest number of prescriptions was:



triphasic | low-dose monophasic | 1/50 formulations

2.

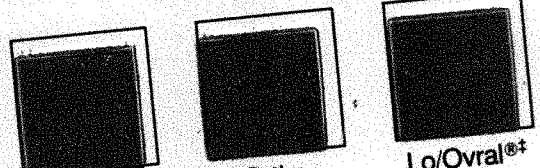
In the last 18 months, more *new* prescriptions were dispensed for:



triphasics | low-dose monophasics

3.

Which monophasic had an increase in new prescriptions 13 times the average of all low-dose monophasics in the last 18 months?



Demulen® 1/35* | Ortho-Novum® 1/35† | Lo/Ovral®‡

Scratch gold squares with a coin to reveal the answers.

*Demulen® 1/35 (ethynodiol diacetate 1 mg, ethinyl estradiol 35 mcg) is a registered trademark of G.D. Searle & Co.

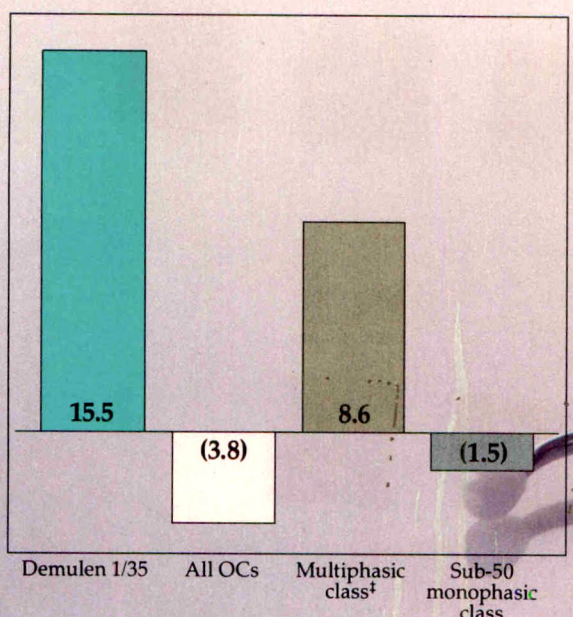
†Ortho-Novum® 1/35 (norethindrone 1 mg, .035 mg ethinyl estradiol) is a registered trademark of Ortho Pharmaceutical Corporation.

‡Lo/Ovral® (norgestrel and ethinyl estradiol tablets) is a registered trademark of Wyeth-Ayerst.

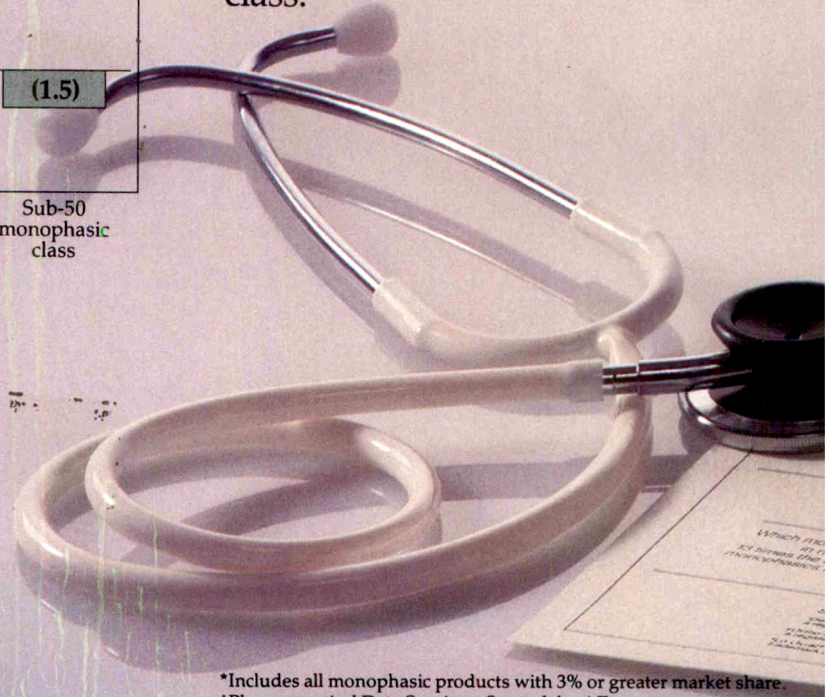
There's an OC ir
and it's not

Now Demulen® 1/35 is the fastest growing monophasic*

New OC Rx's: *Percent change, October 1987 to March 1989*†



Today more and more physicians are taking an increased interest in Demulen 1/35 and prescribing it for their patients on oral contraceptive (OC) therapy. In fact, recent statistics show that new prescriptions for Demulen 1/35 rose by 16% during the last 18 months (October 1987 to March 1989).† That's a significantly greater increase than any other monophasic OC,* and nearly twice the growth rate of the multiphasic class.‡



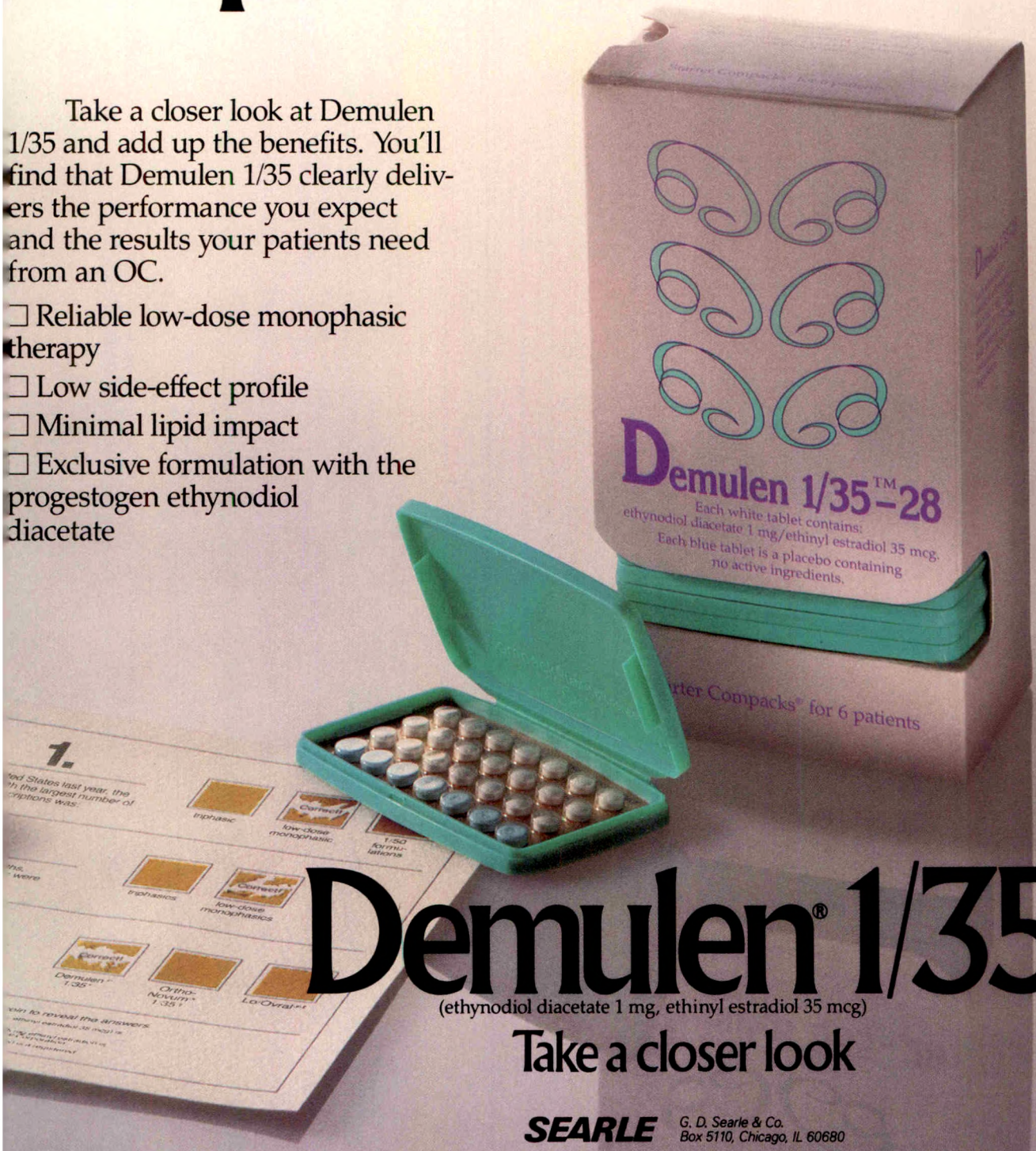
*Includes all monophasic products with 3% or greater market share.
†Pharmaceutical Data Services, Scottsdale, AZ.
‡Data derived from the change (by percentage) in the number of new prescriptions for all multiphasic products combined, from October 1987 to March 1989.

The physician should become familiar with the full prescribing information (labeling) for this product, which discusses reported risks, such as thromboembolic disorders (including strokes and heart attacks) and hepatic lesions, as well as the need to monitor patients for early symptoms of any diseases or medical conditions, so use can be discontinued where appropriate. A brief summary of the prescribing information accompanies this advertisement.

a growth phase—
a triphasic.

Take a closer look at Demulen 1/35 and add up the benefits. You'll find that Demulen 1/35 clearly delivers the performance you expect and the results your patients need from an OC.

- ❑ Reliable low-dose monophasic therapy
- ❑ Low side-effect profile
- ❑ Minimal lipid impact
- ❑ Exclusive formulation with the progestogen ethynodiol diacetate



Demulen® 1/35

(ethynodiol diacetate 1 mg, ethinyl estradiol 35 mcg)

Take a closer look

SEARLE G. D. Searle & Co.
Box 5110, Chicago, IL 60680

© 1989, G.D. Searle & Co.

Please see brief summary of prescribing information on following page.

A89-A7450T

Demulen® 1/35

(ethynodiol diacetate 1 mg, ethinyl estradiol 35 mcg)

Demulen 1/35™ Each white tablet contains ethynodiol diacetate 1 mg and ethinyl estradiol 35 mcg. Each blue tablet in Demulen 1/35-28 is a placebo containing inert ingredients.

Demulen 1/50™ Each white tablet contains ethynodiol diacetate 1 mg and ethinyl estradiol 50 mcg. Each pink tablet in Demulen 1/50-28 is a placebo containing inert ingredients. **Indication and usage:** For prevention of pregnancy. Pregnancy rate for products with ≥35 mcg ethinyl estradiol or ≥50 mcg mestranol is ≤1/100 woman-years. With ≤35 mcg ethinyl estradiol, rate is slightly higher; with progestogen-only oral contraceptives (OCs), rate is about 3. Precise comparisons cannot be made. Rates for other methods have been published. *Dose-related Thromboembolic Risk*: Studies showed positive association between OC estrogen dose and thromboembolic risk. It is in keeping with good therapeutics to minimize estrogen exposure. Prescribe product containing least amount of estrogen compatible with acceptable pregnancy rate and patient acceptance. Start new users on ≤50 mcg estrogen. **Contraindications:** Thrombophlebitis, thromboembolic disorders, past history of deep vein thrombophlebitis or thromboembolic disorders; cerebrovascular disease, myocardial infarction or coronary artery disease, or past history of these; known or suspected breast carcinoma, estrogen-dependent neoplasia, or pregnancy; undiagnosed abnormal genital bleeding; past or present, benign or malignant liver tumors developing during use of estrogenic products. **Warnings:**

Cigarette-smoking increases risk of serious cardiovascular side effects from OC use. Risk increases with age and heavy smoking (15 or more cigarettes/day) and is quite marked in women over 35. OC users should be strongly advised not to smoke. OCs are associated with increased risk of several serious conditions including venous and arterial thromboembolism, thrombotic and hemorrhagic stroke, myocardial infarction, visual disorders, hepatic tumors, gallbladder disease, hypertension, fetal abnormalities. Practitioners should be familiar with the available information relating to these and other risks.

1. *Thromboembolic disorders and other vascular problems*: Increased risk of thromboembolic and thrombotic disease associated with OCs is well established. A British study showed increased risk for fatal venous thromboembolism (TE). British and U.S. studies showed increased risk for nonfatal disease. U.S. studies showed increased risk for stroke. OC users were 2-11 times more likely to manifest these diseases without evident cause. In a study of idiopathic deep vein thrombosis and pulmonary embolism (PE), projected hospitalization rates for ages 16-40 were 47/year/100,000 users and 5 for nonusers. Overall excess mortality from PE or stroke was 1.3-3.4 deaths/year/100,000 users and increased with age. *Cerebrovascular Disorders*: In a study of stroke with and without predispositions, risk of hemorrhagic stroke was 2 times greater and thrombotic stroke 4.0-9.5 times greater in users. Mortality trends in 21 countries indicate that changes in cardiovascular mortality were associated with changes in OC prevalence. The Royal College of GPs (RCGP) reported on its prospective study: "A statistically significant higher rate of reporting of cerebrovascular accidents in Takers is evident, but the numbers are too small to justify an estimation of the degree of risk." A 1981 analysis showed a 4-fold increased mortality in users from circulatory diseases, mainly myocardial infarction (MI) and hemorrhagic stroke. Excess mortality was associated with age and smoking. A 1983 analysis showed elevated cerebrovascular disease, increasing with use to 8 yrs, including cerebral thrombosis or embolism and TIA. Peripheral arterial disease and MI were increased. Women over 30 had more arterial disease, being greatest in smokers. Arterial case-fatality was 2-3 times greater for users who smoked. Family Planning Assoc. (FPA) found significant association between OC use and stroke; increased risk for venous thrombosis and PE was 4-fold and statistically significant. Mortality from nonrheumatic heart disease was greater in ever-users. In the Walnut Creek Study, subarachnoid hemorrhage risk was associated with smoking, age, and OC use. *Myocardial Infarction*: Increased risk of MI associated with OCs was reported in Britain, as previously suspected. The morbidity study found the greater the number of risk factors for coronary artery disease (smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk, regardless of OC use. OCs were an additional risk factor. Excess rate of fatal MI was 3.5/year/100,000 users aged 30-39 and 20 for ages 40-44. (These are based on British statistics with acute MI death rates 2-3 times less than the U.S. Similar estimates for the U.S. are 25.7 for users aged 30-39 with predispositions vs 1.5 without; for ages 40-44, 85.2 vs 5.1.) Excess hospitalization rate for nonfatal MI in Britain was 3.5/year/100,000 users aged 30-39 and 47 for ages 40-44. Smoking major predisposing condition to MI. In terms of relative risk, nonsmoking OC users are twice as likely to have fatal MI as nonsmoking nonusers. Smoking users have a 5-fold greater risk than nonsmoking users, but a 10-12-fold greater risk than nonsmoking nonusers. Amount of smoking is an important factor. In determining importance of these risks, give serious consideration to rates in table below. Importance of other predispositions cited above in determining relative and absolute risks has not been quantified; it is likely the same synergism exists, but perhaps to a lesser extent. U.S. studies similarly relate MI, OCs, smoking, and age.

Estimated Mortality Rate from Myocardial Infarction per 100,000 Women/Year

	Aged 30-39:	*Users*	*Nonusers*	*Aged 40-44:*	*Users*	*Nonusers*
All smokers		10.2	2.6		62.0	15.9
≥15 cigarettes daily		13.0	5.1		78.7	31.3
<15 cigarettes daily		4.7	0.9		28.6	5.7
Nonsmokers		1.8	1.2		10.7	7.4
Smokers and nonsmokers		5.4	1.9		32.8	11.7

Risk of Dose: Reports of TE after use of OCs with ≥50 mcg estrogen received by drug safety committees in Britain, Sweden, and Denmark were compared with distribution expected from market research sales estimates. A positive correlation was found between estrogen dose and reporting of TE, including coronary thrombosis, in excess of that predicted by sales. OCs with ≥100 mcg estrogen were associated with higher TE risk than those with 50-80 mcg. Estrogen quantity may not be the sole factor; influence of progestogens was not considered, which may be responsible for certain discrepancies in the data. No significant differences were noted between OCs containing same estrogen dose nor between ethinyl estradiol and mestranol. In Britain certain TE conditions were associated with progestogen or estrogen dose. In Sweden reports of TE decreased when higher estrogen doses were no longer prescribed. Studies on TE risk with progestogen-only OCs have not been done. Cases have been reported for these; they are not presumed free of risk. In a U.S. case-controlled study, relative risk of OC use 1 month before hospitalization for various types of TE was calculated. If relative estrogenic potency and possible influence of different progestogens are ignored, OCs may be divided into those with <100 mcg and those with ≥100 mcg estrogen. For all cases combined, larger doses were associated with only slightly higher risk; for idiopathic cases risk was doubled with larger doses, but confidence limits overlapped and differences were not significant. Apparently there was less increased risk for cases with predispositions. In FPA study no nonhemorrhagic strokes occurred with <50 mcg estrogen, vs 13 with higher doses. TE risk in users and nonusers increases with age. OCs have been considered an independent risk factor. *Persistence of Risk* after OC discontinuation was reported for circulatory disease, nonrheumatic heart disease, and cerebrovascular disease, including subarachnoid hemorrhage, cerebral thrombosis, and TIA. *Excess Mortality from Circulatory Diseases*: A U.K. prospective study estimated annual mortality from circulatory diseases for OC users and nonusers by age, smoking, and duration of use. Excess mortality was 23/100,000 users (2 and 10 for ages 25-34, 15 and 48 for ages 35-44, 41 and 179 for ages >44, for nonsmokers and smokers, respectively). Risk was significant only for smokers over 34. Most deaths were due to subarachnoid hemorrhage or ischemic heart disease. Risk for ever-users rose with parity. One study estimated risk of death with various contraceptive methods, each estimate including risk of method (eg, TE for OCs) plus risk due to pregnancy or abortion in cases of method failure. This latter risk varies with effectiveness. It concluded mortality with all methods is low and below that of childbirth, except for OC users over 40 who smoke. Heavy smokers have double the risk of smokers, light smokers about half. Mortality with OC use in nonsmokers over 40 is higher than with any other method in that age group. Lowest mortality is with condom or diaphragm backed up by early abortion. Risk of TE and thrombotic disease with OCs increases with age after 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia, and especially cigarette smoking. Based on current data, the chart gives gross estimates of risk of death from circulatory disorders associated with OCs:

Risk is: A—very high; B—high; C—moderate; D—low.	Age:	<30	30-39	≥40
Heavy smokers (≥15/day)		C	B	A
Light smokers (<15/day)		D	C	B
Nonsmokers, no predispositions		D	C,D	C
Nonsmokers, with predispositions		C	C,B	B,A

Be alert to earliest manifestations of TE and thrombotic disorders (eg, thrombophlebitis, PE, cerebrovascular insufficiency, coronary artery disease, MI, retinal thrombosis, mesenteric thrombosis). Should any be suspected, discontinue OCs immediately. A 4-7-fold increased risk of postsurgery TE was reported in users; if feasible discontinue OCs at least 4 weeks before surgery or in prolonged immobilization. Before resuming OCs, weigh risks of postsurgery TE complications against contraceptive needs. The RCGP prospective study reported higher incidence of superficial and deep vein thrombosis in users, the former correlated with progestogen dose. The data suggest varicose veins have little effect on development of deep vein thrombosis but substantially increase risk of superficial vein thrombosis, risk depending on severity of varicosities. **2.** *Ocular lesions*: Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OCs. Discontinue if there is unexplained, gradual or sudden, partial or complete loss of vision; proptosis or diplopia; papilledema; or evidence of retinal vascular lesions. Institute diagnostic and therapeutic measures. **3.** *Carcinoma*: Long-term estrogen in certain animals increases carcinomas and/or nonmalignant neoplasms, such as those of breast, uterus, cervix, vagina, ovary, liver, pituitary. Some synthetic progestogens, none currently in OCs, increase incidence of benign and malignant mammary nodules in dogs. There is evidence that estrogens increase risk of human endometrial carcinoma. In case-controlled studies, increased risk (2.2-13.9 times) associated endometrial carcinoma with prolonged estrogen for menopausal symptoms. Risk was independent of other known risk factors and depended on duration of use and dose. This is supported by increased endometrial cancer incidence since 1969 in areas with cancer-reporting systems, which may be related to rapidly expanding estrogen use. There is no evidence that natural estrogens are more or less hazardous than synthetics at equi-estrogenic doses. Of the first 30 case endometrial carcinoma in OC users under 40, those without predispositions (eg, irregular bleeding before OCs, polycy ovaries) occurred nearly always with no-longer-marketed sequential OCs. No statistical association is reported sugges increased endometrial cancer with combination or progestogen-only OCs, though individual cases were reported. Studies sl no increased breast cancer with OCs or estrogens. However, greater risk was noted in OC users with benign breast dise or long-term (2-4 years) use. One study found greater risk of breast cancer with >4 years OC use prior to first full-t pregnancy. Risk of breast cancer with menopausal estrogens rose and further increased with duration of follow-up. OC estrogen were related to breast cancer in connection with other risk factors. Decrease in benign breast tumors in OC user well known. Some studies suggested increased risk of cervical dysplasia, erosion, and carcinoma in long-term users. Cerv microglandular hyperplasia has been reported. In summary, there is no consistent evidence from human studies of increa cancer risk with OCs. Close surveillance is, however, essential. In undiagnosed persistent or recurrent abnormal vag bleeding, consider nonfunctional causes and take diagnostic measures to rule out malignancy. Carefully monitor users strong family history of breast cancer or with breast nodules, fibrocystic disease, recurrent cystic mastitis, abnor mammograms, or cervical dysplasia. **4.** *Hepatic adenomas, etc*, have been associated with OCs. In 1 study OC formulati with high "hormonal potency" were associated with higher risk, as was age over 30. Though benign, these lesions r rupture and cause fatal hemoperitoneum in short- or long-term users. Two studies related risk to duration of use, being m greater after ≥4 years. Long-term OC users have an estimated adenoma incidence of 3-4/year/100,000. Consider s lesions in users with abdominal pain, tenderness, mass, or shock. About 25% present with abdominal masses, up to with acute hemoperitoneum. Radiologic and lab studies may not be helpful; liver scans may show local defect. Hep arteriography or CAT may be useful. Hepatocellular carcinoma risk is increased 7-20 times in users for 8 or more years. duct cancer may occur with OC use. OCs are contraindicated in case of past or present, benign or malignant liver tum developing during use of estrogenic products. **5.** *Use in or immediately preceding pregnancy, birth defects, malignancy offspring*: Estrogens or progestogens during early pregnancy may seriously damage offspring. Females exposed in uterc DES and other nonsteroidal estrogens may have increased risk of vaginal or cervical cancer. Though OCs may not enha this risk, monitor such patients carefully. From 30-90% of DES-exposed women develop benign vaginal or cervical adeno which could be premalignant. DES-exposed daughters have more unfavorable pregnancy outcomes. Males so exposed develop urogenital and sperm defects. Other estrogens may mimic DES. Reports relate fetal exposure to female sex hormo and congenital anomalies, eg, VACTERL for vertebral, anal, cardiac, tracheoesophageal, renal, and limb defects. There appe preferential expression by males. There was a 4.7-fold increased risk of limb-reduction defects in infants exposed in uterc sex hormones (OCs, hormonal withdrawal pregnancy tests, or treatment for threatened abortion). Some exposures were short, being only a few days. Cardiovascular defects in children exposed to female hormones, including OCs, during e pregnancy occurred in 18.2/1,000 births, compared to 7.8 for those unexposed (P<.05). More twinning was reported conceptions shortly after OC discontinuance. Female sex hormones were used for threatened or habitual abortion, but th is considerable evidence estrogens are ineffective and no evidence progestogens are effective. Triploidy and possibly ot polyploidy may be increased in abortuses from women becoming pregnant soon post-OC. These almost always ab spontaneously. It is unknown if spontaneous abortion is increased soon after stopping OCs. Increased chromosome aberratic have been reported. Safety of OCs in pregnancy has not been demonstrated. Rule out pregnancy in using OCs and alwa consider it if withdrawal bleeding does not occur. If 2 consecutive periods are missed rule out pregnancy before continuii If user deviates from regimen, consider pregnancy at first missed period and withhold OCs. If pregnancy is confirmed, appr patient of potential fetal risks and discuss continuation of pregnancy. Users discontinuing for pregnancy should use alternat contraception for 3 months before attempting to conceive, though this is not based on precise information. Do not progestogen-only or progestogen-estrogen drugs as pregnancy tests. **6.** *Gallbladder disease* risk, surgically confirm appeared after 2 years' use and doubled after 4-5 years in 1 study; it appeared in 6-12 months' use in another. **7.** *Carbohydr and lipid metabolism*: Decreased glucose tolerance occurs in a significant number of users; carefully observe diabetic a prediabetic patients. Triglycerides, total phospholipids, and lipoproteins may increase. **8.** *Elevated blood pressure* (BP) occ in some users in a few months, and degree may relate to progestogen dose. Hypertension prevalence in users is not high in the first year, but increases with longer exposure; in the 5th year it is 2½—3 times the first year. Age is strongly correlat with hypertension in users. Those with history of elevated BP (hypertension), preexisting renal disease, history of toxemia elevated BP in pregnancy, familial tendency to hypertension or its consequences, or history of excessive weight gain or fl retention during menstrual cycles may be more likely to develop elevated BP on OCs and should be monitored closely. Ev though elevated BP may be in "normal" range, do not ignore implications. Follow closely, particularly those with other r factors for cardiovascular disease or stroke. High BP may or may not persist after stopping OCs. **9.** *Headache*: Onset exacerbation of migraine or development of headache of a new pattern that is recurrent, persistent, or severe requires stoppi OCs and evaluating cause. **10.** *Bleeding irregularities*: Breakthrough bleeding (BTB), spotting, and amenorrhea are frequ reasons for discontinuing. In BTB, as in all cases of irregular bleeding, consider nonfunctional causes. In undiagnos persistent or recurrent bleeding, rule out pregnancy or malignancy. If pathology is excluded, time or change to another prod may suffice. Change to a higher-estrogen OC only when necessary as this may increase thromboembolic risk. Those w history of oligomenorrhea or secondary amenorrhea or without established regular cycles may be anovulatory or amenorrh after discontinuing. Those with such problems should be so informed and other methods encouraged. Post-use anovulatic possibly prolonged, may occur in women without previous irregularities. Higher incidence of galactorrhea and of pituit tumors has been associated with postpill amenorrhea. One study reported 16-fold increased prevalence of pituitary prolact secreting tumors in patients with postpill amenorrhea when galactorrhea was present. **11.** *Ectopics*: In failures with combinati OCs, the ratio of ectopic to intrauterine pregnancies is no higher than in nonusers. There may be increased risk of ectopi after discontinuing. **12.** *Breast feeding*: OCs may decrease quantity and quality of milk. A small fraction of OC steroids appea in milk. Effects if any on child are unknown. If feasible defer OCs until after weaning. **13.** *Infertility* may occur after discontinui OCs. While impairment diminishes with time, there is still an appreciable difference in nulliparas aged 25-29 after 30 month difference is negligible after 48 months. In nullips aged 30-34 impairment persists to 72 months and is more severe for year users. In paras aged 25-34, difference is negligible after 30 months. **Precautions:** Conduct complete history and physic prior to OC use and annually thereafter. Include breasts, abdomen, and pelvis, with BP, Pap, and lab tests. Preexisting uteri leiomyomata may enlarge. Mental depression may occur; with positive history, observe carefully and discontinue if seriou recurrence. Fluid retention may occur; prescribe cautiously and monitor carefully in convulsive disorders, migraine, asthm or cardiac, hepatic, or renal dysfunction. History of jaundice in pregnancy increases risk of recurrence on OCs; obser carefully and discontinue if it occurs. Cholestatic jaundice occurred after OC and troleandomycin use. OC/cyclospori combination may be hepatotoxic. Steroids may be poorly metabolized in liver dysfunction and should be given cautious Tryptophan metabolism may be disturbed and result in pyridoxine deficiency; megaloblastic anemia has been reported. Seru folate may be depressed and may be exacerbated in post-OC pregnancy. Advise pathologist of OC use when submittir specimens. Endocrine and liver function tests may be altered; repeat abnormal tests after stopping for 2 months. These ha been observed: increased BSP retention and other abnormalities in liver function tests; increased prothrombin and coagulatic factors VII, VIII, IX, X; decreased antithrombin III, increased platelet aggregability; increased thyroid-binding globulin leadir to increased circulating total thyroid hormone (PBI, T_4); decreased T_3 uptake, unaltered free T_4, decreased pregnanedi excretion; reduced metyrapone response; increased blood transcortin, corticosteroid, triglyceride, phospholipid, reduced seru folate; impaired glucose tolerance; altered plasma trace minerals. Influence of prolonged OC use on pituitary, ovarian, adren hepatic, or uterine function, or on immune response not established. OCs may mask onset of climacteric. Cervical chlamydi prevalence is increased; do not assume OC protects against chlamydial PID. *Information for patients*. See patient labelin *Drug interactions*. OC effectiveness may be decreased and more BTB occur with rifampin, isoniazid, ampicillin, neomyci penicillin V, tetracycline, chloramphenicol, sulfonamides, nitrofurantoin, griseofulvin, barbiturates, phenytoin, carbamazepin primidone, phenylbutazone, analgesics, tranquilizers, antimigraines. OCs may alter effectiveness of oral anticoagulant anticonvulsants, tranquilizers, tricyclic antidepressants, antihypertensives, theophylline, caffeine, vitamins, hypoglycemic clofibrate, glucocorticoids, acetaminophen. **Adverse reactions:** Increased risk of the following serious adverse reactions h been associated with OCs: thrombophlebitis and thrombosis; pulmonary embolism; arterial thromboembolism; Raynaud disease, myocardial infarction; coronary thrombosis; cerebral thrombosis; cerebral hemorrhage; hypertension; gallbladd disease; Budd-Chiari syndrome; benign adenomas and other hepatic lesions, with or without intra-abdominal bleeding, liv cancer, congenital abnormalities. There is evidence of association between the following and OCs, though confirmatory studi have not been done: mesenteric thrombosis; neuro-ocular lesions, eg, retinal thrombosis and optic neuritis. The following a believed drug related: nausea, vomiting, gastrointestinal symptoms, BTB, spotting, change in menstrual flow, dysmenorrhe amenorrhea during and after use, infertility post-OC, edema, chloasma or melasma, which may persist post-OC, brea changes (tenderness, enlargement, secretion), weight changes, change in cervical erosion and secretion, endocervic hyperplasia, lactation decrease, cholestatic jaundice, migraine, enlarged uterine leiomyomata, allergic rash, mental depressio reduced carbohydrate tolerance, vaginal candidiasis, change in corneal curvature (steepening), intolerance to contact lense Association not confirmed or refuted for: premenstrual-like syndrome, cataracts, libido changes, chorea, appetite change cystitis-like syndrome, headache, paresthesia, nervousness, dizziness, auditory disturbances, rhinitis, fatigue, backach hirsutism, scalp hair loss, erythema multiforme or nodosum, hemorrhagic eruption, herpes gestationis, hemolytic uremi malignant hypertension, itching, vaginitis, porphyria, impaired renal function, acute renal failure, sometimes irreversible, PVC abnormal EKG, pulmonary hypertension, TTP, anemia, pancreatitis, hepatitis, colitis, Crohn's disease, gingivitis, dry socke lupus erythematosus, rheumatoid arthritis, pituitary tumors (eg, adenoma) with amenorrhea and/or galactorrhea after us malignant melanoma, endometrial, cervical, breast, and bile duct carcinoma. **Acute overdosage:** Serious ill effects have n been reported after large acute doses of OCs in young children. Nausea and withdrawal bleeding might occur. **Important not Complete prescribing information should be consulted prior to use and is available on request.**

9/21/87 • P87-A4830

SEARLE *Searle & Co. San Juan, PR 00936*

Address medical inquiries to: G.D. Searle & Co. Medical & Scientific Information Department 4901 Searle Parkway, Skokie, IL 60077

Phase II trial of ifosfamide and mesna in mixed mesodermal tumors of the uterus (A Gynecologic Oncology Group Study*)

Gregory P. Sutton, MD,[a] John A. Blessing, PhD,[b] Neil Rosenshein, MD,[c] Guy Photopulos, MD,[d] and Philip J. DiSaia, MD[e]

Indianapolis, Indiana, Buffalo, New York, Baltimore, Maryland, Memphis, Tennessee, and Orange, California

A phase II trial of ifosfamide (isophosphamide, NSC 109724) and mesna (2-mercaptoethane sodium sulfonate, NSC 113891) in women with advanced or recurrent mixed mullerian tumors of the uterus was conducted by the Gynecologic Oncology Group. The starting dose of ifosfamide was 1.5 gm/m^2 daily, intravenously, for 5 days. The starting dose of ifosfamide was reduced 1.2 gm/m^2 daily in patients who had received prior radiotherapy. Mesna was given intravenously immediately and at 4 and 8 hours after the administration of ifosfamide. Each mesna dose was 20% of the total daily dose of ifosfamide. Twenty-nine patients are evaluable for toxicity, and 28 patients are evaluable for response. Twenty-one patients had received prior abdominal hysterectomy, and eight patients had prior radiotherapy. Thirteen tumors were homologous and 15 heterologous. Gynecologic Oncology Group grade 3 or 4 granulocytopenia occurred in seven (25%) patients and two (7.1%) had grade 3 or 4 thrombocytopenia. Two patients (7.1%) had grade 3 or 4 neurotoxicity. One patient experienced lethargy and confusion that responded to discontinuation of the ifosfamide. A second patient developed progressive cerebellar dysfunction, left hemiparesis, and coma. This patient died after 3 days of therapy. Complete responses were seen in five (17.9%) patients and partial responses occurred in four (14.3%) patients for a total response rate of 32.2%. These results indicate that ifosfamide is an unusually active drug in patients with advanced or recurrent mixed mullerian tumors of the uterus. Studies with combination regimens incorporating ifosfamide are warranted. The toxicity of ifosfamide in Gynecologic Oncology Group studies is being evaluated retrospectively. (Am J Obstet Gynecol 1989;161:309-12.)

Key words: Ifosfamide, mesna, mullerian tumors

Although ifosfamide was first synthesized in Germany in the mid-1960s, severe urothelial toxicity prevented widespread evaluation of the drug until the uroprotector mesna (2-mercaptoethane sodium sulfonate) became available. Since the introduction of mesna, ifosfamide has been found to be highly active in the treatment of malignant lymphomas and pancreatic, testicular, and ovarian carcinomas.

With the exception of doxorubicin, ifosfamide possesses unsurpassed activity in the treatment of soft tissue sarcomas. Bramwell et al.[1] compared cyclophosphamide with ifosfamide in advanced soft tissue sarcomas. The response rate in previously untreated patients was 25% with ifosfamide and 13% with cyclophosphamide. Other authors[2] have confirmed response rates of 23% to 65% in previously treated patients with soft tissue sarcomas. Ifosfamide is also active in osteosarcoma and Ewing's sarcoma.

Because of these promising results, the Gynecologic Oncology Group elected to initiate a phase II trial of ifosfamide and mesna in the treatment of patients with uterine sarcomas refractory to surgery and radiotherapy but untreated with previous chemotherapeutic agents. The results of ifosfamide therapy in mixed mullerian tumors are reported herein; results in other uterine sarcomas are pending at this writing.

Methods

Patients with histologically proved advanced or recurrent mixed mesodermal (malignant mixed mullerian) tumors of the uterus not amenable to curative ther-

From the Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, Indiana University Medical School,[a] Roswell Park Memorial Institute,[b] The Johns Hopkins Hospital and Johns Hopkins Oncology Center,[c] the Division of Gynecologic Oncology, University of Tennessee at Memphis,[d] and the Department of Obstetrics and Gynecology, University of California, Irvine Cancer Center.[e]

Received for publication November 6, 1988; revised February 1, 1989; accepted February 17, 1989.

Reprint requests: Gregory Sutton, MD, Department of Obstetrics and Gynecology, Indiana University Hospital, 926 W. Michigan, Indianapolis, IN 46223.

**The following are participating institutions and the National Cancer Institute grants supporting this study: University of Alabama at Birmingham (CA 12484), Duke University Medical Center (CA 12534), University of Mississippi Medical Center (CA 13633), University of Miami School of Medicine (CA 37234), The Milton S. Hershey School of Medicine of Pennsylvania State University (CA 16386), Georgetown University Hospital (CA 16938), Indiana University Medical Center (CA 21720), Bowman Gray School of Medicine of Wake Forest University (CA 21946), The Albany Medical College of Union University (unfunded), University of California Medical Center at Irvine (CA 23765), Tufts New England Medical Center (CA 37569), Illinois Cancer Council (CA 27806), Eastern Virginia Medical School (CA 40296), The Johns Hopkins Oncology Center (unfunded), and State University of New York at Stony Brook (unfunded).*

Table I. Clinical response to ifosfamide

	Response				
	CR	*PR*	*SD*	*Prog*	*Total*
GOG performance status					
0	4	3	2	3	12
1	0	0	5	6	11
2	1	1	3	0	5
Prior pelvic radiotherapy					
Yes	1	0	4	3	8
No	4	4	6	6	20
Prior hysterectomy					
Yes	5	2	7	7	21
No	0	2	3	2	7

CR, Complete response; *PR*, partial response; *SD*, stable disease; *Prog*, progressive disease; *GOG*, Gynecology Oncology Group.

Table II. Response by hematologic toxicity

	Response				
Adverse effect	*CR*	*PR*	*SD*	*Prog*	*Total*
WBC: GOG grade					
0	0	0	1	4	5
1	0	0	0	1	1
2	3	1	3	2	9
3	2	3	1	0	6
4	0	0	5	2	7
Total	5	4	10	9	28
Platelet GOG grade					
0	5	2	7	7	21
1	0	1	2	1	4
2	0	1	0	0	1
3	0	0	1	0	1
4	0	0	0	1	1
Total	5	4	10	9	28

CR, Complete response; *PR*, partial response; *SD*, stable disease; *Prog*, progressive disease; *WBC*, white blood count; *GOG*, Gynecology Oncology Group.

apy but unexposed to prior chemotherapeutic agents were eligible for this study. Additional eligibility criteria included measurable disease by physical examination or medical imaging technique. Ascites and pleural effusions were not considered measurable disease. Also required were Gynecologic Oncology Group performance status 2 or better (Karnofsky 50-100), an interval of at least 3 weeks since any prior tumor-oriented therapy, absence of significant infection, and recovery from recent surgery or radiotherapy. Adequate hematologic (white blood count $\geq 3000/\mu l$; platelet count $\geq 100{,}000/\mu l$), hepatic (serum bilirubin, aspartate aminotransferase, and alkaline phosphatase $\leq$two times normal), and renal (serum creatinine ≤ 2.0 mg/dl or creatinine clearance ≥ 50 ml/min) function tests. Informed consent was obtained from all subjects, and none was treated until the study was approved by the Human Investigations Committee of the participating institution.

Criteria for exclusion were absence of measurable disease, prior chemotherapy, Gynecologic Oncology Group performance status of ≥ 3, presence of a second malignancy excluding squamous or basal skin lesions, or the presence of microhematuria in excess of the institutional lowest normal range.

Ifosfamide was administered intravenously daily, for 5 days, every 4 weeks, at a dose of 1.5 gm/m^2. This dose was reduced to 1.2 gm/m^2 in patients with prior radiotherapy. Mesna (300 mg/m^2) was given intravenously every 4 hours, for three doses, after ifosfamide administration.

History and physical examination with assessment of evaluable lesions, Gynecologic Oncology Group performance status, blood chemistries, blood urea nitrogen, creatinine, and microscopic urinalysis were performed before each cycle of therapy, and complete blood count, differential, and platelet counts were obtained weekly. Abdominal pelvic computed tomography scans were used unless tumor parameters were assessed by examination or chest radiograph.

Drug administration was delayed after hematologic toxicity until white blood count was $>3000/\mu l$, platelet count was $>100{,}000/\mu l$, and granulocyte count was $>1500/\mu l$. Patients were considered evaluable for toxicity if just one course of therapy was given and evaluable for response after a single course of therapy if there was clearcut progression. In other cases patients remained in the study until disease progression was noted or adverse effects prevented further therapy. Response duration was calculated from the date of response until documentation of disease progression. Standard Gynecologic Oncology Group response criteria were used.[3]

Results

Between May 1986 and March 1988, 30 patients were entered into the study. One never received therapy, which left 29 patients evaluable for toxicity. One patient died during the first course of therapy, leaving 28 patients evaluable for response. The median age of evaluable patients was 68 years (range, 36 to 86 years). Twenty-one patients had undergone prior hysterectomy, one had laparotomy only, and six had no surgery. Eight patients had received prior pelvic radiotherapy. Fifteen patients had heterologous tumors, and 13 had tumors that contained homologous elements.

Response. Table I outlines the clinical results of therapy with ifosfamide. Five patients (17.9%) had complete responses and four (14.3%) had partial responses, for an overall response rate of 32.2%. In addition, 10 patients, or 35.7%, had stable disease. Response duration was 1.4+ to 8.6 months, with a median response duration of 3.8 months. Three patients are progression

free at 1.4, 3.0, and 4.8 months. Response by Gynecologic Oncology Group performance status is shown in Table I. Responses were equally divided between heterologous and homologous tumors. Sites of responding lesions included vagina (four), pelvis (two), lung (two), and suprapubic skin (one). Median age for complete responders was 72 years (range, 36 to 76 years) and 66½ years (range, 63 to 76 years) for partial responders. Only one response occurred in a patient receiving prior radiotherapy; the other eight responders had not received radiotherapy. Complete responders received a median of six courses of chemotherapy (range, five to 16 courses), and partial responders were treated with a median of 6½ courses (range, four to 10 courses). Responses by adverse hematologic effects are detailed in Table II.

Toxicity. Twenty patients were treated at an initial dose of 1.5 gm/m² for 5 days; the remainder had an initial dose of 1.2 gm/m² because of prior radiotherapy. Thirteen patients (46.4%) experienced Gynecologic Oncology Group grade 3 or 4 leukocytopenia (white blood count ≤200/μl), and seven (25%) had granulocyte counts of <1000/μl. The median white blood count nadir for all patients with leukocytopenia was 1700/μl (range, 200 to 3400). White blood count and platelet adverse effects are outlined in Table II. Two patients (7.1%) developed thrombocytopenia of <50,000/μl. The median nadir for patients with thrombocytopenia was 117,000/μl (range, 4000 to 147,000 μl). No patient experienced granulocytopenic fever or thrombocytopenic hemorrhage.

Other adverse effects are listed in Table III. One patient each developed grade 3 or 4 neurotoxicity. The first patient became lethargic and disoriented but responded within 24 hours to the withdrawal of ifosfamide and mesna. A second patient developed cerebellar dysfunction, left hemiparesis, progressive coma, and death during the first course of therapy with ifosfamide and mesna after 3 days. Neurologic evaluation, including a brain computed tomography scan and hepatic and renal function studies, was normal before the patient's death. Lumbar puncture was not performed, and permission for autopsy was not granted.

Hematuria was not observed in this patient population despite prior radiotherapy in many subjects. There was no grade 3 or 4 renal toxicity.

Table III. Other adverse effects

Adverse effect	*Grade* 0	1	2	3	4	*Total*
Leukopenia	6	1	9	6	7	29
Thrombocytopenia	22	4	1	1	1	29
Granulocytopenia	20	1	1	3	4	29
Gastrointestinal	10	4	15	0	0	29
Dermatologic	18	0	1	10	0	29
Renal	16	11	2	0	0	29
Cardiac	26	2	0	1	0	29
Neurologic (central)	24	2	0	2	1	29
Pulmonary	27	1	0	1	0	29
Fever	27	1	0	1	0	29
Anemia	27	1	1	0	0	29

Comment

Although hysterectomy may be curative in a small proportion of patients with early mixed mullerian tumors of the uterus, therapy of these sarcomas has been largely unsuccessful. Reported 5-year survival rates in Stage I disease seldom exceed 50%.[4] Although some authors[5] have recommended adjunctive pelvic radiotherapy in early disease, the benefit of this type of treatment remains controversial.[6] Adjunctive chemotherapy is also unproved; patients with Stage I disease treated with doxorubicin after surgery had no better survival than control subjects undergoing surgery alone in a study conducted by the Gynecologic Oncology Group.[7]

In advanced or recurrent disease, treatment with chemotherapy may produce responses, but they are rarely complete or of long duration. Evaluation of chemotherapeutic agents in mixed mullerian tumors has been hampered because most studies fail to differentiate these neoplasms from other uterine or soft tissue sarcomas. The Gynecologic Oncology Group first demonstrated the differential sensitivity of uterine sarcomas to drug therapy.[8] In this report there was a response rate of 25% in patients having uterine leiomyosarcomas treated with doxorubicin, whereas subjects with mixed mesodermal sarcomas demonstrated only a 15% response rate to the same drug. The addition of dacarbazine to doxorubicin yielded no improvement in either group of patients. Cisplatin has definite activity as a second-line agent in refractory mixed mullerian tumors.

Thigpen et al.[9] reported an 18% response rate in previously treated patients given a dose of 50 μg/m² of cisplatin intravenously every 3 weeks. Two patients developed complete responses and had progression-free intervals of 14 and 16 months. No other single chemotherapeutic agent studied by the Gynecologic Oncology Group has produced results superior to those of cisplatin or ifosfamide.

Combination therapy may produce results more favorable than those obtained with single drugs. Piver et al.[10] reported a 23% response rate, with 12% complete responses, with a combination of cyclophosphamide, vincristine, doxorubicin, and dacarbazine. Hannigan et al.[11] also reported a 29% response rate (13.3% complete) in patients with uterine sarcomas treated with cyclophosphamide, dactinomycin (Actinomycin D), and vincristine. However, there is no current evidence from randomized trials that combination therapy

offers an advantage as single drug therapy in the management of uterine sarcomas.

Ifosfamide-based combination regimens are beginning to show promise in the management of soft tissue sarcomas. This drug has been combined effectively with doxorubicin in phase II studies. Schutte et al.[12] reported a 36% response rate, including 6.3% complete responses in 126 evaluable patients treated with a 1-day regimen of ifosfamide and doxorubicin. Sledge et al.[13] reported that 16 of 39 responders (41%) used a similar regimen. Elias et al.[14] added dacarbazine to these two drugs and observed responses in 13 of 29 patients (45%), with 3 patients or 10% having complete responses. The Gynecologic Oncology Group is currently performing a phase III study using ifosfamide with or without cisplatin in the therapy of advanced uterine sarcomas.

The incidence of neurotoxicity (7.1%) in the present study is lower than that observed by others but may have been underreported in the early portion of this study. Mild neurotoxicity consisting of a decreased level of arousal and somnolence was difficult to detect in the presence of lorazepam, and antiemetic was used frequently during this trial. The person who had progressive confusion and stupor responded rapidly to the discontinuation of ifosfamide. Although lethal toxicity of the central nervous system has been reported after single doses of ifosfamide >3 gm/m^2, this complication is previously unreported in patients receiving doses fractionated over 5 days. Moreover, the pattern of neurotoxicity in the one death in the present study is atypical for adult patients. Most often a decreased level of arousal is noted, although other central nervous system manifestations, such as confusion, hallucinations, forgetfulness, cerebellar signs, weakness, incontinence, and seizures, have been reported.[15] Hemiparesis as an isolated neurologic finding is unreported to the best of the authors' knowledge but may be attributable to exposure to ifosfamide.

The neurotoxicity of ifosfamide is not well understood but may be related to the accumulation of the metabolite chloracetaldehyde. Oxidation of ifosfamide may release 10-fold more chloracetaldehyde than metabolism of similar amounts of cyclophosphamide. This metabolite is related to both acetaldehyde and trichloracetaldehyde, breakdown products of ethanol and chloral hydrate, respectively. Differences in neurotoxicity observed between patients or reported in various trials may be attributable to diverse rates of osfamide administration, variations in hepatic or renal clearance of chloracetaldehyde, or differential sensitivity of the central nervous system to this compound.[16] The Gynecologic Oncology Group is presently reviewing neurologic toxicity among patients treated in a variety of ifosfamide trials to identify factors that may predispose to this side effect of therapy. During treatment with ifosfamide, an effort should be made to minimize concomitant medication with active agents of the central nervous system, such as antiemetics, hypnotics, or antihistamines.

REFERENCES

1. Bramwell VH, Mouridsen HT, Santoro A, et al. Cyclophosphamide versus ifosfamide: final report of a randomized phase II trial in adult soft tissue sarcoma. Eur J Cancer Clin Oncol 1987;23:311-21.
2. Kroner T, Marti C, Cavalli F. High dose ifosfamide in advanced osteosarcoma and soft tissue sarcoma: preliminary results of two prospective studies. Presented at the Thirteenth International Congress of Chemotherapy, Vienna, Austria, Aug. 13, 1983.
3. Blessing JA. Design, analysis and interpretation of chemotherapy trials in gynecologic cancer. In: Deppe G, ed. Chemotherapy of gynecologic cancer. New York: Alan R Liss, 1984:49-83.
4. Kahanpaa KV, Wahlstrom T, Grohn P, et al. Sarcomas of the uterus: a clinicopathologic study of 119 patients. Obstet Gynecol 1986;67:417-24.
5. Spanos WJ, Wharton JT, Gomez L, et al. Malignant mixed mullerian tumors of the uterus. Cancer 1984;53:311-6.
6. Hornback NB, Omura G, Major FJ. Observations on the use of adjuvant radiation therapy in patients with Stage I and II uterine sarcoma. Int J Radiat Oncol Biol Phys 1986;12:2127-30.
7. Omura GA, Blessing JA, Major FJ, et al. A randomized clinical trial of adjuvant Adriamycin in uterine sarcomas: a Gynecologic Oncology Group Study. J Clin Oncol 1985;3:1240-5.
8. Omura GA, Major FJ, Blessing JA, et al. A randomized study of Adriamycin with and without dimethyltriazenoimidazole carboxamide in advanced uterine sarcoma. Cancer 1983;52:626-32.
9. Thigpen JT, Blessing JA, Orr JW. Phase II trial of cisplatin in the treatment of patients with advanced or recurrent mixed mesodermal sarcomas of the uterus. Cancer Treat Rep 1986;70:271-4.
10. Piver MS, DeEulis TG, Lele SB, et al. Cyclophosphamide, vincristine, Adriamycin and dimethyltriazenoimidazolecarboxamide (CYVADIC) for sarcomas of the female genital tract. Gynecol Oncol 1982;14:319-23.
11. Hannigan EV, Freedman RS, Elder KW, et al. Treatment of advanced uterine sarcoma with vincristine, Actinomycin-D, and cyclophosphamide. Gynecol Oncol 1983;15:224-9.
12. Schutte J, Dombernowsky P, Santoro A, et al. Adriamycin and ifosfamide, a new effective combination in advanced soft tissue sarcoma; preliminary report of a phase II study of the EORTC soft tissue and bone sarcoma group. Proc Am Soc Clin Oncol 1986;5:145.
13. Sledge G, Loehrer R, Brenner D, et al. Treatment of advanced soft tissue sarcomas with ifosfamide and Adriamycin. Proc Am Soc Clin Oncol 1988;7:1060.
14. Elias AD, Antman K, Ryan L. Doxorubicin, ifosfamide, and DTIC with mesna uroprotection for advanced untreated sarcoma. Proc Am Soc Clin Oncol 1986;5:134.
15. Pratt CB, Green AA, Horowitz ME, et al. Central nervous system toxicity following the treatment of pediatric patients with ifosfamide/mesna. J Clin Oncol 1986;4:1253-61.
16. Goren MP, Wright RK, Pratt CB, et al. Dechloroethylation of ifosfamide and neurotoxicity. Lancet 1986;2:1219-20.

Ceramide lactoside in amniotic fluid: High concentration in chorioamnionitis and in preterm labor

Mikko Hallman, MD, DrMedSci, Kristiina Bry, MD, and Olli Pitkänen, MD
Helsinki, Finland

The mechanisms responsible for the onset and progression of preterm labor are poorly understood. In the present study a total of 115 amniotic fluid specimens were analyzed for a lipid that has not previously been detected in amniotic fluid. This glycolipid was identified as ceramide lactoside. It was found in two-dimensional thin-layer chromatograms for evaluation of lung maturity and quantified by gas chromatography. Ceramide lactoside concentrations in amniotic fluid were low in spontaneous labor at term (1.7 ± 0.7 nmol/ml) and in pregnancies that were not associated with spontaneous preterm labor (1.4 ± 0.6 nmol/ml). The concentrations were high in chorioamnionitis with signs of infection (11.8 ± 5.8 nmol/ml) and in preterm labor without clinical signs of chorioamnionitis (5.4 ± 4.0 nmol/ml). A high ceramide lactoside (≥5 nmol/ml) predicted chorioamnionitis with signs of infection at a sensitivity and a specificity of 94% and 95%, respectively. A moderately high ceramide lactoside concentration (≥2.5 nmol/ml) predicted spontaneous preterm labor: sensitivity, 82%; specificity, 95%. Little, if any, ceramide lactoside was present in urine, vernix, normal fetal membranes, or lung effluent, whereas this glycolipid was present in large amounts in granulocytes and in inflamed fetal membranes. We propose that phagocytosing granulocytes release ceramide lactoside into amniotic fluid. (AM J OBSTET GYNECOL 1989;161:313-8.)

Key words: Preterm labor, diagnosis of chorioamnionitis, amniotic fluid

Preterm birth is the primary cause of neonatal death and morbidity. The identity of the signals involved in the onset and progression of normal and premature labor is still controversial.[1-3] Our deficient understanding of the pathogenetic mechanisms involved has inhibited the development of effective methods to identify high-risk cases or to prevent and treat premature labor. According to the current hypothesis, mobilization of arachidonic acid and synthesis of prostaglandins in fetal membranes play important roles in initiation, propagation, or modulation of human parturition.[4]

Preterm labor is often associated with infection or chorioamnionitis. Bacterial phospholipases have been suggested to release arachidonic acid from the fetal membrane–associated phospholipids and so promote preterm labor.[5] In theory, the inflammatory cells within the fetal membranes could help to precipitate premature labor. During inflammation, products of polymorphonuclear leukocytes accumulate in the transcellular space (for instance, in pulmonary alveolar fluid).[6] This may also apply to the amniotic fluid.

The fetal lung is a source of amniotic fluid phospholipids, which serve as reliable indices of lung maturity. Additionally, amniotic fluid lipids may be derived from fetal membranes, skin, blood, and excretions or from cells in the amniotic fluid. We have recovered amniotic fluid during preterm labor for evaluation of fetal lung maturity. Surprisingly, most of these specimens contained large quantities of a lipid that was identified as ceramide lactoside. We additionally describe the possible source and the concentrations of ceramide lactoside in amniotic fluid in normal and complicated pregnancies.

Patients and methods

A total of 115 amniotic fluid specimens were studied. In 80 cases the specimen was obtained by transabdominal amniocentesis. Fourteen samples were recovered during speculum examination of patients with rupture of the fetal membranes, 17 by amniocentesis during cesarean section, and 4 by amniocentesis after delivery of a fetus with intact membranes. The indications for transabdominal amniocentesis were preeclampsia (13 cases), isoimmunization (9 cases), maternal diabetes (32 cases), premature labor (5 cases), suspected fetal abnormality (12 cases), premature rupture of the fetal membranes (5 cases), and unknown gestational age that was verified by examination of the newborn (4 cases). None of the specimens were visibly contaminated with blood or meconium. The amniotic fluids were centrifuged for 10 minutes at 150 *g*, and the supernatant stored at −20° C before the analysis.

The diagnosis of preterm labor (<37 weeks) was

From the Department of Pediatrics, University of Helsinki, and the Departments of Obstetrics and Gynecology, University of Helsinki.
Supported by funds from the Sigrid Jusélius Foundation, the Foundation for Pediatric Research, and the Finnish Academy of Sciences.
Received for publication February 9, 1988; revised December 13, 1988; accepted February 8, 1989.
Reprint requests: Mikko Hallman, MD, DrMedSci, Department of Pediatrics, UC Irvine Medical Center, 101 City Dr. South, Bldg. 29A, Orange, CA 92668.

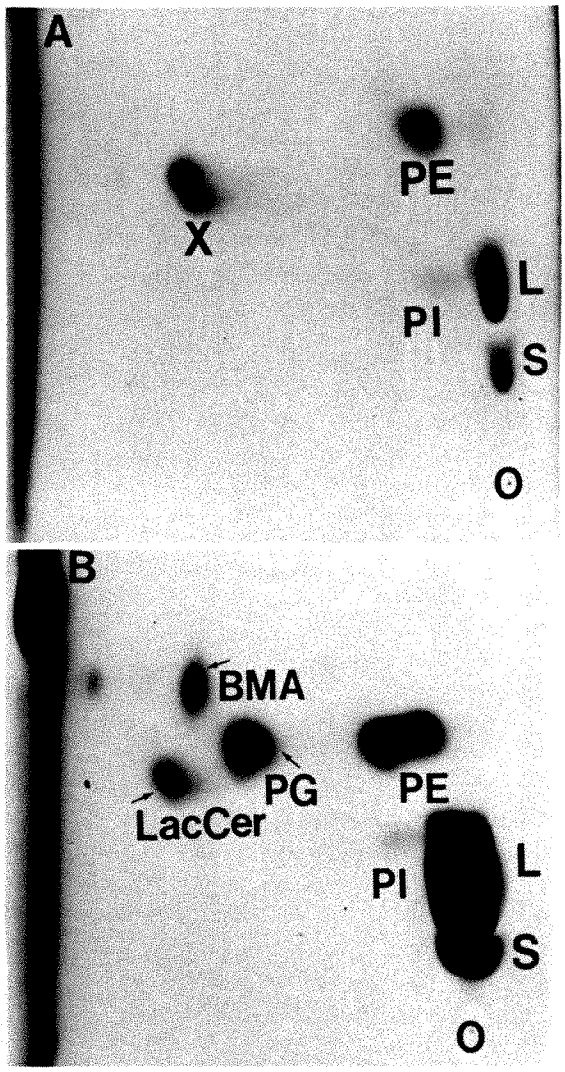


Fig. 1. Thin-layer chromatogram of amniotic fluid illustrating various phospholipids and ceramide lactoside *(LacCer)*. Plates were developed in two directions with specific solvents[10] and stained with iodine. **A,** Chorioamnionitis. *X* was identified as ceramide lactoside. **B,** Normal term pregnancy, ceramide lactoside standard added. *L*, Lecithin; *S*, sphingomyelin; *PI*, phosphatidylinositol; *PE*, phosphatidylethanolamine; *PG*, phosphatidylglycerol, *BMA*, *bis*-(monoacylglycerol) phosphate; *O*, origin.

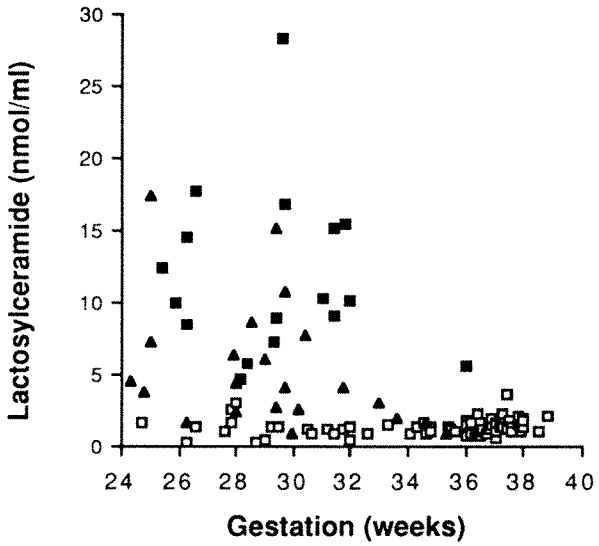


Fig. 2. Concentration of ceramide lactoside in the amniotic fluid. *Open squares*, No preterm labor; *solid triangles*, preterm labor without clinical signs of chorioamnionitis; *solid squares*, chorioamnionitis with signs of infection and preterm labor. None had symptomatic chorioamnionitis without labor.

made when the following symptoms either were present (35 cases)[7] or became evident within 3 days (4 cases): (1) uterine contractions (4 times per 20 minutes or 8 times per 60 minutes) and cervical changes (effacement of ≥80% or dilatation of ≥2 cm) or (2) uterine contractions and rupture of the fetal membranes before 37 weeks. In each case the preterm delivery took place within 0 to 10 days after the specimen was taken.

Chorioamnionitis was suspected when there was rupture of the fetal membranes, when there were signs of infection (maternal fever, elevated C-reactive protein level, leukocytosis, positive maternal cultures), or when the fetal membranes were malodorous and opaque. In these cases the placentas and fetal membranes were examined microscopically by a pathologist. Chorioamnionitis was diagnosed when the fetal membranes, intervillous space, or chorionic plate showed clusters of polymorphonuclear leukocytes (>5 per high-power field) migrating into the chorionic plate on the fetal side of the intervillous space.

The presence of ceramide lactoside in amniotic fluid was verified essentially as described previously.[8] The main features are pointed out briefly here. The lipid was isolated by thin-layer chromatography as a doublet characteristic of ceramide lactoside from extraneural tissues. It contained equimolar quantities of galactose and glucose. According to gas chromatographic analysis,[9] the ratio of sphingosine to glucose and galactose was 1.00:1.05:1.09 (n = 4).

For quantification of ceramide lactoside, the total lipids were isolated by chloroform-methanol extraction, and the glycolipids were "separated" on high-performance thin-layer chromatography plates (Merck, Darmstadt). The chromatograms were developed in one direction with chloroform-methanol–2.5 mol/L ammonium hydroxide, 60:35:8 (vol/vol/vol). Alternatively, we used two-dimensional thin-layer chromatography, as described previously for fetal lung maturity.[10] The lipids were visualized with iodine. Hexose-containing lipids were stained with orcinol in sulfuric acid, and those containing neuraminic acid with resorcinol in hydrochloric acid. The area of the chromatogram that corresponded to the ceramide lactoside standard was stained with iodine and orcinol. It was scraped

Table I. Concentrations of ceramide lactoside in amniotic fluid

Condition	*N*	*Ceramide lactoside (nmol/ml)*	*Gestation (wk)*
Preterm gestation, no labor	52	1.6 ± 0.9	31.6 ± 3.8
Preterm labor, no chorioamnionitis, with signs of infection	22	5.4 ± 4.0	29.5 ± 3.1
Preterm labor, symptomatic chorioamnionitis	17	11.8 ± 5.8	29.3 ± 3.8
Term gestation, no labor	20	1.5 ± 0.6	37.9 ± 0.6
Term labor	4	1.7 ± 0.7	38.2 ± 1.1

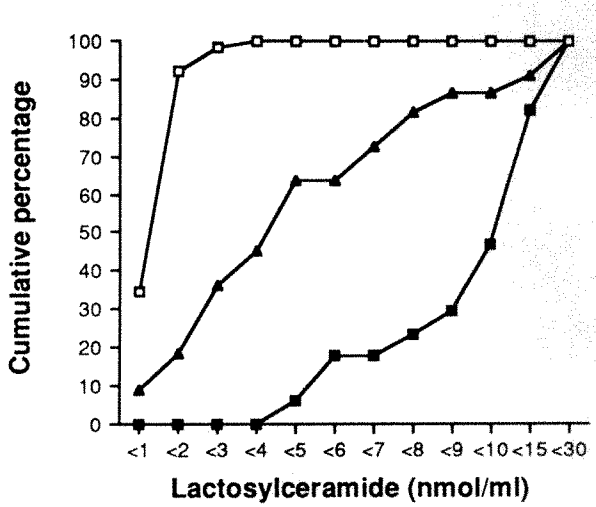


Fig. 3. Percentage distribution of ceramide lactoside concentration in amniotic fluid from preterm pregnancies. *Open squares*, No labor (n = 52); *solid triangles*, spontaneous labor without clinical signs of chorioamnionitis (n = 22); *solid squares*, symptomatic chorioamnionitis and spontaneous labor (n = 17).

from the gel and eluted with chloroform-methanol-water, 5:5:1.1 (vol/vol/vol). The eluate was dried, and the glycolipid degraded in hydrochloric acid–methanol.[8] The carbohydrates released were analyzed with a Hewlett-Packard 5890 gas chromatograph. α-Methylmannoside was used as internal standard. The temperature of the capillary column (cross-linked methyl silicone, 25 m, Hewlett-Packard) was programmed from 80° to 240° C. Hydrocarbons were detected with a flame ionization detector and quantified with a Hewlett-Packard 3390 A integrator. The presence of equimolar quantities of galactose and glucose in all specimens confirmed the specificity of the assay. The concentration of ceramide lactoside was calculated on the basis of the quantity of galactose, which was corrected for the recovery of ceramide lactoside, 69% ± 12%.

The lecithin/sphingomyelin ratio and phosphatidylglycerol were measured semiquantitatively after isolation of individual phospholipids by two-dimensional thin-layer chromatography as described previously.[10] Peripheral blood granulocytes were isolated by density gradient centrifugation and subsequent Ficoll-Paque centrifugation.[11] For isolation of amnion, reflected parts of the fetal membranes were removed from the placentas and washed with isotonic saline solution. The amnion was peeled from the chorion laeve.

The statistical analyses were performed with a Macintosh SE computer and a Stat View 512+ program (Brain Power, Inc, Calabasas, Calif.). Linear regression analysis was used to test whether the concentration of ceramide lactoside was correlated with gestational age. The dependence of ceramide lactoside on additional qualitative factors (sex, complications of pregnancy) was evaluated by multiple regression analysis. The results were expressed as means ± SD. The concentrations of ceramide lactoside were compared by the t test or by the Wilcoxon rank sum test.

Results

Amniotic fluid was recovered from preterm pregnancies or, in some cases, at preterm birth for evaluation of fetal lung maturity and the need of surfactant therapy. The amniotic fluid at term was obtained either during vaginal delivery or during elective cesarean section and used for isolation of human surfactant. Surprisingly, the specimens recovered during preterm labor often contained a lipid, which in the other specimens was either indistinguishable or barely detectable. In two-dimensional thin-layer chromatography the lipid migrated rather closely to phosphatidylglycerol (Fig. 1). In contrast to phosphatidylglycerol, it contained neither phosphorus nor neuraminic acid but stained for hexose.

Specimens of amniotic fluid that contained large quantities of the unknown lipid were therefore developed by one-dimensional thin-layer chromatography for glycolipids, with the appropriate standards. The major glycolipid was identified as ceramide lactoside (see Patients and methods section). It migrates as a doublet, because the fatty acids of ceramide lactoside are not uniform.

An accurate method was designed for quantification of ceramide lactoside, involving both thin-layer and gas-liquid chromatography. The interassay coefficient of variation was 12%. The two-dimensional thin-layer chromatography[10] method is more practical because it allows simultaneous evaluation of both ceramide lactoside and lung maturity.

Fig. 2 shows the concentration of ceramide lactoside in amniotic fluid as a function of gestational age. Table I additionally shows the concentrations of ceramide lactoside in amniotic fluid from term and preterm pregnancies. In pregnancies without labor, the concentrations of ceramide lactoside were low and there were no

Table IIA. Sensitivity and specificity of ceramide lactoside in predicting chorioamnionitis with signs of infection

Ceramide lactoside	*Chorioamnionitis (n = 17)**		*No chorioamnionitis (n = 95)**		*Sensitivity† (%)*	*Specificity† (%)*	*Overall accuracy† (%)*
	No.	*%*	*No.*	*%*			
≥5 nmol/ml	16	94.1	5	5.3	94.1	94.7	94.6

*Three cases of spontaneous preterm labor were excluded since placenta and fetal membranes were not evaluated for chorioamnionitis by a pathologist despite prolonged rupture of the fetal membranes or maternal infection.

†Sensitivity: Correct positive test results/All positive cases × 100. Specificity: Correct negative test results/All negative cases × 100. Overall accuracy: Correct test results/All test results × 100.

Table IIB. Sensitivity and specificity of ceramide lactoside in predicting chorioamnionitis in spontaneous preterm labor

Ceramide lactoside	*Preterm labor (n = 39)*		*No preterm labor (n = 76)*		*Sensitivity (%)*	*Specificity (%)*	*Overall accuracy (%)*
	No.	*%*	*No.*	*%*			
≥2.5 nmol/ml	33	84.6	4	5.3	84.6	94.7	91.3

differences between the various diagnostic categories (normal, maternal diabetes, preeclampsia, isoimmunization). Regression analysis showed that there was no significant correlation between the concentration of ceramide lactoside and the length of gestation ($p = 0.078$). In term labor ceramide lactoside was also low. In contrast, during spontaneous preterm labor without chorioamnionitis, the concentration of ceramide lactoside was signficantly higher than in preterm gestation without labor ($p = 0.102$). There was no detectable correlation between the concentration of glycolipid and the length of gestation in preterm labor ($p = 0.102$). The concentration of ceramide lactoside was also high (4.9 ± 1.8 nmol/ml, $n = 4$) in impending preterm labor (labor evident within 3 days after amniocentesis). Both preterm labor in twin pregnancy and "idiopathic" preterm labor were associated with high ceramide lactoside concentrations (6.2 ± 5.0 nmol/ml, $n = 8$; 4.9 ± 3.8 nmol/ml, $n = 14$). The ceramide lactoside concentration was significantly higher in preterm labor with chorioamnionitis than in preterm labor without chorioamnionitis ($p < 0.01$).

For a further study of the factors that influence the concentration of ceramide lactoside, multiple regression analysis was used. In addition to the length of gestation, the influence of the following qualitative factors was studied: fetal sex, isoimmunization, preeclampsia, maternal diabetes, term labor, preterm labor without chorioamnionitis, and chorioamnionitis. Only the last two emerged as significant variables (preterm labor, no chorioamnionitis: $p = 0.002$; chorioamnionitis: $p = 10^{-4}$).

Fig. 3 shows the percentage distribution of ceramide lactoside concentration in amniotic fluid in preterm pregnancy. The group with no labor was entirely different from the group with chorioamnionitis. However, the group with spontaneous labor but no suspicion of chorioamnionitis overlapped with both the other groups.

Tables IIA and IIB show the sensitivity, specificity, and accuracy of ceramide lactoside in predicting chorioamnionitis and preterm labor. The concentration limits for ceramide lactoside were adjusted to attain maximum overall predictive accuracy. Ceramide lactoside was a sensitive and specific index of chorioamnionitis. Its sensitivity in predicting spontaneous preterm labor was 84.6%. The gestational age of the six infants who had low ceramide lactoside concentrations and spontaneous preterm delivery was 31.3 ± 3.7 weeks.

To evaluate the possible source of the glycolipid, the tracheal aspirate, amnion, vernix, urine, and human polymorphonuclear neutrophils were analyzed. In each case cultures of the amniotic fluids and the tracheal aspirates were negative for bacteria. Only the granulocytes, neutrophil-laden fetal membranes, and corresponding amniotic fluids had prominent ceramide lactoside (Table III).

Comment

According to the present study, the concentration of ceramide lactoside in amniotic fluid is strikingly and consistently increased in premature labor during chorioamnionitis. In preterm labor without symptomatic chorioamnionitis, the concentration of ceramide lactoside was higher than in term labor or no labor. These findings suggest that ceramide lactoside in amniotic fluid could be used as a biochemical indicator of the imminence of preterm labor. It is uncertain what the

relationship is between ceramide lactoside and the specific event causing the onset of preterm labor. This issue is important in terms of prevention of spontaneous preterm labor and of detection of high-risk patients.

Ceramide lactoside, consisting of a ceramide moiety and two neutral sugars (galactose-glucose-ceramide), is both a precursor and a catabolic product of neutral glycosphingolipids. The lipophilic ceramide moiety of glycosphingolipids anchors the molecule to the cell membrane, and the hydrophilic sugars may serve as receptors of bioactive agents. Plasma membrane–associated glycolipids play a role in cell-to-cell interaction, differentiation, and growth.[12] Ceramide lactoside, however, forms an exception, being found mainly in intracellular membranes.[13]

Only small quantities of ceramide lactoside, if any, were detected in urine, vernix, bacteria, normal fetal membranes, or lung effluent. However, human granulocytes[13] and the granulocyte-laden amnion found in chorioamnionitis contained large quantities of ceramide lactoside. This glycolipid is additionally released into the supernatant by granulocytes during phagocytosis in vitro (unpublished observations). In neutrophils ceramide lactoside is remarkably abundant.[12, 13] It is therefore possible that in spontaneous preterm labor an increase in ceramide lactoside is indicative of inflammation, although this was not always verified by pathologic examination, since there was no clinical suspicion of chorioamnionitis. However, since there is often no maternal fever in pathologic chorioamnionitis,[14] it is possible that the cases with high ceramide lactoside concentrations actually had pathologic chorioamnionitis without signs of infection. In addition, chorioamnionitis may not be the only inflammatory source of ceramide lactoside. In our series there were two cases of spontaneous preterm labor with high amniotic ceramide lactoside concentrations and an inflammatory disease without pathologic chorioamnionitis: one fetus had funisitis and the other pneumonia. According to present figures the incidence of pathologic chorioamnionitis at term is only 4% to 10%.[14, 15] This is consistent with our finding of low ceramide lactoside concentrations suggesting a paucity of inflammation. The present data do not exclude the possibility that this glycolipid is released from other sources besides inflammatory cells. However, they do imply that preterm labor is associated with a unique enhancement of ceramide lactoside in amniotic fluid.

Bacteria are a source of phospholipases that promote prostaglandin synthesis by releasing arachidonic acid from fetal membranes. Some microbes may have low phospholipase activity,[5] and often no bacteria were cultured in amniotic fluid or fetal membranes in chorioamnionitis. Although microbes are not the only cause of inflammation,[16] there are very few data at present to suggest that nonmicrobial factors[15] are responsible for the onset and progression of chorioamnionitis. In the present study nine amniotic fluid samples were cultured for pathogenic bacteria. Cultures for mycoplasma, frequently found in chorioamnionitis,[15] were not available. Of the six specimens in chorioamnionitis, two were positive for bacteria; both culture-positive (8.7 and 14.4 nmol/ml) and culture-negative (9.9 ± 3.4 nmol/ml) amniotic fluid samples had high ceramide lactoside concentrations.

Table III. Ceramide lactoside in amniotic fluid, lung effluent, and amnion*

	Ceramide lactoside/total phospholipid × 100 (mol/mol)	
	No preterm labor (n = 4)	*Chorioamnionitis and preterm labor (n = 4)*
Amniotic fluid	0.9 ± 0.4	9.9 ± 3.4
Tracheal aspirate at birth	0.4 ± 0.3	0.7 ± 0.4†
Amnion	0.5 ± 0.4	3.0 ± 2.0

*Granulocytes from human blood contained ceramide lactoside (8.9 ± 4.4, $n = 4$), but this glycolipid was not detected in urine or in vernix.

†None of these infants had pneumonia.

The accumulation of ceramide lactoside in the amniotic fluid may in itself have no direct functional importance in the progression of preterm labor but appears to reflect the activity of inflammatory cells. On the other hand, phagocytosis by leukocytes produces other mediators that are potentially important in triggering preterm labor. Granulocytes release arachidonic acid–specific phospholipase A_2 activity and platelet-activating factor.[17] The former conceivably activates prostaglandin synthesis and the latter increases intracellular calcium ions. Phosphoinositide-specific phospholipase C and phospholipase A_2 in fetal membranes,[4] as well as myometrial contractility,[18] are dependent on calcium ions. Second, proteinases released from phagocytosing cells effectively degrade collagen and elastin[6] and may contribute to weakening of the fetal membranes and to biochemical changes that take place during cervical maturation in preterm labor.[18] Finally, the free oxygen radicals formed during phagocytosis may enhance prostaglandin synthesis, promote degradation of collagen, and increase chemotactic substance.[19] The possible role of inflammatory cells and their products as mediators in the onset or progression of preterm labor needs further study.

REFERENCES

1. MacDonald PC, Porter JC, Schwartz BE, Johnston JM. Initiation of parturition in the human female. Semin Perinatol 1978;2:273-86.
2. Liggins GC, Forster CS, Grieves SA, Schwartz AL. Control of parturition in man. Biol Reprod 1977;16:39-56.
3. Fuchs AR, Fuchs F. Endocrinology of human parturition: a review. Br J Obstet Gynaecol 1984;91:948-67.

4. Bleasdale JE, Johnston JM. Prostaglandins and human parturition: regulation of arachidonic acid mobilization. Rev Perinat Med 1985;5:151-91.
5. Bejar R, Curbelo V, Davis C, Gluck L. Premature labor. II. Bacterial sources of phospholipase. Obstet Gynecol 1981;57:479-82.
6. Revak SD, Rice CL, Schraufstätter IU, Halsey WA, Bohl BP. Experimental pulmonary inflammatory injury in the monkey. J Clin Invest 1985;76:1182-92.
7. Gonik B, Creasy RK. Preterm labor: its diagnosis and management. Am J Obstet Gynecol 1986;154:3-8.
8. Rauvala H, Hallman M. Glycolipid accumulation in bronchoalveolar space in adult respiratory distress syndrome. J Lipid Res 1984;25:1257-62.
9. Bhatti T, Chambers RE, Clamp JR. The gas chromatographic properties of biologically important N-acetylglucosamine derivatives, monosaccharides, disaccharides, trisaccharides, tetrasaccharides and pentasaccharides. Biochim Biophys Acta 1978;222:339-47.
10. Hallman M, Teramo K. Measurement of the lecithin/sphingomyelin ratio and phosphatidylglycerol in amniotic fluid: an accurate method for the assessment of fetal lung maturity. Br J Obstet Gynaecol 1981;88:806-13.
11. Boym A. Isolation of mononuclear cells and granulocytes from human blood. Scand J Clin Lab Invest 1968; 97(suppl):77-89.
12. Hakomori S. Glycosphingolipids in cellular interaction, differentiation, and oncogenesis. Ann Rev Biochem 1981; 50:733-64.
13. Symington FW, Murray WA, Bearman SI, Hakomori S. Intracellular localization of lactosylceramide, the major human neutrophil glycosphingolipid. J Biol Chem 1987; 262:11356-63.
14. Guzick DS, Winn K. The association of chorioamnionitis with preterm delivery. Obstet Gynecol 1985;65:11-5.
15. Quinn PA, Butany J, Taylor J, Hannah W. Chorioamnionitis: its association with pregnancy outcome and microbial infection. Am J Obstet Gynecol 1987;156:379-87.
16. Ryan GB, Manjo G. Acute inflammation. Am J Pathol 1977;86:185-276.
17. Alonso F, Henson PM, Leslie CC. A cytosolic phospholipase in human neutrophils that hydrolyzes arachidonoyl-containing phosphatidylcholine. Biochim Biophys Acta 1986;878:273-80.
18. Huzar G, Naftolin F. The myometrium and cervix in normal and preterm labor. N Engl J Med 1984;311:571-81.
19. Hertz F, Cloarec A. Pharmacology of free radicals; recent views on their relation to inflammatory mechanisms. Life Sci 1984;34:713-20.

Cardiac isoenzymes and electrocardiographic changes during ritodrine tocolysis

Hamid A. Hadi, MD, and Sabah J. Albazzaz, MD

Augusta, Georgia

To investigate the potential myocardial ischemic effects of ritodrine, we studied 36 singleton and four twin preterm pregnancies during ritodrine therapy. We serially determined serum creatinine phosphokinase (CPK-MB fraction) and lactic dehydrogenase isoenzymes and performed electrocardiography before and during ritodrine infusion and again within the first 24 hours of oral drug therapy. We observed that serum CPK-MB and lactic dehydrogenase isoenzymes remained within the normal range during therapy periods. The incidence of sinus tachycardia and non-specific T wave changes were 100% and 25%, respectively. In three of four twin pregnancies, ST-T segment depression in leads I, V_4, V_5, and V_6 of the electrocardiogram was noted. Our study suggests that (1) the recommended ritodrine regimen does not produce direct myocardial damage, and (2) ritodrine may cause cardiac ischemia as determined by electrocardiography, which theoretically would progress to myocardial damage if not treated properly. (Am J Obstet Gynecol 1989;161:318-21.)

Key words: Preterm labor, ritodrine, cardiac isoenzymes, electrocardiography

From the Departments of Obstetrics and Gynecology and Internal Medicine, Section of Cardiology, Medical College of Georgia.
Received for publication September 21, 1988; revised January 4, 1989; accepted February 21, 1989.
Reprint requests: Hamid A. Hadi, MD, Department of Obstetrics and Gynecology, Division of Maternal-Fetal Medicine, East Carolina University School of Medicine, Greenville, NC 27858-4354.

Ritodrine hydrochloride, a β-adrenergic agonist, was approved by the Food and Drug Administration in 1980 and has been widely used in the treatment of preterm labor as a potent tocolytic agent. Its use has been associated with frequent and occasionally severe maternal cardiovascular effects.[1, 2] One of the most im-

Table I. Clinical data of study patients

	Mean ± SD	*Range*
Maternal age (yr)	25 ± 3.2	19-32
Parity	2 ± 1.3	1-3
Gestational age (wk)	28 ± 1.5	25-34
Total dose of IV ritodrine (mg/12 hr)	142 ± 18	128-162
Total dose of oral ritodrine (mg) until end of study period (average, 16 hr)	210 ± 18	10-280
Fluids (IV) (ml)	1320 ± 80	1200-1510

IV, Intravenous.

portant side effects of ritodrine is potential myocardial toxicity. Animal studies indicate that β-mimetics in dosages not comparable with human dosages result in myocardial ischemia, presumably by causing intracellular adenosine monophosphate depletion.[3, 4] Human studies have reported transient electrocardiographic changes during ritodrine infusion, which also suggests its myocardial ischemic effect.[5] However, at the present time well-controlled prospective data to serially examine changes in sensitive cardiac isoenzymes with simultaneous electrocardiography during ritodrine therapy are lacking. In an attempt to elucidate the myocardial ischemic effect of ritodrine, this study was designed to serially measure serum creatinine phosphokinase (CPK-MB), lactic dehydrogenase subunits (LDH), and LDH_1/LDH_2 ratio and perform electrocardiography before and during ritodrine therapy.

Material and methods

The study was done on 40 patients (36 singleton and four twin pregnancies) undergoing suppression of preterm labor with ritodrine hydrochloride and undertaken after patients signed a consent form approved by the institutional review committee. Maternal age, parity, and race were recorded, and gestational age was determined from patients' last menstrual period or by ultrasound examination. Criteria for enrollment included: (1) preterm labor as defined by regular uterine contractions recurring at less than 10-minute intervals, along with cervical dilation and/or effacement, (2) gestational age between 20 and 36 weeks, and (3) intact membranes. Exclusions from the study included patients with medical complications during pregnancy, such as hypertension, diabetes mellitus, and heart disease.

Ritodrine dosage. Intravenous infusion of ritodrine was administered according to a previously reported protocol.[6] The starting dose was 100 μg/min and increased every 10 minutes by increments of 50 μg/min until labor was inhibited, side effects occurred, or the maximum dose of 350 μg/min was reached. The infusion period was 12 hours, to be followed by 10 mg of ritodrine orally every 2 hours for first 24 hours and then 10 to 20 mg every 4 to 6 hours for subsequent days.

Study periods. There were three study periods: before infusion or control (A), during infusion (B), and oral therapy (C). Serum CPK and MB fraction, total and subunits of LDH isoenzymes, and a standard electrocardiogram were obtained during the preinfusion period and served as patients' own control. These were repeated at 6- and 12-hour intervals during infusion and again within 24 hours of oral ritodrine therapy. An aliquot of blood for CPK isoenzymes was immediately frozen, and isoenzymes were separated by electrophoresis in an agarose medium. Serum samples for LDH subunit (LDH_1 to LDH_5) measurement were collected and stored at room temperature. The cardiac isoenzymes were measured by an automatic clinical analyzer kit (Dupont Company, Clinical Systems Division, Wilmington, Del.). This method is based on a coupled enzyme reaction with the generation of reduced nicotinamide-adenine dinucleotide phosphate, which consists of the Rosalki modification of the original Oliver's assay.[7] The CPK-MB fraction and LDH isoenzymes were interpreted as abnormal if the CPK-MB fraction was >5% and the ratio of LDH_1 to LDH_2 was ≥1.

Maternal systolic and diastolic blood pressures, pulse rate, and serum electrolytes were also measured during study periods. Although the preinfusion period served as the control period, the study was initially designed to use paired *t* tests and compare the mean values of isoenzymes in the infusion and oral periods with those of the control period and consider *p* values <0.05 statistically significant.

Results

Forty patients completed the three study periods. There were 36 singleton pregnancies and four sets of twin gestation. Clinical and laboratory data on study patients are shown in Table I. The results of isoenzyme values during study periods are illustrated in Table II. Total serum CPK, CPK-MB, and LDH isoenzymes re-

Table II. Isoenzyme data on study patients

Isoenzymes (normal values)	*Study periods*			
	A Before infusion	*B During infusion*		*C Oral 24 hr*
		6 hr	*12 hr*	
Total CPK (21-215 mU/ml)	83 ± 15	77 ± 22	89 ± 14	79 ± 18
CPK-MB (%) (0%-5%)	3.1 ± 1.1	3.2 ± 1.3	3.3 ± 1.4	3.1 ± 0.6
LDH_1 (20%-28%)	21	26	25	28
LDH_2 (30%-40%)	32	37	40	40
LDH_3 (18%-24%)	19	23	25	23
LDH_4 (6%-12%)	7	11	12	8
LDH_5 (5%-10%)	6	8	14	13
LDH_1/LDH_2 ratio (<1)	0.6	0.7	0.6	0.7

mained within the normal range during ritodrine study periods. Mean values of CPK-MB isoenzymes during the control periods, 6 and 12 hours of the infusion period, and during oral therapy were 3.1% ± 1.1%, 3.2% ± 1.3%, 3.3% ± 1.4%, and 3.1% ± 0.6%, respectively. Mean values for LDH isoenzymes and LDH_1/LDH_2 ratios were within normal limits. There was no change in mean systolic blood pressure during study periods. However, 18 patients (45%) had a diastolic blood pressure ≤40 mm Hg, including two sets of twins and 10 patients with nonspecific T wave changes on electrocardiography. Serum potassium level declined from a preinfusion rate of 3.5 ± 0.72 to 2.71 ± 0.39 mEq/L during infusion of ritodrine and returned to 3.13 ± 0.02 mEq/L during the oral phase of the study ($p < 0.05$).

Electrocardiographic changes. All patients (100%) had sinus tachycardia during infusion. Ten of 40 patients (25%) had nonspecific (ST < 1 mm above or below baseline) ST-T wave changes during intravenous ritodrine therapy. The QRS axis remained within normal limits throughout the study. In three of four twin pregnancies, significant ST-T segment depression in leads I, V_4, V_5, and V_6 were noted (>1 mm depression). Since the isoenzyme values remained within the normal range during study periods, we did not perform statistical analysis except for comparison of serum potassium values, which showed significant decline during the infusion period.

Comment

Myocardial ischemia has been suggested by the development of chest pain and electrocardiographic changes during β-agonist tocolytic therapy.[5-8] However, controversy exists whether β-mimetics exert a direct cardiotoxic effect or symptoms of ischemia are the result of a fall in mean arterial blood pressure and a shortened diastolic filling time, leading to reduced blood flow to the left ventricular myocardium. These two theories will be addressed in this study.

There is suggestive evidence that as with catecholamines, β-adrenergic tocolytic agents may have cardiotoxic effects. A well-characterized myocardiopathy with subendocardial ischemia has been previously reported in patients with pheochromocytoma.[9] β-Mimetics also produce subendocardial ischemia and necrosis in rats, presumably as a result of an increase in mitochondrial calcium content and depletion of high-energy phosphate stores caused by excessive breakdown of adenosine triphosphate by calcium-dependent adenosine triphosphatase and resultant impaired myocardial oxidative phosphorylation.[10-12] To examine this hypothesis in humans during ritodrine tocolysis, in this study we serially measured total serum CPK, CPK-MB fraction, and LDH isoenzymes. CPK-MB is an energy-transferring enzyme and found most abundantly in high energy–consuming tissues, that is, myocardium. Abundant CPK-MB enzyme level in serum and its rapid release in association with irreversible myocyte injury account for its high diagnostic sensitivity.[13] Serum CPK-MB isoenzyme rises within 4 to 6 hours after chest pain. None of our patients in this series had chest pain, and the CPK-MB isoenzymes during ritodrine infusion and oral therapy remained within the normal range. The LDH isoenzymes appear later than CPK in myocardial infarcts. The ratio of LDH_1 to LDH_2 of ≥1 has become the standard for confirmation of myocardial damage.[13] This ratio remained <1 during our study periods, rejecting the hypothesis of direct myocardial injury with therapeutic ritodrine dosage. Since patients did not have symptoms during the first 24 hours of oral drug therapy and CPK isoenzymes remained within normal limits, we did not extend measurements of LDH subunits beyond this period. Thus we can only categorically state that enzyme studies during the first 24 hours of oral ritodrine therapy did not reveal myocyte damage.

Benedetti[1] and Hankins[14] suggested that β-mimetic–related ischemia appears to be subendocardial in origin. Under physiologic conditions, the ratio of suben-

docardial to epicardial blood flow is 1:1. The combination of pregnancy and β-mimetics diminishes aortic diastolic blood pressure and induces tachycardia, which result in a shortened diastole.

Increased heart rate and myocardial oxygen demands that exceed supply will produce signs of ischemia and may progress to myocyte damage. This intriguing notion is supported by our study, in which a decrease in diastolic blood pressure and electrocardiographic changes, especially in twin pregnancies, were associated with normal cardiac isoenzymes.

In summary, with the use of CPK and LDH isoenzymes and the LDH_1/LDH_2 ratio, this study did not show a direct cardiotoxic effect of ritodrine used for the suppression of preterm labor after a standard protocol. However, manifestations, such as chest pain and electrocardiographic changes, are suggestive of myocardial ischemia caused by ritodrine-related hemodynamic changes, leading to decreased blood flow to the myocardium and may lead to myocyte damage if not properly treated. Increased heart rate and contractility in twin pregnancies compared with singleton pregnancies suggest reduced cardiac reserve.[15] Therefore in the presence of any symptoms of ischemia or electrocardiographic changes, ritodrine should be discontinued. In our institution routine electrocardiography is performed before the administration of ritodrine therapy. Since current ritodrine infusions yield unnecessary excessive plasma concentration and result in significant cardiovascular symptoms, the new low-dose infusion regimen that was recently proposed by Caritis[16] may be beneficial in reducing cardiovascular side effects without adversely affecting the ability of the drug to inhibit labor.

REFERENCES

1. Benedetti TJ. Maternal complications of parenteral beta-sympathomimetic therapy for premature labor. AM J OBSTET GYNECOL 1983;145:1-6.
2. Bieniarz J, Ivankovich A, Scommegna A. Cardiac output during ritodrine treatment in premature labor. AM J OBSTET GYNECOL 1974;188:910-20.
3. Magnusson G, Hansson E. Myocardial necrosis in the rat: a comparison between isoprenaline, orciprenaline, salbutamol, and terbutaline. Cardiol 1973;58:147-7.
4. Zimmer HG, Ibel H, Steinkopff G. Reduction of the isoproterenol-induced alterations in cardiac adenine nucleotides and morphology by Ribose. Science 1980;207:319-21.
5. Michalak D, Klein V, Marquette GP. Myocardial ischemia: a complication of ritodrine tocolysis. AM J OBSTET GYNECOL 1983;146:861-2.
6. Barden TP, Peter JB, Merkatz IR. Ritodrine hydrochloride: a beta-mimetic agent for use in preterm labor. Obstet Gynecol 1980;56:1-6.
7. Rosalki SB. Improved procedure for serum creatinine phosphokinase determination. J Lab Clin Med 1967;69:696-8.
8. Tye K, Desser K, Benchimal A. Angina pectoris associated with the use of terbulaline for premature labor. JAMA 1980;244:692-4.
9. Vliet V, Burchell AB, Tilus JL. Fetal myocarditis associated with pheochromocytoma. N Engl J Med 1966;274:1102-5.
10. Rona G, Chappel CL, Balazs T, et al. An infant-like myocardial lesion and other toxic manifestations produced by isoproterenol in the rat. Arch Pathol 1959;67:443-5.
11. Nerdlinger EL, Bramante PO. Subcellular myocardial ionic shifts and mitochondrial alterations in the course of isoproterenol-induced cardiopathy of the rat. J Mol Cell Cardiol 1974;6:49-52.
12. Takenaka F, Higuchi M. High energy phosphate contents of subepicardium and subendocardium in the rat treated with isoproterenol and some other drugs. J Mol Cell Cardiol 1974;6:123-6.
13. Roberts R. Diagnostic assessment of myocardial infarction based lactate dehydrogenase and creatinine kinase isoenzymes. Heart Lung 1981;10:486-92.
14. Hankins GDV. Complications of beta-sympathomimetic tocolytic agents. Critical care obstetrics. 1st ed. Oradell, N.J.: Medical Economics Books, 1987:192-204.
15. Veille JC, Morton MJ, Burry KJ. Maternal cardiovascular adaptations to twin pregnancy. AM J OBSTET GYNECOL 1985;153:261-3.
16. Caritis SN. A pharmacological approach to the infusion of ritodrine. AM J OBSTET GYNECOL 1988;158:380-4.

Elevated liver enzymes and thrombocytopenia in the third trimester of pregnancy: An unusual case report and a review of the literature

Mary E. Hannah, MSc, MDCM,[a] Ron Gonen, MD,[a] Eva J. M. Mocarski, MSc, MD,[a] Ross Cameron, MD,[b] Laurence Blendis, MD,[c] and Michael Glynn, MD[c]

Toronto, Ontario, Canada

A woman presented in the third trimester of pregnancy with epigastric pain, elevated liver enzymes, and thrombocytopenia. The frozen-section liver biopsy findings were compatible with acute fatty liver of pregnancy. The light and electron microscopic findings were those of preeclampsia. All clinical and laboratory abnormalities resolved before delivery. (AM J OBSTET GYNECOL 1989;161:322-3.)

Key words: Preeclampsia, thrombocytopenia, acute fatty liver of pregnancy

Acute epigastric pain, elevated liver enzymes, and thrombocytopenia occurring in the third trimester of pregnancy may be a manifestation of preeclampsia, acute fatty liver of pregnancy, or a non-pregnancy-induced liver disorder such as hepatitis. The following is an unusual case in which a transjugular liver biopsy raised more questions than it answered.

Case report

A 32-year-old white primigravid woman was transferred to a tertiary care perinatal unit at 27 weeks' gestation with premature rupture of the membranes and twin gestation. Five days later she developed severe epigastric pain, nausea, and vomiting.

Blood pressure was 110/70 mm Hg, reflexes were normal, and there was no proteinuria. The platelet count was 103 × 10^9/L, serum aspartate aminotransferase 298 U/L (normal <40), alkaline phosphatase 93 U/L (normal <80), and total bilirubin 14 μmol/L (normal <20). There was no evidence of hemolysis. During the subsequent 24 hours, the platelet count fell and the serum aspartate aminotransferase level rose (Fig. 1). To distinguish between acute fatty liver of pregnancy, atypical preeclampsia, or some other liver disorder, a transjugular liver biopsy was performed. Frozen sections stained with hematoxylin and eosin and with oil red O showed many small orangeophilic globules considered to be fat in hepatocytes, compatible with acute fatty liver of pregnancy.

Light and electron microscopic examinations revealed distinctive zone 1 changes, namely, fibrin thrombi filling sinusoids, hepatocytic necrosis, and focal hemorrhages around all portal tracts with minimal or absent fatty change in any zone. This abnormality is characteristic of preeclampsia or eclampsia.

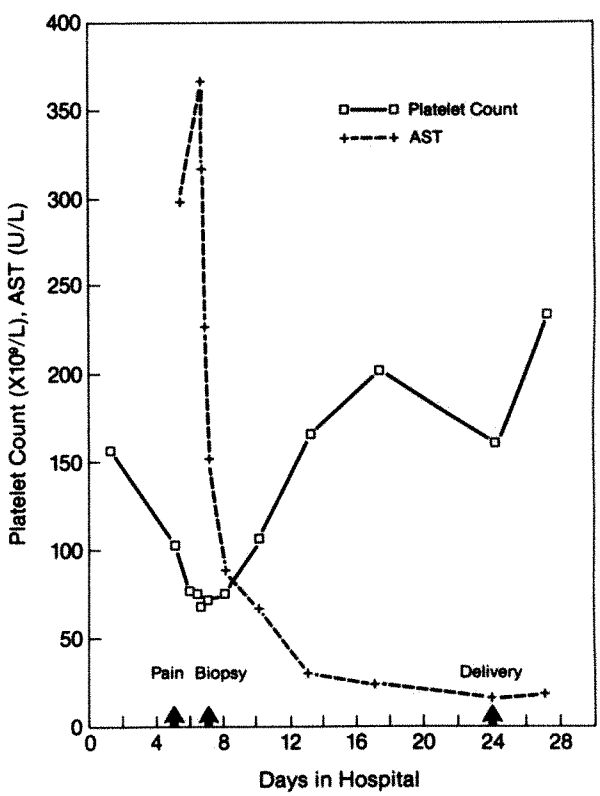


Fig. 1. Changes in serum aspartate aminotransferase *(AST)* level and platelet count during hospital stay.

Laboratory values normalized with expectant management (Fig. 1). On day 26 the patient developed chorioamnionitis and was delivered by cesarean section of two live female infants, weighing 1605 and 1230 gm. The patient and the infants had uneventful postoperative and neonatal courses, respectively.

From the Department of Obstetrics and Gynaecology,[a] Women's College Hospital, and the Departments of Pathology[b] and Medicine,[c] Toronto Hospital Corporation, University of Toronto.

Received for publication November 23, 1988; revised February 14, 1989; accepted March 13, 1989.

Reprint requests: Dr. M. Hannah, Women's College Hospital, 76 Grenville St., Toronto, Ontario, Canada M5S 1B2.

Comment

The liver abnormalities seen in acute fatty liver of pregnancy and in preeclampsia are considered to be quite distinct. The most characteristic microscopic feature of acute fatty liver of pregnancy is the marked deposition of centrilobular microvesicular fat. This is detected on fixed sections stained with hematoxylin and eosin or on frozen section stained with oil red O. In contrast, in preeclampsia-eclampsia, fibrin deposition, hemorrhage, and hepatocellular necrosis in periportal areas are seen.

Many authors have diagnosed acute fatty liver of pregnancy only on the basis of a frozen section stained with oil red O.

In this case the technique proved to be misleading. The histologic features of the liver on the fixed sections were compatible with those found in preeclampsia. The absence of hypertension, proteinuria, and hemolysis and the spontaneous clinical recovery before delivery make the case unusual. Similar cases, however, have been reported.[1, 2]

It is possible that the "preeclamptic" disorder represents a pathologic continuum and that there are atypical presentations that are self-limiting. It is also possible that these cases are being diagnosed more frequently now by liver biopsy.

The treatment of choice for severe preeclampsia and acute fatty liver of pregnancy remains expeditious delivery. However, when the clinical disturbance is atypical and the fetus preterm, consideration should be given to an expectant management approach, provided that the patient is closely monitored and her condition is stable.

REFERENCES

1. Aarnoudse JG, Houthoff HJ, Weits J, Vellenga E, Huisjes HJ. A syndrome of liver damage and intravascular coagulation in the last trimester of normotensive pregnancy. A clinical and histopathological study. Br J Obstet Gynaecol 1986;93:145-55.
2. Goodlin RC, Holdt D. Impending gestosis. Obstet Gynecol 1981;58:743-5.

A complete list of references is available from the authors on request.

Continuous tissue pH monitoring in the term fetus

Mary L. Small, MD, Marie Beall, MD, Lawrence D. Platt, MD, Diane Dirks, RN, and Howard Hochberg, MD

Los Angeles, California

Since Saling's work in 1961, many investigators have attempted to perfect methods to detect fetal acidosis during labor. A system for continuous tissue pH monitoring of the fetus is evaluated in this study of 59 patients. There was poor correlation of tissue pH values and capillary scalp pH values in 19 cases (r = 0.487), and no significant correlation of tissue pH values to the outcome measurements of Apgar scores and cord gas levels. The benefits realized were those of monitoring the trend of tissue pH over time. We believe that a continuous pH monitoring system offers several advantages over intermittent fetal scalp sampling. Further refinement of the technology employed may offer such a system in the future. (Am J Obstet Gynecol 1989;161:323-9.)

Key words: Tissue pH, fetal scalp pH, fetal monitoring

The advent of electronic fetal monitoring in the late 1960s made it possible for obstetricians to evaluate the fetus in the intrapartum period. Occasionally the analysis of the instantaneous fetal heart rate (FHR) was not straightforward. The search for explanations for variance between FHR patterns and the fetal condition led to development and refinement of methods to detect fetal acidosis, first introduced by Saling in 1962.[1] Obtaining a sample of capillary blood from the presenting part by fetal scalp sampling has gained wide but grudging acceptance. Reasons for lack of universal use of fetal scalp sampling include lack of ongoing experience by the attending obstetrician, discomfort to the patient, technical difficulty in obtaining the sample, and numerous chances for error in performance and interpretation of the test. The physician is generally reluctant to repeat this cumbersome procedure, and this limits the ability to discover trend in the pH.

From the Department of Obstetrics and Gynecology, University of Southern California School of Medicine, and the Los Angeles County/University of Southern California Medical Center.
Received for publication April 13, 1988; revised January 25, 1989; accepted March 8, 1989.
Reprint requests: Mary L. Small, MD, Department of Obstetrics and Gynecology, Loma Linda University Medical Center, Loma Linda, CA 92354.

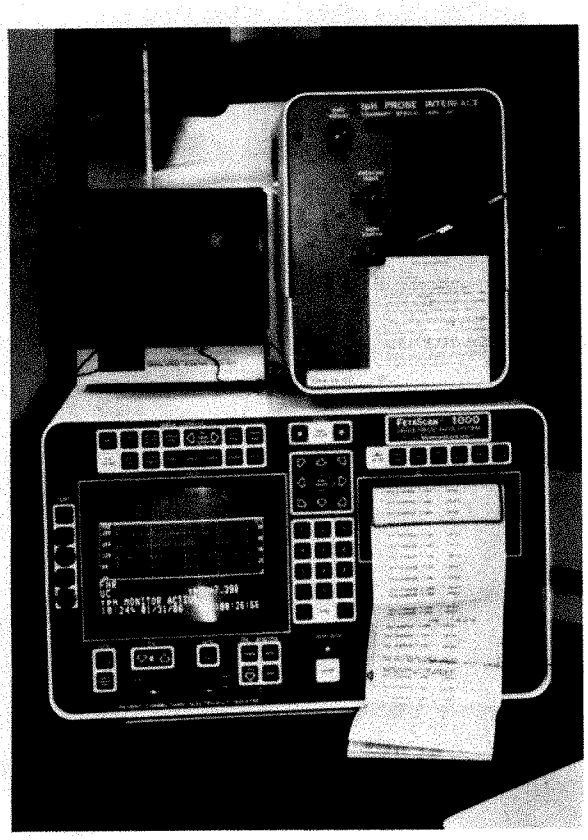


Fig. 1. Fetascan 1000 Obstetrical Data System.

To overcome these difficulties, many investigators have attempted to develop a continuous pH system that initially would determine fetal pH and ultimately be able to measure other respiratory parameters. Objection to some pH systems in the past has concentrated on the use of glass electrodes because of the potential for breakage within the fetal scalp.

This study was undertaken to investigate the use of a new tissue pH (tpH) and fetal monitoring system (Fetascan 1000 Obstetrical Data System, Biomedics International, Bothell, Wash.) that utilizes a spiral needle electrode, similar to the widely used spiral electrode for FHR monitoring. The pH values obtained in our study were to be compared with capillary pH values obtained by fetal scalp sampling to determine if there is a consistent relationship between the two systems. Additionally, the results were to be compared with fetal outcome (judged by 5-minute Apgar score) and cord blood gas levels at delivery.

Material and methods

Study patients were selected from those seen in the labor and delivery area of Women's Hospital at Los Angeles County/University of Southern California Medical Center. Candidates for the study were those patients whose fetuses displayed abnormal or suspicious intrapartum FHR tracings and thus were candidates for scalp pH determinations. After the patient was identified, informed consent was obtained and the patient was prepared for both the traditional fetal scalp sampling and the placement of the tissue pH probe-electrode for use with the tissue pH–FHR monitoring system.

Table I. Characteristics of 59 patients undergoing continuous tissue pH monitoring

	Minimum	*Maximum*	*Mean*
Maternal age (yr)	17	43	26
Estimated gestational age (wk)	35	46	40.7
Duration probe attachment (hr)	0.25	5.5	2.3
Interval between probe removal and delivery (hr)	0.05	11.5	1.6

The monitoring system consists of the following: a combined FHR and tissue pH monitor and a uterine pressure monitor (Fig. 1). The tissue pH is monitored by means of a fiberoptic probe connected by a flexible cable to the tissue pH machine. At the distal end of the probe is a 22-gauge needle formed into a spiral that penetrates into the fetal scalp 3.5 to 4.0 mm (Figs. 2 and 3), just exceeding the usual neonatal scalp thickness (excluding subcutaneous tissue) of 3.0 mm.[2,3] Just proximal to the end of the needle is a window that is covered by a semipermeable membrane through which tissue fluids can pass. The end of the needle under this window is packed with pH-sensitive dye particles of phenol red polyacrylamide.

Changing tissue pH causes a color change in the dye, and this color change is interpreted by the fiberoptic probe as a change in pH. Red and green light passes through the fibers of the cable. Because red light absorbance is not affected by pH in this range, red light reflectance is used to measure probe integrity, and the green light absorbance changes reflect the pH changes. The tissue pH machine provides a continuous printout representing every-1-minute updated pH measurements (Fig. 4). In addition to fetal heart rate and uterine contractions, tissue pH is displayed on an oscilloscope screen.

The probe was sterilized by immersion in activated glutaraldehyde (Omnicide, American Hosp. Corp., McGaw Park, Ill.) for at least 10 hours before each use. To date this equipment has been designed to be reusable with proper cleaning and resterilization between cases. Before application, the sterile probe is calibrated in pH 7.00 and pH 7.40 buffer solutions kept at 37° C in a heating block. This calibration requires approximately 30 minutes.

The spiral probe is applied to the fetal scalp with the

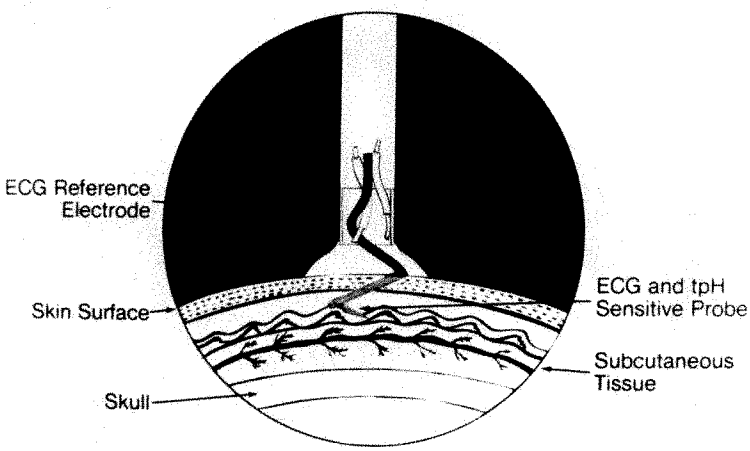


Fig. 2. Diagram of fiberoptic tissue pH probe in situ.

aid of a plastic applicator tool similar to the guide tube used for ordinary scalp electrodes. The two types of electrodes are compared in Fig. 3. On insertion of the probe into the fetal scalp, a fetal electrocardiogram is obtained and integrated into the electronics of the system that serves as FHR monitor and tissue pH monitor. Uterine contractions can be monitored by either an external tocodynamometer or an intrauterine pressure catheter connected to the tissue pH machine. Probe applications were done by three of the authors (M. S., M. B., or L. D. P.) on most of the patients, whereas fetal scalp sampling was usually done by the house staff on duty. The calibration and monitoring were done by the research nurse (D. D.) or by one of the other authors.

After the system was applied and a stable pH reading was attained (approximately 10 minutes), fetal scalp sampling was performed. One specimen was tested immediately for pH on the machine in the labor room (Corometrics 220, Corometrics Medical Systems, Inc., Wallingford, Conn.). An additional one to three specimens were sent to the blood gas laboratory for pH, Po_2, Pco_2, and bicarbonate determinations, completed by the blood gas analyzer method. Acceptable data pairs were those in which fetal scalp sampling was done during the tissue pH monitoring period and in which the tissue pH values were between 7.09 and 7.44 (the values defined as the limits of reliability of the machine). In most cases the patients were monitored until they were transferred to the delivery room. In the delivery room cord samples were obtained immediately after delivery for additional blood gas and pH determinations.

After the probe was removed, a drift check was done to compare the prestudy and poststudy response of the probe to pH 7.00 and 7.40 buffers. If the drift check for probe stability deviated by more than 0.04 pH unit, the probe was considered unstable and data collected with it were discarded.

The newborn infants were examined in the nursery on the second or third day for signs of scalp infection, laceration, or other injury as a result of the tissue pH monitoring.

In analysis of the data, tissue pH and capillary pH values were compared with each other in matched samples, and tissue pH was compared with several outcome measures. Pearson's correlation coefficient was calculated for each comparison with an Epistat statistical package (Gustafson, Round Rock, Tex.); $p < 0.05$ was required for significance.

Results

Fifty-nine patients were studied during a 14-month period between November 1984 and March 1986. The mean patient age was 26 years and the mean gestational age was 40.7 weeks (Table I).

In 39 of the 59 cases, satisfactory simultaneous capillary pH–tissue pH data points were obtained. The other 20 cases were unsuccessful because of technical problems discussed below. The scatterplot of these 39 cases (93 points) is shown in Fig. 5, with $r = 0.188$. Comparison of capillary pH with tissue pH showed an algebraic difference ranging from 0.002 to 0.351 (mean, 0.126); tissue pH was lower in all but five cases.

Correlations were then made between tissue pH and various outcome parameters (Table II) that had valid measurements in all 39 cases. However, only one significant correlation was found, between 5-minute Apgar score and the last tissue pH value when tissue pH was obtained within 30 minutes of delivery. Twelve newborns had 1-minute Apgar scores ≤ 7; one of these had a 5-minute Apgar score <7.

Because of the wide range and poor overall correlation between the simultaneous capillary pH–tissue pH data points (Fig. 5), more detailed analysis was done of the data to exclude the less reliable measurements. Data points were excluded if capillary pH–tissue pH

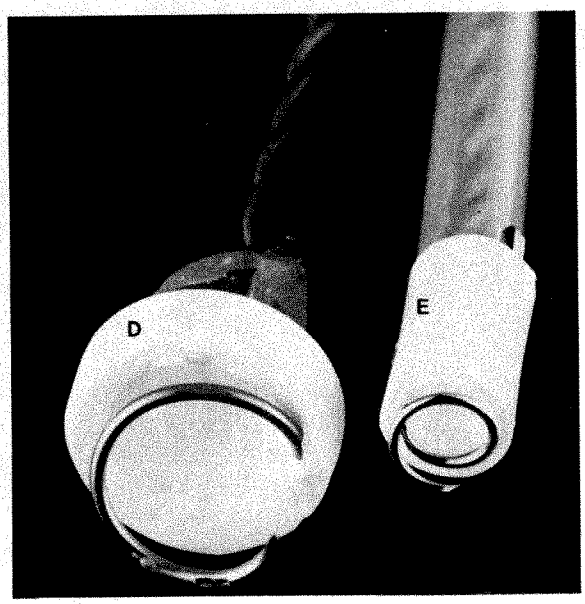


Fig. 3. Comparison of tissue pH fiberoptic probe *(D)* to electronic fetal monitoring scalp electrode *(E)*. (Original magnification ×9.)

pairs were not within 1 minute of each other, if the tissue pH reading had not been stable for 2 minutes before and 2 minutes after the reading was taken, or if capillary pH values were obtained from the labor room pH machine. When exclusions were made for these additional reasons, only 19 pairs of capillary pH–tissue pH data remained that were thought to be reliable points for final statistical analysis. In this group the correlation of capillary pH–tissue pH was $r = 0.487$, $p < 0.05$ (Fig. 6). By comparison, the capillary pH–capillary pH correlation for simultaneous specimens tested in the blood gas laboratory was $r = 0.811$, $p < 0.001$ (Fig. 7).

Follow-up of neonates showed one infant with erythema of the probe puncture site and two infants with scabbing. No neonatal fever or scalp abscesses were noted.

Comment

Because many obstetricians are reluctant to use fetal scalp sampling because of its inconvenience for both doctor and patient, the search continues for a means of continuous pH monitoring in cases in which FHR patterns are suspicious for fetal compromise and the fetal acid-base status needs to be assessed.

From this study it is seen that correlation between continuous tissue pH and capillary pH was modest at best ($r = 0.487$), even when the data were refined to the optimal data pairs. In searching for an explanation of this relatively low correlation, it must be acknowledged that there is a lag time of approximately 6 to 13 minutes between a change in capillary blood pH and the corresponding change in tissue pH, although there is no delay in recording the new tissue pH. This lag is inherent in the design of the machine. Since it is not a constant length of time, it is difficult to assign the appropriate tissue pH for a particular fetal scalp sampling specimen. The relationship between FHR patterns and tissue pH trends has not yet been studied.

When the fetal scalp sampling cone was applied to the fetal scalp, it was often noted that there was an FHR deceleration. It is possible that this FHR change temporarily affected the capillary pH value, such that capillary pH–tissue pH correlation would not be a true measure of the fetal condition. Additionally, an immediate drop in tissue pH was sometimes observed, thus making simultaneous comparison of capillary pH and tissue pH suspect.

It would be reassuring if the difference between capillary pH and tissue pH were constant, which would allow calculation of capillary pH from tissue pH. However, the range of capillary pH–tissue pH difference is too wide in this study (0.002 to 0.351) to be reliable.

Eight probes provided valid data on more than one case each, but since the capillary pH–tissue pH difference between multiple uses of the same probe is greater than the average capillary pH–tissue pH difference (0.126) among cases, this seems to indicate that there is no advantage in using a probe more than once. It would be functionally easier to use a presterilized disposable probe.

Table II shows the lack of correlation between last recorded tissue pH and the outcome variables of Apgar score, umbilical artery pH, and umbilical vein pH. When the cases were limited to those infants who were delivered within ≤30 minutes or ≤15 minutes from probe removal, all variables except last tissue pH versus 5-minute Apgar score showed the same lack of correlation. The explanation for this could be that the in-

Table II. Comparison of last tissue pH with outcome measures

	n	r	p
Last tpH vs. UApH	27	0.3199	0.1038
Last tpH vs. UVpH	27	0.3463	0.0768
Last tpH vs. 1 min Apgar score	27	−0.0847	0.674
Last tpH vs. 5 min Apgar score	27	−0.171	0.3935
Last tpH (30 min) vs. 1 min Apgar score	16	0.1288	0.6344
Last tpH (30 min) vs. 5 min Apgar score	16	−0.5264	0.0362*
Last tpH (15 min) vs. 1 min Apgar score	7	0.1640	0.7253
Last tpH (15 min) vs. 5 min Apgar score	7	−0.4706	0.2865

tpH, Tissue pH; *UApH*, umbilical artery pH; *UVpH*, umbilical vein pH.
*$p < 0.04$.

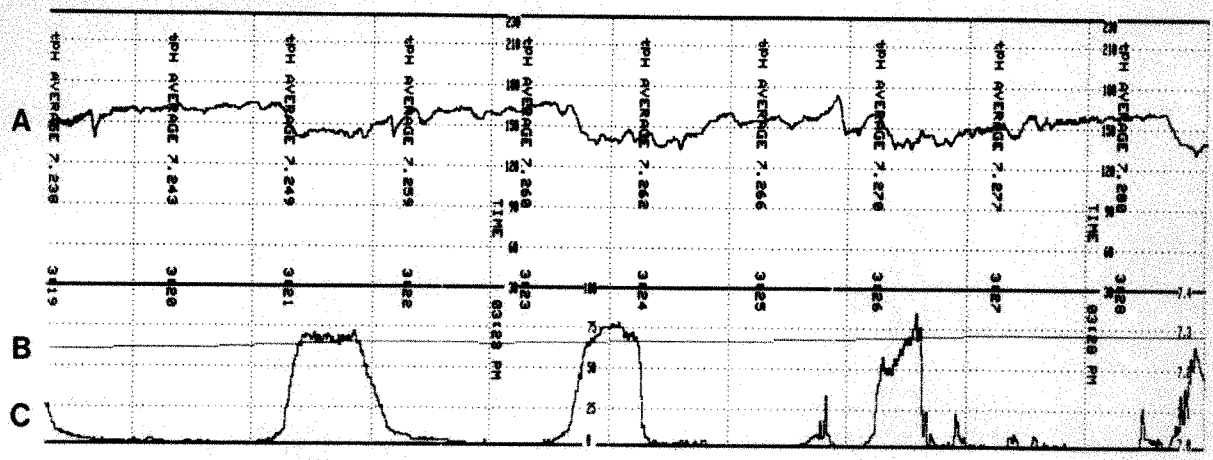


Fig. 4. Portion of tissue pH monitor strip shows record of **(A)** FHR, **(B)** tissue pH, and **(C)** uterine contractions.

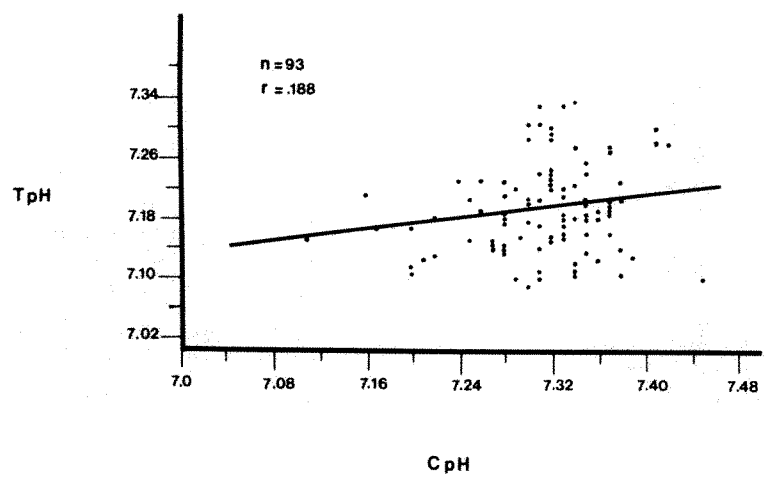


Fig. 5. Scatterplot of 93 simultaneous capillary pH–tissue pH data pairs.

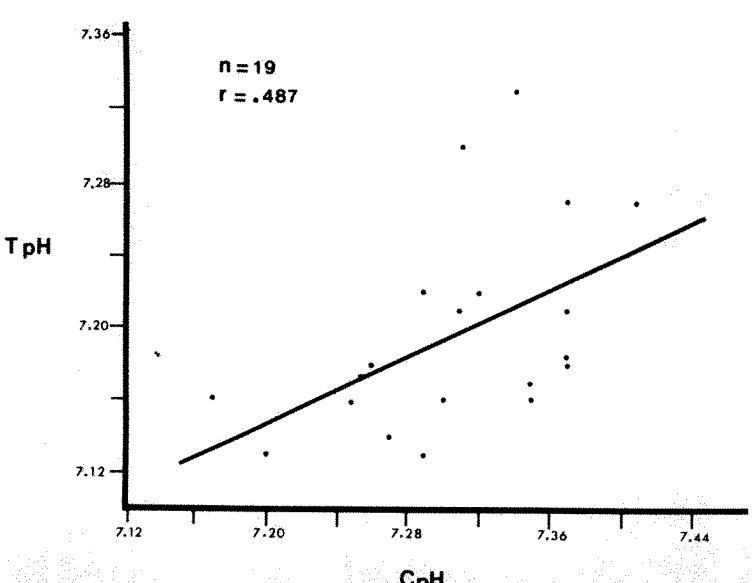


Fig. 6. Scatterplot of 19 simultaneous capillary pH–tissue pH data pairs considered reliable for statistical analysis.

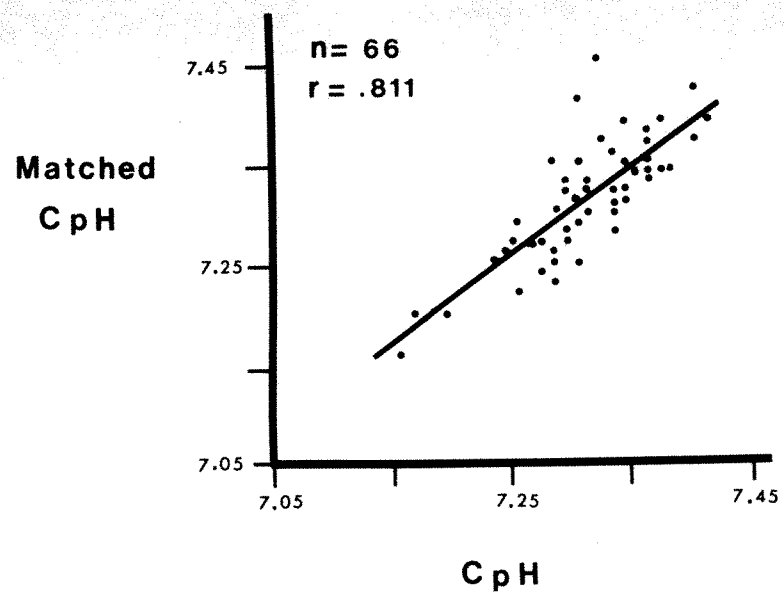


Fig. 7. Scatterplot of 66 simultaneous capillary pH–capillary pH data pairs analyzed in blood gas laboratory.

Table III. Usefulness of continuous tissue pH monitoring in seven cases

Case No.	*Benefit*
1	Detected steady downward drift
2	Extra fetal scalp sampling was avoided
3	Despite normal FHR, tissue pH showed steady downward trend
4	Variable decelerations; tissue pH trend allowed labor to continue
5	In presence of tachycardia, tissue pH revealed stability of fetus
6	Tissue pH trend led to repeat fetal scalp sample
7	Trend was helpful

Table IV. Technical objections to fetal scalp sampling

1. Requires cervical dilatation of 3-4 cm
2. Usually done with patient supine, a position not optimal for uteroplacental perfusion
3. Uncomfortable for patient and awkward for personnel
4. Time-consuming
5. Multiple opportunities for error in specimen collection and transport:
   - Inadequate sample because of poor capillary flow or poor technique
   - Air bubbles in capillary tube
   - Contamination with amniotic fluid
   - Inadequate contact of capillary tube with ice in transport
   - Delay in getting specimen to laboratory, with resultant temperature change

terval from probe removal to the outcome measurement was long enough that metabolic readjustment had already occurred. For this reason a system that allows continuous monitoring until the moment of delivery is essential and is currently being developed. However, with the present state of technology the inconvenience of monitoring until delivery (i.e., having to transport the apparatus to the delivery room) restricts the investigation of correlations to Apgar scores or cord blood gas levels. An alternative is to have labor and delivery in the same room, which is being done in many centers.

A further limitation of the system is the necessity to reject tissue pH values <7.09. This has been designated as the lower limit of reliability of the machine and can be a problem if pH values in the fetus are very low, especially if the fetus is acidotic at the time of probe application.

Sixty-two probes were calibrated during the study period. Some of the calibrated probes could not be used because of technical problems, and other probes were substituted. User-related problems included a probe that became loose or fell off, broken cables, recalibration of the machine by mistake, dislodgment of the probe by the fetal scalp sampling cone, unplugging of the machine (which caused loss of calibration), and difficulty in removing the probe from the fetal scalp. There were also certain system-related problems that included the following: repeated gain sets required during a study, leakage of fluid at the Y-joint of the cable, migration of the probe application site posteriorly under the edge of the cervix, slow stabilization of the probe, bent probe, unstable heat block, necessity to recalibrate the probe, and refusal of the machine to display fetal electrocardiogram or tissue pH.

Twenty probes were used in more than one case after appropriate resterilization. In three cases more than

Table V. Detection of acidosis

	Capillary pH acidotic (<7.20)	*Capillary not acidotic (>7.20)*
Tissue pH acidotic (<7.17)	100% Sensitivity	22% False positive
Tissue pH not acidotic (>7.17)	0% False negative	78% Specificity

one probe was used because of difficulty in calibration, placement, or recording of the tissue pH. Eight probes provided valid data on more than one case each.

The 40 cases excluded from final analysis reflect the complexity of this monitoring system. These problems are being addressed, and in the future it is anticipated that there will be precalibrated, sterile, disposable probes that provide automatic drift checks after the studies.

Despite the many difficulties, continuous tissue pH monitoring was considered helpful in patient management in seven cases (Table III). Results of tissue pH monitoring led to repeat fetal scalp sampling in one case, and in four cases a stable tissue pH reassured the physicians about the stability of the fetus and thus avoided extra fetal scalp sampling or premature or unnecessary cesarean section. Each of the fetuses had a 5-minute Apgar score >6. A system of continuous tissue pH monitoring can avoid the multiple opportunities for error inherent in repeated fetal scalp sampling with its isolated data points (Table IV). It also allows the laboring patient to remain on her side while pH monitoring is being accomplished, a feature not always preferred by persons doing fetal scalp sampling with the cone but one that is important in optimizing uteroplacental perfusion.[4]

After collation of data from several centers involved in studying the Fetascan 1000 monitor, one author (H. H.) found that tissue pH was consistently 0.03 pH unit lower than capillary pH. Because of this offset the boundary between acidotic and preacidotic states can be shifted downward from 7.20 to 7.17.[5] With these criteria the Fetascan 1000 model detects acidosis with 100% sensitivity and 78% specificity (Table V).

It seems likely that tissue pH monitoring will have its own unique value, not necessarily that of fetal scalp sampling. We believe there are enough advantages to a continuous monitoring system to warrant pursuit of the technical improvements necessary to make it rapid to apply and convenient to use (Table VI). Our recent experience with a modified system supports this position.

Table VI. Comparison of continuous tissue pH to intermittent fetal scalp sampling

Advantages of tissue pH	*Disadvantages of tissue pH*
Minute-by-minute update	Fetal head can change position relative to cervix and dislodge tissue pH probe
Shows trend in tissue pH	Time lag 6-13 min (90% response in 4 minutes)
Can afford an early warning of problems	No large data base of tissue pH measurements compared to fetal outcome
Problems during monitoring will be advised on screen	
Can apply probe to patients whose cervical dilatation does not allow fetal scalp sampling	
Patient can remain in optimum position without disturbing tissue pH monitoring	
Not affected by presence of amniotic fluid	

This study was part of a multicenter trial of the equipment described; further reports of the same apparatus are anticipated.

In conclusion, a study of 59 patients who had continuous tissue pH monitoring showed poor correlation of capillary pH–tissue pH, no correlation of tissue pH with outcome measures, and absence of significant morbidity for fetus or mother. It was helpful in patient management because it revealed trends in tissue pH. With certain technologic improvements it can be a useful tool when it is desirable to know the pH of the fetus.

We thank Paul Roby, MD, for his help in statistical analysis of the data.

REFERENCES

1. Saling E. A new method for examination of the child during labor. Introduction, technique, and principles. Arch Gynekol 1962;197:108.
2. Lauersen NH. Clinical methods and use of fetal acid-base measurements. In: Lauersen NH, Hochberg HM, eds. Clinical perinatal biochemical monitoring. Baltimore: Williams & Wilkins, 1981.
3. Ogita S, Kamei T, Sugawa T. Estimation of fetal weight by fetography. Am J Obstet Gynecol 1977;127:37.
4. Humphrey MD, Chang A, Wood C, Morgan S, Hounslow D. A decrease in fetal pH during second stage labor, when conducted in the dorsal position. J Obstet Gynaecol Br Commonw 1974;81:600.
5. Fetal blood sampling. In: American College of Obstetricians and Gynecologists, Technical Bulletin 42, Oct. 1976.

Noninvasive management of isolated bilateral fetal hydrothorax

Leen Pijpers, MD, PhD, Annette Reuss, MD, Patricia A. Stewart, NDCT, CCPT, and Juriy W. Wladimiroff, MD, PhD

Rotterdam, The Netherlands

Fetal outcome was studied in eight cases of isolated bilateral fetal hydrothorax. All patients were referred because of polyhydramnios. Spontaneous resolution of pleural effusion was observed twice. No remarkable change in the degree of hydrothorax was demonstrated in the remaining six cases. All eight cases resulted in the birth of a live infant without other abnormalities. Postnatal intubation, which was carried out in all six fetuses with hydrothorax, was always followed by spontaneous respiration. Subsequent thoracocentesis resulted in the collection of 50 to 500 ml of serous fluid. Sustained intubation (7 days) was necessary in only two infants because of developing respiratory distress as a result of prematurity or recurrent pleural fluid accumulation. All eight infants were alive and well at the age of 1 month. Although in the present study noninvasive management of isolated fetal hydrothorax seems to have been justified, a larger multicenter study is needed to compare survival with and without pleuroamniotic shunting. (Am J Obstet Gynecol 1989;161:330-2.)

Key words: Ultrasonography, isolated fetal hydrothorax, polyhydramnios

Bilateral fetal hydrothorax is a pathologic finding that usually occurs as a part of fetal hydrops. Occasionally, however, it may be observed in an isolated form.

A relationship between pleural effusions and the development of lung hypoplasia has been suggested in various reports; the principal goal of thoracocentesis and pleuroamniotic shunting is normal pulmonary development.[1-5] In the fetal lamb progressive inflation of an intrathoracic balloon from a gestational age of 100 days resulted in respiratory insufficiency at birth caused by pulmonary hypoplasia, whereas prenatal decompression at day 120 was associated with normal neonatal respiratory function.[6] However, variable success rates of pleuroamniotic shunting in bilateral hydrothorax in the human fetus have been reported.[3-5] Often the study described was not limited to isolated pleural effusions but also included fetuses with associated hydrops and/or other structural anomalies. Moreover, data on the natural history of isolated bilateral fetal hydrothorax are lacking.

We report the outcome of eight such fetuses with isolated bilateral pleural effusions in whom a conservative approach was adopted.

From the Department of Obstetrics and Gynecology, Academic Hospital Rotterdam-Dijkzigt, and Erasmus University Rotterdam.
Received for publication September 28, 1988; revised January 6, 1989; accepted February 8, 1989.
Reprint requests: L. Pijpers, MD, PhD, Department of Obstetrics and Gynecology, Academic Hospital Rotterdam-Dijkzigt, Erasmus University Rotterdam, Dr. Molewaterplein 40, 3015 GD Rotterdam, The Netherlands.

Material and methods

Between August 1986 and August 1988, 1890 pregnant women were referred for a fetal anomaly scan because of an abnormal ultrasound finding elsewhere. In 278 cases a fetal structural anomaly was diagnosed, eight of which were documented as isolated bilateral hydrothorax. In all eight cases the reason for referral was polyhydramnios. Maternal age ranged between 22 and 32 years (median, 28 years), maternal parity varied between 1 and 4 (median, 2), and gestational age ranged between 25 and 39 weeks (median, 33 weeks). The ultrasound equipment used was a Diasonics Cardio Vue 100 mechanical sector scanner with a 3.5 or 5.0 MHz transducer. Fluid in the fetal pleural cavity produces a sonolucent image with the lateral and inferior borders shaped by the thoracic wall and diaphragm. The fetal lung appears in the shape of a butterfly wing as an echo-dense area.[7] A diagnosis of isolated bilateral fetal hydrothorax was made if pleural effusions were present in the absence of hydropic changes and other structural malformations. In this series of cases polyhydramnios was defined as a large fluid pocket of 8 cm or more.[8]

When isolated fetal hydrothorax is present, chylothorax and intrauterine infections must be considered.[9] After the initial diagnosis, maternal blood samples were examined for cytomegalovirus, *Toxoplasma gondii*, and rubella infections. Serial ultrasound scans were performed at 2-week intervals to (1) measure the degree of pleural effusion and (2) monitor fetal growth by means of measurement of the upper abdominal cir-

cumference,[10] and fetal well-being according to the biophysical profile score.[11]

Data on fetal and neonatal outcome were obtained in all eight cases of isolated bilateral fetal hydrothorax.

Results

A diagnosis of isolated bilateral fetal hydrothorax and polyhydramnios was confirmed in all eight cases. Maternal blood samples showed no evidence of recent infections.

Serial ultrasound scans revealed spontaneous resolution of pleural effusion in two cases (at 33 and 37 weeks), whereas in the remaining six, no remarkable change in the degree of bilateral hydrothorax was observed. Fetal growth was normal, and the biophysical profile demonstrated that except for polyhydramnios, there was no sign of fetal compromise. All eight pregnancies resulted in the delivery of a live infant (two males and six females). Two infants were delivered at 33 and 36 weeks, respectively. The remaining six infants were born between 38 and 41 weeks of gestation, four spontaneously and two by cesarean section, because of breech presentation or cervical dystocia. Fetal birth weights were situated between the tenth and ninetieth percentiles according to the tables of Kloosterman[12] and were corrected for maternal parity and fetal sex. No congenital anomalies were present. Apgar score at five minutes was 8 or more in seven of eight cases and six in the remaining case. Neonatal development was uneventful in the two infants with spontaneous resolution of pleural effusion. All six infants with isolated bilateral hydrothorax at birth were intubated as a preventive measure immediately after delivery. This was always followed by spontaneous respiration, reflecting lung expansion. After confirmation of isolated bilateral hydrothorax by ultrasonography or chest x-ray films, thoracocentesis was performed under sedation by means of silicone rubber diversion catheters under ultrasonic guidance. The amount of serous fluid collected varied between 50 and 500 ml. In four of six infants, intubation was only maintained for about 30 minutes because of normal lung expansion.

In two infants intubation was sustained for 7 days because of signs of developing respiratory distress as a result of prematurity in one and recurrent fluid accumulation in the pleural cavity necessitating drainage over a 5-day period in the other infant. Chylothorax was established in two cases (one male and one female), including the infant with long-term ventilation support and pleural drainage. A diagnosis of chylothorax was made in the presence of 20 to 50 leukocytes per high-power field, with 90% or more consisting of lymphocytes, and fluid and protein content approaching that of plasma.[13] In the remaining four infants, no signs of chylothorax could be documented. All eight infants were alive and well at the age of 1 month.

Comment

When fluid is found in the fetal chest, a number of other fetal problems must be considered. These include fetal hydrops, whether caused by erythroblastosis or nonimmunologic causes, as well as intrauterine infections.[9] The present study only deals with isolated bilateral fetal hydrothorax. Chylothorax was present in two cases, whereas in the remaining six no cause for pleural effusion could be established. Polyhydramnios was documented in all cases. It has been hypothesized that the onset of polyhydramnios may represent decompensation of amniotic fluid homeostasis caused by collapse of the fetal lung.[9] This is not supported by our observations of normal lung development after birth. Another explanation may be interference with normal swallowing due to increased intrathoracic pressure.[9] Although a few reports on successful prenatal chest drainage have appeared,[2, 3, 5] it should be realized that there is a risk of fetal and maternal harm, such as bleeding after puncture of fetal or maternal vessels, fetal organ damage, premature rupture of the membranes, and premature labor. Moreover, pleuroamniotic shunting may result in technical failure as a result of shunt instability, with the latter risk greatly reduced during the neonatal period. Our conservative approach to isolated bilateral fetal hydrothorax has led to two important observations: (1) that isolated bilateral hydrothorax may resolve spontaneously during fetal life and (2) that fetal outcome may be good without prenatal invasive procedures. The essence of a prenatal diagnosis of isolated bilateral fetal hydrothorax seems to be determined more by the possibility of forewarning the neonatologist, thereby allowing effective diagnostic and therapeutic measures to be taken as soon as delivery has occurred.

To determine the efficacy of prenatal thoracocentesis and pleuroamniotic shunts, several issues must be addressed. First, no large study is available on the natural history of isolated bilateral fetal hydrothorax, although in one report,[14] the mortality rate of chylothorax has been estimated at 5%. Second, better criteria for selecting the fetus with normal pulmonary development should be developed. Third, if an infant survives after successful prenatal chest drainage, it does not necessarily follow that the procedure was beneficial.

Although we do not suggest that conservative management of isolated bilateral fetal hydrothorax will always result in a successful fetal outcome, our results seem to justify a larger study on the natural history of this particular disorder. Because of the risks attached to any invasive intrauterine procedure, a prospective randomized trial should be carried out to compare sur-

vival with and without prenatal shunting in the fetus with isolated bilateral fetal hydrothorax. Since the prevalence of this disorder is very low, the answer can only be obtained through a multicenter study.

REFERENCES

1. Lange IR, Manning FA. Antenatal diagnosis of congenital pleural effusion. AM J OBSTET GYNECOL 1981;140:839.
2. Schmidt W, Harms E, Wolf D. Successful prenatal treatment of non-immune hydrops fetalis due to congenital chylothorax. Case report. Br J Obstet Gynaecol 1985; 92:685.
3. Benacerraf BR, Frigoletto FD, Wilson M. Successful midtrimester thoracocentesis with analysis of the lymphocyte population in the pleural effusion. AM J OBSTET GYNECOL 1986;155:398.
4. Seeds JW, Bowes WA. Results of treatment of severe fetal hydrothorax with bilateral pleuroamniotic catheters. Obstet Gynecol 1986;68:577.
5. Blott M, Nicolaides KH, Greenough A. Pleuroamniotic shunting for decompression of fetal pleural effusions. Obstet Gynecol 1988;71:798.
6. Harrison MR, Bressack MA, Churg AM, et al. Correction of congenital diaphragmatic hernia in utero. Simulated correction permits fetal lung growth with survival at birth. Surgery 1980;20:260.
7. Vintzileos AM, Campbell WA, Nochimson DJ, Weinbaum PJ. Antenatal evaluation and management of ultrasonically detected fetal anomalies. Obstet Gynecol 1987; 69:640.
8. Manning FA, Hill HM, Platt LD. Qualitative amniotic fluid volume determination by ultrasound: antepartum detection of intrauterine growth retardation. AM J OBSTET GYNECOL 1981;139:254.
9. Petres RE, Redwine FO, Cruikshank DP. Congenital bilateral chylothorax. Antepartum diagnosis and successful intrauterine surgical management. JAMA 1982;248: 1360.
10. Campbell S, Wilkin D. Ultrasonic measurement of fetal abdominal circumference in the estimation of fetal weight. Br J Obstet Gynaecol 1975;82:689.
11. Manning FA, Platt LD, Sipos L. Antepartum fetal evaluation: development of a fetal biophysical profile. AM J OBSTET GYNECOL 1980;136:787.
12. Kloosterman G. On intrauterine growth. The significance of prenatal care. Int J Gynaecol Obstet 1970;8:895.
13. Puntis JWL, Roberts KD, Handy D. How should chylothorax be managed? Arch Dis Child 1987;62:593.
14. Broadman RF. Congenital chylothorax. NY State J Med 1975;75:553.

Relapsing polychondritis in pregnancy: A case report and review

Martin L. Gimovsky, MD, and Mark Nishiyama, MD
Los Angeles, California

A case of relapsing polychondritis is reported. This woman was delivered of two normal infants at term.
(AM J OBSTET GYNECOL 1989;161:332-4.)

Key words: Chronic pulmonary disease, pregnancy

Relapsing polychondritis is an uncommon inflammatory disease, characterized by secondary degenerative changes in cartilaginous tissue. Symptoms are diverse, and pathologic changes in cartilage may result in serious pulmonary and cardiovascular sequelae. In 1956 a case was reported of an infant born to a woman with relapsing polychondritis who manifested symptoms identical to those of the mother, suggesting a humorally mediated process. A case is presented here in which a woman diagnosed as having severe relapsing polychondritis was delivered of two normal term infants.

From the Department of Obstetrics and Gynecology, White Memorial Medical Center.
Received for publication May 17, 1988; revised November 10, 1988; accepted March 8, 1989.
Reprint requests: Martin L. Gimovsky, MD, 830 San Vicente Road, Arcadia, CA 91007.

Case report

A 26-year-old woman, gravida 2, para 1, was first seen by the Obstetrical Service at the White Memorial Medical Center at an estimated gestational age of 17 weeks. Her past medical history was significant for episodic shortness of breath with increasing wheezing over the past 7 years. She was initially diagnosed as "asthmatic" and treated with bronchodilators with little improvement in her symptoms. Two years afterward, she underwent a successful term pregnancy with the delivery of a normal female infant, 7 pounds 10 ounces, with subsequent normal neonatal course. In the 4 years that followed, her condition steadily worsened and she was first seen at White Memorial Medical Center 2 years before her second pregnancy for acute exacerbation of

"chronic asthma." At that time ear and nose abnormalities were noted, including saddle nose deformity and auricular chondritis. Consultation with an ear, nose, and throat specialist was obtained. Further history confirmed a 1-year history of pain and swelling in both helices. Neurosensory hearing loss was present but minimal. The suspected diagnosis of relapsing polychondritis was confirmed by biopsy (of auricular cartilage). The biopsy specimen contained an infiltrate of lymphocytes and plasma cells at the cartilage–connective tissue interface. Chest x-ray film revealed marked stenosis of the left mainstem bronchus and tracheal narrowing from the level of T4 to the carina. The patient required intubation and high-dose corticosteroids through several successive hospitalizations and thereafter was managed on a regimen of varying doses of prednisone and dapsone.

At the beginning of her second pregnancy physical examination revealed a cushingoid white female with bilateral auricular chondritis and a saddle nose deformity. Inspiratory and expiratory wheezes were evident. There was no evidence of ocular involvement. A grade II/VI systolic ejection murmur was present. Uterine size was small for dates and an initial ultrasonography was performed. Arterial blood gas measurements while she was breathing room air showed a P_{O_2} of 77 mm Hg, P_{CO2} of 44 mm Hg, and pH of 732. Medications included prednisone USP 5 mg daily, lorazepam 0.5 mg twice a day, and dapsone USP 50 mg daily.

Pulmonary function testing revealed a forced expiratory volume at 1 second of 1.1 L (43% of predicted value). The forced expiratory volume at 1 second/forced vital capacity was 71% of the predicted value. Maximum voluntary ventilation was 32 L/min, 30% of the predicted value. Test results for antinuclear antibody and rheumatoid factor were negative. A 24-hour total urine collection was within normal limits. An echocardiogram was normal.

Fetal growth was evaluated by serial ultrasonography. At 17 weeks the patient complained of increasing shortness of breath with productive cough. History revealed the patient had taken herself off prednisone. She was restarted on a regimen of prednisone, 5 mg daily, and was given a course of ampicillin. At 23 weeks of gestation, increased wheezing was noted and a blood gas analysis while she was breathing room air revealed a P_{O_2} of 74 mm Hg, P_{CO_2} of 26 mm Hg, and pH of 747. The patient was treated with an inhalational bronchodilator and was noted to be much improved 3 days later. At 26 weeks prednisone was increased to 10 mg daily and dapsone was discontinued. A repeat diabetic screen was within normal limits. A productive cough recurred at 36 weeks and was again treated successfully with ampicillin. At 40½ weeks' gestation the patient underwent an uneventful labor and spontaneous delivery. She was given additional hydrocortisone peripartum (100 mg hydrocortisone every 8 hours for three doses). She was delivered of a 3189 gm male infant with Apgar scores of 7 and 9.

Evaluation of the infant failed to disclose any abnormalities. Follow-up of the infant at age 1 and 2 years demonstrated a normal course.

Table I. Clinical and histopathologic features of relapsing polychondritis[1]

Clinical
1. Recurrent chondritis of the auricles
2. Inflammation of ocular structures, including conjunctivitis, keratitis, scleritis, and/or uveitis
3. Chondritis of laryngeal and/or tracheal cartilage
4. Cochlear and/or vestibular damage manifest by neurosensory hearing loss, tinnitus, and/or vertigo
5. Seronegative inflammatory polyarthritis
Histopathologic
1. Loss of basophilic staining of cartilage matrix
2. Perichondral inflammation
3. Cartilage destruction and replacement with fibrous tissue
4. Cellular damage to chondrocyte

Comment

Episodic dyspnea coupled with inspiratory and expiratory wheezing in a woman of child-bearing age immediately suggests the diagnosis of reversible bronchoconstriction ("asthma"). The inability to obtain a satisfactory response to bronchodilators suggests other forms of obstructive pulmonary disorders seen in the child-bearing years such as cystic fibrosis and α_1-antitrypsin deficiency. Both of these disorders include a significant fetal risk in addition to the potential for serious maternal sequelae.

Relapsing polychondritis is a multisystem disorder characterized by the potential for destruction of cartilage with fibrous tissue replacement. The diagnosis is based on a combination of clinical criteria with pathologic confirmation (Table I). These criteria are both clinical and histopathologic. When three or more of these criteria are met, including biopsy confirmation, the diagnosis may be considered "certain."[1]

Involvement of the respiratory tract is reported as the cause of most of the deaths in relapsing polychondritis. The patient manifests dyspnea, wheezing, cough, a choking sensation, and tenderness over the thyroid cartilage or anterior cervical trachea. Airway obstruction and pulmonary infection are common.

The arthritis of relapsing polychondritis may involve large or small joints. Initially the symptoms may be migratory and resemble rheumatoid arthritis.

Cardiovascular manifestations occur in approximately 25% of patients with relapsing polychondritis. Dilatation of the ascending aorta with destruction of the aortic valve cusps may result in aortic regurgitation or aortic aneurysm.

Renal involvement is less common. Biopsy specimens show glomerulonephritis with immunoglobulin G, immunoglobulin M, and C 3 deposits consistent with an immune complex etiology.

Laboratory findings are inconsistent. More than half of the patients have anemia resulting from chronic disease and an elevated sedimentation rate. A minority will have an increase in quantitative immunoglobins, a positive rheumatoid factor, and a false-positive serologic test result.

An autoimmune pathogenesis is suggested by the presence of circulating antibodies to type II collagen in patients with relapsing polychondritis. These antibodies appear to correlate with the clinical severity of symptoms. Cellular mediated immunity has been demonstrated by lymphocyte transformation in response to proteoglycans in relapsing polychondritis. In a rodent model in which autoimmunity to type II collagen is induced, inflammatory ear lesions and acute arthritis have been produced.[2] The pathologic changes include loss of basophilic staining of cartilage matrix, inflammatory cell infiltration, phagocytosis of chondrocytes, and total destruction of cartilage with fibrous replacement. In the case report[3] of an infant born to a woman with relapsing polychondritis who manifested symptoms at birth, the infant's symptoms, which included anemia of uncertain cause and transient arthritis, cleared in the first year of life, which is consistent with a transplacental humorally mediated process.

Treatment has been primarily symptomatic, with both corticosteroids and nonsteroidal antiinflammatory drugs used during active episodes. Dapsone has been used extensively because of its ability to inhibit lyzosomal enzymes in hypervitaminosis A–induced chondritis, a condition histologically similar to relapsing polychondritis. Because of its folic acid antagonism, in addition to the risk of Stevens-Johnson syndrome and neutropenia, use of dapsone is a significant risk during pregnancy.

Relapsing polychondritis may be an example of a humorally mediated autoimmune disease that may affect both the fetus and newborn by transplacental antibody passage. Chronic respiratory insufficiency in a gravid woman that is not responsive to standard therapy should always be further investigated for less likely causes, which include systemic disorders such as relapsing polychondritis.

We acknowledge the help of Jean Lawler, Craig Knecht, and Lawrence Spencer-Smith in the preparation of this manuscript.

REFERENCES

1. McAdam LP, O'Hanlan MA, Bluestone R, et al. Relapsing polychondritis: a prospective study of 23 patients and a review of the literature. Medicine 1976;55:193.
2. Foidart JM, Abe S, Martin GR, et al. Antibodies to type II collagen in relapsing polychondritis. N Engl J Med 1978; 299:1203.
3. Arundell FW, Jaserick JR. Familial chonic atrophic polychondritis. Arch Dermatol 1960;82:439.

A complete list of references is available from the authors on request.

Abscess of allantoic duct remnant

I. Cori Baill, MD, G. William Moore, MD, PhD, and Lora A. Hedrick, MD
Baltimore, Maryland

A recently submitted placenta was found to be histopathologically unique. Funicitis was present at the early gestational age of 22 1/7 weeks, and an abscessed allantoic duct remnant was present. The latter finding had not previously been seen at this pathology department, nor could reference to it be found in the literature. (Am J Obstet Gynecol 1989;161:334-6.)

Key words: Allantoic duct remnant, funicitis, placenta pathology

A 17-year-old para 0-0-1-0 woman registered for prenatal care at dates equal to examination at 8 4/7 weeks' gestation. Her registration cervical culture was positive for gonococcus. Subsequent to treatment with oral ampicillin 3.5 gm and oral probenecid 1.0 gm, her culture was negative. She also was prophylactically treated for *Chlamydia* with standard doses of erythromycin. Ampicillin was prescribed 5 days before her admission, secondary to a positive cervical culture for group B streptococci.

The patient was admitted with a 21-hour history of

From the Departments of Gynecology and Obstetrics and Pathology, The Johns Hopkins Medical Institutions.
Received for publication November 18, 1988; accepted March 20, 1989.
Reprint requests: G. William Moore, MD, PhD, Department of Pathology, The Johns Hopkins Medical Institutions, 600 N. Wolfe St., Baltimore, MD 21205.

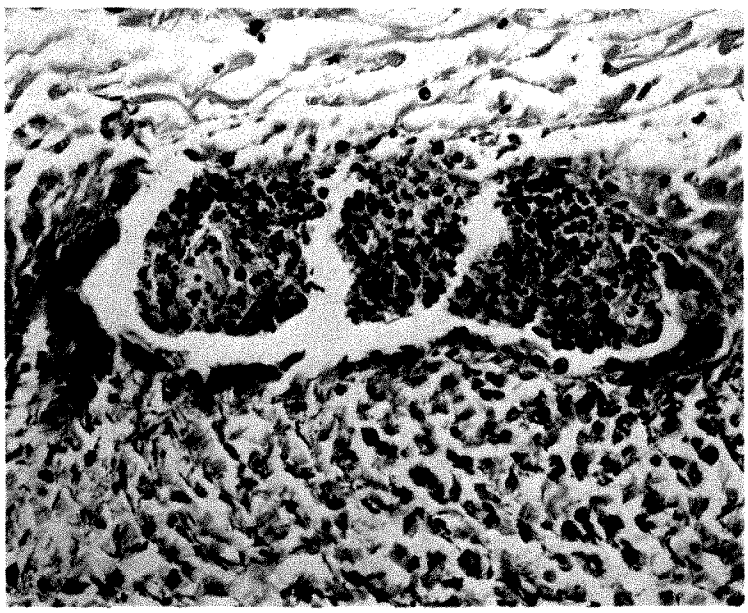


Fig. 1. Abscess of allantoic duct remnant. Typical type of transitional epithelial lining of allantoic duct remnant enclosed pool of polymorphonuclear leukocytes. (Hematoxylin and eosin stain; original magnification ×400.)

cramps, lower abdominal pain, and back pain. Her initial physical examination revealed the following: temperature, 37.7° C; fetal heart rate, 160 to 170 beats/minute; maternal heart rate, 102 beats/minute; respirations, 18; blood pressure, 110/67; fundal height, 21 to 22 cm; cervix, 3 to 4 cm; 50% effaced; fern negative. The best obstetric estimate at admission was 22 1/7 weeks' gestation, established by her early examination that was consistent with the last menstrual period, and an ultrasonographically derived gestational age of 19.9 weeks at dates of 19 weeks. Ritodrine tocolysis failed, and 7 hours after her admission the patient was delivered of a 540 gm girl with Apgar scores of 1 at 1 minute and 1 at 5 minutes.

Findings

The placenta weighed 210 gm and was grossly normal for gestational age. Histopathologic evidence of chorioamnionitis and funicitis with allantoic duct abscess were present. The chorioamnionic membrane showed massive infiltration with polymorphonuclear leukocytes and gram-negative bacterial overgrowth that extended from the decidua into the amnionic membrane. The amnionic epithelium was intact, but the chorionic cells were largely necrotic.

The umbilical vein showed marked infiltration by polymorphonuclear leukocytes that extended beyond the muscular wall and into the Wharton's jelly. The two umbilical arteries had leukocytes within, but not beyond, the muscular wall. The allantoic duct remnant showed a small abscess that consisted of leukocytes with a fragmented transitional lining (Fig. 1). There was mild condensation of Wharton's jelly around the duct remnant.

Comment

Funicitis in this case presumably resulted from margination and subsequent migration of fetal leukocytes into the media around the umbilical vessels. The sequence of cord inflammatory lesions tends to be a progression from vein involvement, to the arteries, and finally to the cord substance.[1] Allantoic duct remnant involvement in this case may have resulted from contiguous spread.

Lavery[1] states that inflammatory lesions of the cord are quite unusual at less than 26 weeks' gestation, and this observation is supported by Chellam and Rushton's[2] study of chorioamnionitis and funicitis in the placentas of 200 neonates that weighed <2500 gm. They found inflammatory lesions present in 48.5% of all specimens, but only 9.5% involved the cord. Allantoic duct remnants were not noted to be involved.

During the third to fifth weeks' gestation the allantois is responsible for fetal blood formation. Its blood vessels comprise the umbilical vessels of the forming umbilical cord. At 3 to 4 weeks' gestation, cephalocaudal flexion of the embryo leads to fusion of the yolk sac projection and its vitelline vessels to the connecting stalk that continues the allantois and umbilical vessels, and thus forms the cord at 5 to 6 weeks' gestation.[2]

Sections taken from just beyond the fetal end of the cord, but rarely at the maternal end, commonly reveal

allantoic duct remnants.[1] The allantoic duct remnant is usually situated between the two umbilical arteries and is generally lined by flattened transitional epithelium. The duct wall may have condensation of Wharton's jelly. In contrast, yolk sac (omphalomesenteric duct) remnants are situated at the margin of the cord, are usually cubical or columnar epithelium, and may contain mucus. These rarely differentiate toward gastrointestinal-type structures. Cysts, polyps, a duct lined by gastric-type mucosa, and nodules that contain pancreatic or small bowel tissue have been described.

In summary, this is a case of an abscess in an allantoic duct remnant, secondary to the presence of funicitis in a 22 1/7 weeks' gestation umbilical cord.

REFERENCES

1. Lavery JP. Appendages of the placenta. In: Lavery JP, ed. The human placenta: clinical perspectives. Baltimore: Aspen Publishers. 1987:49-50.
2. Chellam VG, Rushton DI. Chorioamnionitis and funiculitis in the placentas of 200 births weighing less than 2.5 kg. Br J Obstet Gynaecol 1985;92:808-14.

Infection and labor

IV. Cachectin–tumor necrosis factor in the amniotic fluid of women with intraamniotic infection and preterm labor

Roberto Romero, MD, Kirk R. Manogue, PhD, Murray D. Mitchell, DPhil, Ying King Wu, MD, Enrique Oyarzun, MD, John C. Hobbins, MD, and Anthony Cerami, PhD

New Haven, Connecticut, New York, New York, and Salt Lake City, Utah

A growing body of evidence supports a causal link between subclinical intrauterine infection and preterm labor. The mechanisms responsible for the onset of parturition in this setting have not been elucidated. The conventional view has been that bacterial products increase prostaglandin biosynthesis by intrauterine tissues and this, in turn, leads to the onset of labor. An alternative or complementary mechanism is that microbial products activate the host monocyte-macrophage system and that cytokines released during this process signal the initiation of parturition by stimulating prostaglandin biosynthesis by intrauterine tissues. This study was conducted to determine if cachectin–tumor necrosis factor is present in the amniotic fluid of women with intraamniotic infection and whether this cytokine can alter the rate of prostaglandin biosynthesis by intrauterine tissues. Amniotic fluid from 54 women was assayed for tumor necrosis factor. Tumor necrosis factor was not detectable in the amniotic fluid of women without intraamniotic infection regardless of the presence or absence of term or preterm labor. On the other hand, the amniotic fluid of 11 of 15 women with preterm labor and intraamniotic infection had measurable tumor necrosis factor. This cytokine stimulated prostaglandin E_2 biosynthesis by amnion cells in monolayer culture in a dose-dependent fashion. These data support the concept that macrophage activation is involved in the onset of human parturition in the setting of infection. We propose that the host (fetus and/or mother) signals the onset of parturition through the secretion of inflammatory cytokines released in response to bacterial invasion. (Am J Obstet Gynecol 1989;161:336-41.)

Key words: Tumor necrosis factor, intraamniotic infection, labor, parturition, preterm labor, prostaglandins, cytokines, interleukin-1, prematurity

From the Department of Obstetrics and Gynecology, Yale University School of Medicine, New Haven, the Laboratory of Medical Biochemistry, Rockefeller University, New York, and the Department of Obstetrics and Gynecology, University of Utah, Salt Lake City.
Supported by grants from the Walter Scott Foundation for Medical Research and National Institutes of Health grant HD 20779. Dr. Romero is the recipient of a Physician Scientist Award from the National Institute of Child Health and Human Development.
Received for publication September 18, 1988; accepted February 15, 1989.
Reprint requests: Roberto Romero, MD, Yale University School of Medicine, Department of Obstetrics and Gynecology, P.O. Box 3333, New Haven, CT 06510.

Prematurity is the leading cause of perinatal mortality and morbidity worldwide.[1] Accumulating evidence indicates that preterm labor is causally related to subclinical intrauterine infection.[2] Increased biosynthesis of prostaglandins by intrauterine tissues is widely accepted as a key event in the initiation of parturition.[3] Mechanisms responsible for increased bioavailability of prostaglandins in preterm labor associated with infection have not been defined. The conventional view has been that bacteria and their products are responsible

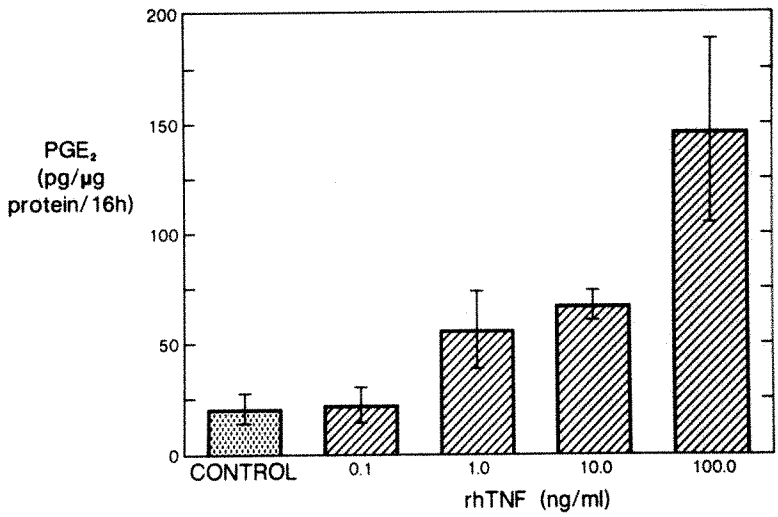


Fig. 1. Effect of different concentrations of rHuTNF on prostaglandin production by amnion cells in primary culture (representative experiment). Mean and SEM are depicted.

for the increased biosynthesis of prostaglandins that results in the onset of labor.[4,5] However, in recent years, it has been recognized that many of the metabolic derangements associated with microbial infection are mediated by endogenous factors.[6] Cachectin–tumor necrosis factor (TNF), a monokine produced in response to bacterial lipopolysaccharide, is an inflammatory mediator implicated in the pathogenesis of shock and cachexia associated with infection.[6] TNF stimulates prostaglandin production by several cell types.[7] Thus we hypothesize that bacterial products stimulate fetal and/or maternal cells to produce a network of interacting cytokines (including interleukin-1 and TNF), which in turn increase prostaglandin biosynthesis by intrauterine tissues and results in the onset of labor. To test this hypothesis, the concentration of TNF was measured in amniotic fluid of women in preterm labor, and the effect of this monokine on prostaglandin biosynthesis by human amnion was determined.

Material and methods

Study design. Amniotic fluid was collected from 54 women classified into four groups: group 1, women with term pregnancies without labor or infection (n = 14); group 2, women with term pregnancies in active labor (cervical dilatation >6 cm) but without infection (n = 13); group 3, women with preterm labor leading to preterm delivery within 24 hours of admission but without infection (n = 12); group 4, women with preterm labor and intraamniotic infection (n = 15). An intraamniotic infection was defined as the presence of a positive amniotic fluid culture whether or not clinical signs of chorioamnionitis were present. Labor was defined as the presence of regular uterine contractions that led to cervical dilatation and spontaneous vaginal delivery. Amniotic fluid was obtained by transabdominal amniocentesis from all women except for those in group 2. In our institution, amniocentesis is performed routinely on all women with preterm labor and/or premature rupture of membranes to screen for intraamniotic infection. Patients in group 1 had amniocentesis for evaluation of lung maturity before an elective cesarean section. Patients in group 2 had a transvaginal amniotomy when they were in active labor at >6 cm of dilatation. No serial samples from the same patient were included in this study.

Amniotic fluid culture technique. Amniotic fluid was transported to the laboratory in a capped plastic syringe immediately after amniocentesis. Amniotic fluid was plated within 30 minutes of collection in all cases. Cultures for aerobic and anaerobic bacteria, *Mycoplasma hominis* and *Ureaplasma urealyticum* were performed. Methods of bacterial culture and identification have been described previously in detail.[8] *Mycoplasma* species were cultured with a commercially available system (Mycotrim-GU, Berkeley, Calif.).

Quantitation of TNF. The cachectin-TNF concentration in amniotic fluid was measured by modification of the standard L-929 cell cytotoxicity assay in the presence of actinomycin D.[9] Aliquots of amniotic fluid were thawed and diluted 1 : 10 in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and filter sterilized. Aliquots of 50 μl were then added in triplicate to microtiter plate wells already containing subconfluent (1×10^5) L-929 cells under 50 μl fresh Dulbecco's modified Eagle's medium–fetal bovine serum with actinomycin D (2 μg/ml) and with or without TNF-neutralizing monoclonal antibody (MAb 2-2-3E3, a gift from Chiron Corp., Emeryville, Calif.) at a final antibody concentration of 10 μg/ml. This concentration of anti-TNF antibody is sufficient to neutralize more than 5 ng of recombinant human cachectin–TNF

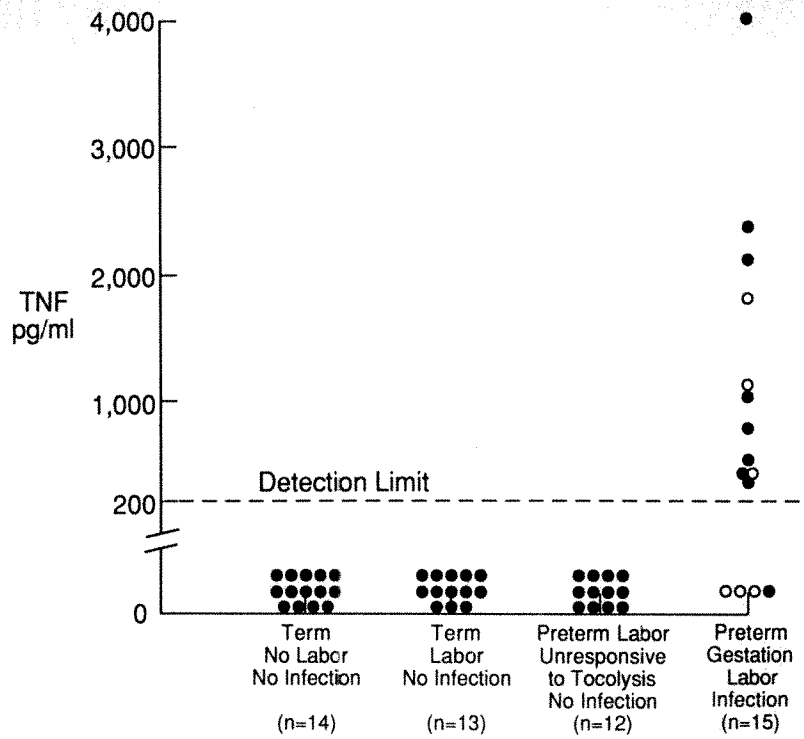


Fig. 2. Amniotic fluid concentrations of TNF in different study groups. *Open circles* represent women with premature rupture of membranes. *Solid circles* represent women with preterm labor and intact membranes.

[(rHuTNF), also donated by Chiron Corp.] per well (500 units). A series of triplicate standards was included on each assay plate. Final concentrations were no TNF; 41 ng rHuTNF/ml; and 4.1 ng/ml to 8 pg/ml in a ×2 dilution series. After an 18-hour incubation at 37° C in a humidified 5% carbon dioxide incubator, viable cells were estimated by their ability to reduce thiazolyl blue (MTT; Sigma Chemical Co., St. Louis) to a colored formazan[10, 11] during an additional 4-hour incubation after the addition of 10 μl MTT (5 mg/ml) to each well. Wells were then aspirated dry and cells lysed with 100 μl of 0.04N hydrochloric acid in isopropanol and metabolized dye further solubilized with 100 μl water. Optical densities were read in a dual wavelength 96-well microtiter plate reader (Bio-Tek Instruments, Burlington) at 570 and 690 nm. Mean sample (or standard) optical densities were converted to percent cells dead using the mean optical density of control (no rHuTNF added) wells as 0% cells dead and the mean optical density of 41 ng rHuTNF/ml wells as 100% cells dead for each assay plate. Percent cells dead is linearly related to the log concentration of rHuTNF with good agreement over the range 10% to 90% cells dead (data not shown). For each sample (in both presence and absence of a neutralizing antibody), an equivalent concentration of rHuTNF was interpolated from the mean rHuTNF dilution curve of cytotoxicity. These interpolated values were multiplied by 20 to account for dilution of the original samples. The difference (equivalent picograms per milliliter without antibody minus equivalent picograms per milliliter with antibody) was used to represent TNF-specific cytotoxicity. In general, 10% cells dead corresponds to approximately 10 pg rHuTNF per milliliter assay medium. Thus the lower limit of detection is approximately 200 equivalent pg rHuTNF per milliliter of undiluted amniotic fluid. At 1:20 final dilution, only three of 54 samples had >10% cytotoxicity that was not neutralized by the anti-TNF antibody. These samples were considered negative for TNF. Cytotoxicity in all other samples was reduced to <10% in the presence of the TNF-neutralizing antibody.

Prostaglandin production by human amnion. Experiments to evaluate the effect of TNF on prostaglandin biosynthesis by intrauterine tissues were conducted on human amnion in primary culture. This system has been shown to be morphologically, enzymatically, and biochemically similar to fresh amnion.[12] Amnion was taken from patients having elective cesarean section at term, before the onset of labor. Amnion cultures were established as previously described.[12] Purified rHuTNF (>98% pure by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, 1×10^8 U/mg, 25 ng of endotoxin per milligram of protein by *Limulus* amebocyte lysate test; Chiron Corp.) was added to amnion cells with final concentrations of 0.1, 1, 10, and 100 ng/ml in Dulbecco's modified Eagle's medium.

Table I. Clinical, microbiologic, and amniotic fluid TNF data in women with preterm labor and intraamniotic infection

Patient identification no.	*Gestational age (wk)*	*Organism*	*Colony count (cfu/ml)*	*Amniotic fluid TNF (pg/ml)*	*Membrane status*
1	34	*Haemophilus influenzae*	—	ND	R
2	24	*Bacteroides neolyticum*	—	357	I
3	25	*Streptococcus viridans*	$>10^5$	1798	R
4	31	*Gardnerella vaginalis*	$>10^5$	414	R
5	26	*Enterococcus* sp.	<100	2370	I
6	26	*Fusobacterium* sp.	2000	765	I
7	29	*Streptococcus viridans*	10^5	4016	I
8	30.5	*Streptococcus agalactiae*	+	1130	R
9	34	Group A streptococci	2000	ND	R
10	27	*Klebsiella* sp. *Ureaplasma urealyticum*	$>10^5$	2110	I
11	33	Mixed anaerobic *Proteus vulgaris*	$>10^5$ $>10^5$	ND	R
12	29	*Streptococcus viridans*	10^5	ND	I
13	22	*Bacteroides* sp.	5000	507	I
14	26	*Bacteroides fragilis*	$>10^5$	1016	I
15	32	*Haemophilus influenzae*	$>10^5$	414	I

cfu/ml, Colony-forming units per milliliter of amniotic fluid; *ND*, nondetectable; *R*, premature rupture of membranes; *I*, intact membranes (preterm labor).

Incubations were conducted for 16 hours at 37° C in a humidified incubator. Each condition of testing was repeated in a given primary amnion culture in replicates of four. The effect of rHuTNF on amnion production of prostaglandin E_2 (PGE_2) was examined in primary cultures from five separate patients. PGE_2 is the main product of arachidonic acid metabolism in amnion. PGE_2 was measured by a specific and sensitive radioimmunoassay previously described in detail.[13] Protein quantification was performed by the method of Lowry et al.

Statistical analysis. The effect of TNF on PGE_2 biosynthesis by amnion cells was examined by analysis of variance with a factorial design. A logarithmic transformation of PGE_2 output by amnion cells data was performed before analysis.

Results

Fig. 1 illustrates a representative experiment of the effect of TNF on the production of PGE_2 by human amnion. A concentration-dependent stimulation was observed in all of the five amnion cell cultures A significant increase in PGE_2 biosynthesis occurred at TNF final concentrations of 1, 10, and 100 ng/ml ($p < 0.001$). A significant variability existed in the basal rate and in the maximum rate of PGE_2 production in response to TNF among different amnion cell cultures ($p = 0.0001$).

Fig. 2 displays the concentrations of TNF in amniotic fluid of women in the four study groups. No detectable TNF was found in the amniotic fluid of women at term with or without labor (groups 1 and 2). Similarly, TNF was not detected in the amniotic fluid of women with preterm labor and without intraamniotic infection (group 3). Of 15 patients with intraamniotic infection and preterm labor, 11 had detectable TNF. Table I displays the microbiologic features and TNF concentrations in each patient with intraamniotic infection (group 4).

Comment

The mechanisms responsible for the onset of labor in patients with intraamniotic infection are poorly understood. A role for prostaglandins is suggested by their increased concentration in amniotic fluid in women with intraamniotic infection.[14] The signals responsible for their increased bioavailability in this setting have not been elucidated. To date, research has focused on the role of bacteria on arachidonic acid metabolism by human amnion. Microorganisms indigenous to the genital tract have been shown to have phospholipase activity,[4] and microbial products can stimulate the production of prostaglandin E by amnion cells in culture.[5]

We have also demonstrated that endotoxin stimulates prostaglandin production by intrauterine tissues (amnion and decidua) in a dose-dependent manner.[15] However, the concentrations of endotoxin required to elicit a significant stimulation in prostaglandin biosynthesis in vitro is often higher than that found in patients with intraamniotic infection. This suggests that a direct endotoxin effect may not be the sole mechanism by which microorganisms lead to the onset of human parturition. It is possible that microbial products other than endotoxin may play a role.

An alternative hypothesis is that the signals for the onset of human parturition are of host origin. Bacterial endotoxin can induce TNF production by a host cell(s)

that in turn stimulates prostaglandin production by intrauterine tissues. Our data clearly show that TNF stimulates PGE_2 biosynthesis by human amnion over a range of TNF concentrations detected in amniotic fluid of women with intraamniotic infection and preterm labor. These observations are consistent with the effects of TNF on arachidonic acid metabolism reported in other cell types.[7] The stimulatory effect of rHuTNF on the biosynthesis of PGE_2 by amnion cells cannot be attributed to contamination of the recombinant material with endotoxin because nanogram quantities of this bacterial product are generally required to stimulate PGE_2 biosynthesis by human amnion.[16] Our data suggest that amniotic fluid TNF does not play a role in the onset of physiologic labor at term or in preterm labor not associated with intraamniotic infection because TNF could not be detected in amniotic fluid from women with these clinical conditions (groups 2 and 3). TNF was present in women with intraamniotic infections and preterm labor (group 4). This suggests that a host-mediated response, evidenced by TNF concentrations, is associated with the onset of parturition in this particular subset of patients.

Although amniotic fluid TNF concentrations have been measured as an index of host response to microbial infection, this is not the only host product capable of stimulating prostaglandin biosynthesis by human intrauterine tissues. Interleukin-1[17] and platelet activating factor (PAF) have been identified in the amniotic fluid of women with intraamniotic infection (Hoffman D, Romero R, Johnston J, unpublished observations) and can also increase prostaglandin biosynthesis by human amnion[18] and decidua (unpublished observations). Thus TNF may be only one of several mediators utilized by the host to signal the initiation of labor. Interleukin-1 or PAF may be involved synergistically or predominantly in the cases of preterm labor with intraamniotic infections in which amniotic TNF either is not detectable or is not in the range required for significant stimulation of prostaglandin production. Indeed, synergism between interleukin-1 and TNF in the stimulation of prostaglandin production by synovia has been demonstrated. TNF produced by human decidua may play a role in the initiation of labor in cases of extraamniotic infection (deciduitis). Macrophages in decidua produce TNF in response to bacterial products and this cytokine stimulates prostaglandin production by decidual cells.[19] The relative importance of the different components of the cytokine network in human parturition has not been established. We have recently determined that interleukin-1 is more potent than TNF and endotoxin in stimulating prostglandin biosynthesis by intrauterine tissues (unpublished observations). Furthermore, interleukin-1 is present more frequently than TNF in the amniotic fluid of women with intraamniotic infection and preterm labor. Therefore it is tempting to conclude that interleukin-1 may participate to a greater extent than TNF in this process.

On the basis of these data, we propose a model in which the initiation of human parturition in the presence of infection is controlled by the host. Systemic maternal infections such as pyelonephritis or localized infections such as deciduitis could trigger parturition by activating the maternal monocyte-macrophage system. A second host, the fetus, may also signal the onset of labor when the infection has extended to the intraamniotic cavity and activates the fetal immune system. Preterm labor can therefore be viewed as an event occurring when the intrauterine or maternal environment is hostile and threatens the well-being of the fetus and/or mother.

REFERENCES

1. van den Berg BJ, Oechsli FW. Prematurity. In: Bracken MB, ed. Perinatal epidemiology. London: Oxford University Press, 1984:69-85.
2. Romero R, Mazor M, Wu YK, et al. Infection in the pathogenesis of preterm labor. Semin Perinatol 1988;12:262-79.
3. Novy MJ, Liggins GC. Role of prostaglandins, prostacyclin and thromboxanes in the physiologic control of the uterus and in parturition. Semin Perinatol 1980;4:45-66.
4. Bejar R, Curbelo V, Davis C, et al. Premature labor. II. Bacterial sources of phospholipases. Obstet Gynecol 1981;57:479-82.
5. Lamont RF, Rose MP, Elder MG. Effect of bacterial products on prostaglandin E production by amnion cells. Lancet 1985;2:1331-3.
6. Beutler B, Cerami A. Cachectin: more than a tumor necrosis factor. N Engl J Med 1987;316:379-85.
7. Dayer JM, Beutler B, Cerami A. Cachectin/tumor necrosis factor stimulates collagenase and prostaglandin E_2 production by human synovial cells and dermal fibroblasts. J Exp Med 1985;162:2163-8.
8. Romero R, Emamian M, Wan M, et al. The value of the leukocyte esterase test in diagnosing intraamniotic infection. Am J Perinatol 1988;5:64-9.
9. Aggarwal BB, Kohr WJ, Hass PE, et al. Human tumor necrosis factor: production, purification, and characterization. J Biol Chem 1985;260:2345-54.
10. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods 1983;65:55-63.
11. Green LM, Reade JL, Ware CF. Rapid colorimetric assay for cell viability: application to the quantitation of cytotoxic and growth inhibitory lymphokines. J Immunol Methods 1984;70:257-68.
12. Okita JR, Sagawa N, Casey ML, et al. A comparison of human amnion tissue and amnion cells in primary culture by morphological and biochemical criteria. In Vitro 1983;19:117-26.
13. Mitchell MD, Flint AP. Prostaglandin production by intrauterine tissues from periparturient sheep: use of a superfusion technique. J Endocrinol 1978;76:111-21.
14. Romero R, Emamian M, Wan M, Hobbins JC, Mitchell MD. Prostaglandin concentrations in amniotic fluid of women with intraamniotic infections and preterm labor. Am J Obstet Gynecol 1987;157;1461-7.
15. Romero R, Kadar N, Hobbins JC, Duff GW. Infection and labor. II. The detection of endotoxin in amniotic fluid. Am J Obstet Gynecol 1987;157:815-9.

16. Romero R, Hobbins JC, Mitchell MD. Endotoxin stimulates prostaglandin E_2 biosynthesis by human amnion. Obstet Gynecol 1988;71:227-8.
17. Romero R, Brody DT, Oyarzun E, et al. Infection and labor. III. Interleukin-1: a signal for the onset of parturition. Am J Obstet Gynecol. 1989;160:1117-23.
18. Billah MM, DiRenzo GC, Ban C. Platelet-activating factor metabolism in human amnion and the responses of this tissue to extracellular platelet-activating factor. Prostaglandins 1985;30:841-50.
19. Romero R, Mazor M, Wu YK, et al. Bacterial endotoxin and tumor necrosis factor stimulate prostaglandin production by human decidua. Prostaglandins Leukot Essent Fatty Acids (In press).

Pulsed Doppler flow-velocity waveforms in the prediction of fetal hematocrit of the severely isoimmunized pregnancy

Joshua A. Copel, MD, Peter A. Grannum, MD, Jacqueline J. Green, MS, RDMS, Kathy Belanger, PhD, and John C. Hobbins, MD

New Haven, Connecticut

We previously reported a significant relationship between the hematocrit levels of anemic fetuses and information derived from ultrasonographic and Doppler flow-velocity waveforms. In this study we prospectively tested two formulas for the prediction of hematocrit values, by use of gestational age, presence or absence of hydrops, and Doppler indices. Although one of the two formulas predicted hematocrit values significantly, the only component of the formula that made a significant contribution was fetal hydrops. We conclude that currently available fetal Doppler measurements are unable to be applied in the prospective prediction of hematocrit values in anemic fetuses of isoimmunized pregnancies. (Am J Obstet Gynecol 1989;161:341-4.)

Key words: Prenatal diagnosis, ultrasonography, Doppler ultrasonography, fetal treatment, erythroblastosis fetalis, isoimmunization

Management of the severely isoimmunized pregnancy has recently evolved from indirect to direct means of fetal evaluation and treatment. Amniotic fluid optical density measurements of bilirubin may now be supplemented with fetal phlebotomy and direct hematocrit determinations, and intraperitoneal transfusion is rapidly being replaced by fetal intravascular transfusion.

A continuing question in the management of such patients has been the proper place of fetal blood sampling. Although valuable information may be gained, the procedure presently carries a higher inherent risk than amniocentesis and can be performed with limited frequency. A noninvasive means of determining the fetal hematocrit would obviously be preferable in the evaluation of patients, before the initiation of a course of transfusions and to determine the optimal timing of follow-up transfusions.

The use of fetal Doppler flow velocity waveforms has been proposed as an indirect means of gaining information concerning the fetal hematocrit. Based on the reports of other investigators,[1-3] we previously reported that equations could be derived that relate Doppler waveforms to fetal hematocrit.[4] We now report a prospective trial of the ability of Doppler information to predict hematocrit.

Material and methods

Between August 1, 1987, and March 1, 1988, 13 patients were referred to the Yale Perinatal Unit because of severe isoimmunization for intravascular transfusion therapy. Gestational ages varied from 20 to 32 weeks (mean, 27 weeks). All patients had evidence of erythroblastosis on the basis of indirect Coombs tests and either fetal hydrops or amniotic fluid optical density measurement in zone III or upper zone II by Liley curves.

Before initial intravascular transfusion in all patients had full ultrasonographic evaluation of gestational age

From the Section on Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Yale University School of Medicine.
Received for publication September 25, 1988; accepted February 15, 1989.
Reprint requests: Joshua A. Copel, MD, Department of Obstetrics and Gynecology, Yale University School of Medicine, 333 Cedar St., P.O. Box 3333, New Haven, CT 06510-8063.

Table I. Comparison of actual hematocrit value and Doppler predictions

Patient no.	*Actual hematocrit value (%)*	*Formula I*	*Formula II*
1	17	24	19
2*	10	13	8
3	27	25	29
4	27	28	32
5	18	22	20
6	13	24	27
7*	11	11	16
8*	14	15	33
9	29	24	20
10*	14	14	23
11	32	22	19
12	30	25	22
13	21	26	27

*Hydropic fetuses.

and gross anatomy, including fetal echocardiography. Fetal weight was estimated according to Shepard et al.,[5] except in cases of gross fetal hydrops, when a mean weight for estimated age was used since abdominal distention would invalidate the Shepard formula. Four of the fetuses in this report were hydropic at the time of study.

Pulsed Doppler waveforms were obtained from the fetal umbilical artery and descending aorta during periods of fetal apnea (General Electric model RT 3600, GE, Milwaukee). Three consecutive umbilical waveforms were analyzed by means of the Pourcelot index, systolic/diastolic ratio, and pulsatility index[2] and the mean value was calculated. The fetal descending aortic signal was angle corrected at the time it was obtained. Both pulsatility index and peak aortic velocity were calculated, with the mean of three values used.

Fetal transfusions were performed as previously described.[6, 7] Before blood infusion, sufficient fetal blood was removed for measurement of hemoglobin and hematocrit values by standard automated technique. Fetal origin of the blood was confirmed by Kleihauer-Betke staining.

Doppler results were stored on a spreadsheet in a personal computer and previously reported formulas relating Doppler information to fetal hematocrit were calculated.[4] The two formulas used were:

$$\text{Hematocrit} = 7.778 - (0.088 \times \text{peak aortic velocity}) + (0.968 \times \text{gestational age}) - (10.911 \text{ if hydropic}) \quad \text{(I)}$$

$$\text{Hematocrit} = 45.312 - (56.261 \times \text{umbilical artery Pourcelot index}) - (0.128 \times \text{peak aortic velocity}) + (1.042 \times \text{gestational age}) \quad \text{(II)}$$

Results

The predicted hematocrits were compared to the actual hematocrits as shown (Table I, Fig. 1). Whereas formula I continued to be significantly correlated with the actual hematocrit value ($r = 0.706$, $p < 0.007$), formula II was not ($r = 0.286$, $p < 0.35$). Patients 2, 7, 8, and 10 were hydropic at the time of presentation; the others were not hydropic.

Formula I was derived from three factors: the gestational age, the peak velocity in the descending aorta, and a factor for hydrops. Each of these was independently compared to the actual hematocrit level to determine whether each was significantly related, or whether one factor was responsible for the significant correlation observed. The only significant correlation was between hydrops and hematocrit level ($p < 0.007$). The gestational age and peak aortic velocity were not significantly correlated with hematocrit value ($p = 0.51$, and $p = 0.26$, respectively).

Comment

The relationship between Doppler flow-velocity waveforms and fetal anemia has been the focus of examination for several years. Kirkinen et al.[1] first reported a close correlation between umbilical venous blood velocities and postpartum hematocrits. Griffin et al.[2] reported a weak relationship ($r = 0.5$) of uncertain clinical significance between the time-averaged maximal velocity in the descending aorta and the hematocrit. Rightmire et al.[3] similarly found in a retrospective study that a relationship was present between Doppler waveform indices and hematocrit. No prospective test of the reported relationships was performed.

An effect of anemia on Doppler waveforms might be anticipated from the pregnant ewe model. Tenenbaum et al.[8] reported that sudden increases in fetal hematocrit resulted in sustained alterations in umbilical artery flow. Our previous report failed to show any significant changes in Doppler waveforms before and after transfusion. In contrast, Fumia et al.[9] reported that fetal flow to a variety of organs, including the placenta, failed to change over a wide range of induced hematocrits. Although in cases of severe erythroblastosis the anemia and hypoproteinemia may result in reduced blood viscosity, we have not attempted such measurements. The only Doppler consequence of such a change may be reduced spectral broadening. Woo et al.[10] reported reduced spectral broadening in anemic fetuses with homozygous α-thalassemia, although this was based on subjective criteria because no generally accepted measurement is available for spectral broadening and no comparisons to fetal hematocrit were presented.

In our earlier report, we retrospectively examined the relationship between selected Doppler indices and fetal hematocrit.[4] The regression equations derived from patients before initial transfusion were highly sta-

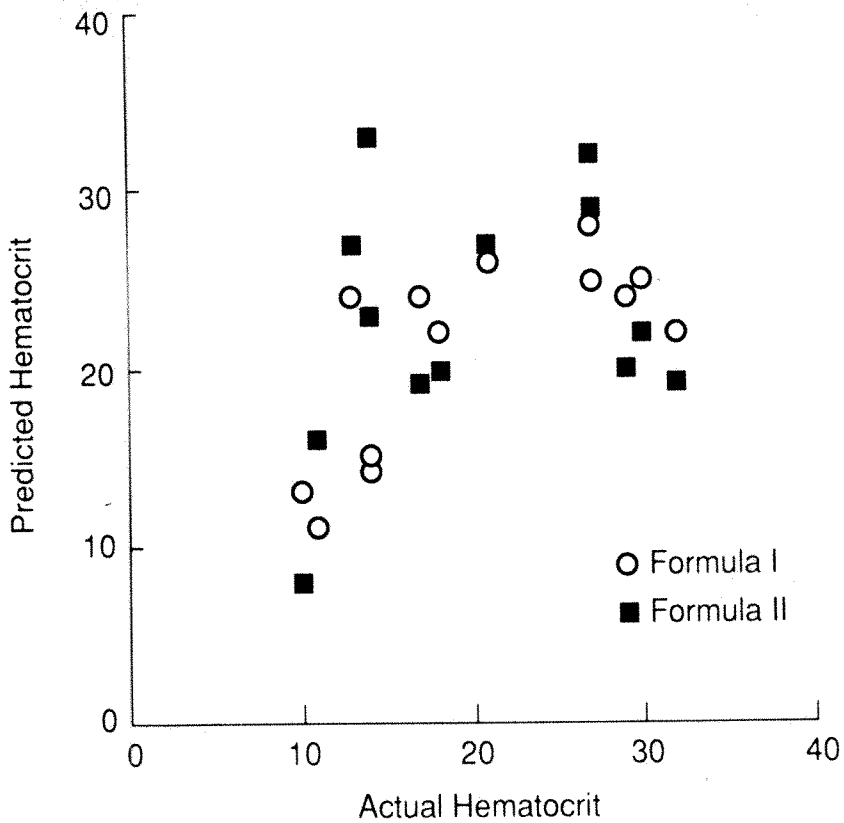


Fig. 1. Predicted and actual hematocrits. Horizontal axis shows actual hematocrits and vertical axis shows level predicted by two formulas.

tistically significant. These same equations were unable to predict hematocrit level before subsequent transfusions. Similarly, a new equation derived from the second transfusion data was unable to predict the hematocrit at third or later transfusions. This latter equation included the uterine artery pulsatility index, a surprising finding.

Our current study used the equations derived from the "first transfusion" group to examine their ability to predict the hematocrit level in a prospective fashion in patients about to undergo primary transfusions. Although the previously reported relationship between one of the Doppler-based formulas and fetal hematocrit remained significant, the second formula failed to demonstrate any statistical significance. The difference between statistical significance and clinical usefulness must be considered in further detail.

When the components of the equation were each examined for their contribution to the overall predicted hematocrit value, only the factor for presence or absence of hydrops retained significance. The correlation of hydrops and hematocrit was $r = 0.7$, whereas for hematocrit and peak aortic velocity and gestational age, poor correlation was observed ($r = 0.38$, and $r = 0.14$, respectively).

Thus it appears that the presence or absence of hydrops was the only factor responsible for the significant relationship between the formula being tested and the true hematocrit. Further confirmation of this conclusion is provided by the results obtained from the three nonhydropic fetuses included in this series whose hematocrits fell below 20% (patients 1, 5, and 6, Table I). In none of these fetuses did the predicted hematocrit value fall below 20% by formula I.

We conclude that our previously described formulas that used Doppler flow indices in the severely isoimmunized fetuses were unable to assist in the prediction of hematocrit. Prospective evaluation of any Doppler information must be an integral part of the evaluation of this new technique before its acceptance as a standard component of obstetric care. At present Doppler flow-velocity waveform indices appear to be of limited usefulness in the prediction of which isoimmunized pregnancies will require fetal transfusion, or in the timing of series of transfusions.

REFERENCES

1. Kirkinen P, Jouppila P, Eik-Nes S. Umbilical vein blood flow in rhesus isoimmunization. Br J Obstet Gynaecol 1983;90:640-3.
2. Griffin D, Cohen-Overbeek T, Campbell S. Fetal and utero-placental blood flow. Clin Obstet Gynecol 1983; 10:565-602.

3. Rightmire DA, Nicolaides KH, Rodeck CH, Campbell S. Fetal blood velocities in Rh isoimmunization: relationship to gestational age and to fetal hematocrit. Obstet Gynecol 1986;68:233-6.
4. Copel JA, Grannum PA, Belanger K, et al. Pulsed Doppler flow velocity waveforms before and after intrauterine intravascular transfusion for severe erythroblastosis fetalis. Am J Obstet Gynecol 1988;158:768-74.
5. Shepard MJ, Richards VA, Berkowitz RL, et al. An evaluation of two equations for predicting fetal weight by ultrasound. Am J Obstet Gynecol 1982;142:47-54.
6. Grannum PA, Copel JA, Plaxe SC, et al. In utero exchange transfusion by direct intravascular injection in severe erythroblastosis fetalis. N Engl J Med 1986;314:1431-4.
7. Grannum PAT, Copel JA, Moya FR, et al. The reversal of hydrops fetalis by intravascular intrauterine transfusion in severe isoimmune fetal anemia. Am J Obstet Gynecol 1988;158:914-9.
8. Tenenbaum DG, Piasecki GJ, Oh W, et al. Fetal polycythemia and hyperviscosity: effect on umbilical blood flow and fetal oxygen consumption. Am J Obstet Gynecol 1983;147:48-51.
9. Fumia FD, Edelstone DI, Holzman IR. Blood flows and oxygen delivery to fetal organs as functions of fetal hematocrit. Am J Obstet Gynecol 1984;150:274-82.
10. Woo JSK, Liang ST, Lo RLS, Chan FY. Doppler blood flow velocity waveforms in alpha-thalassemia hydrops fetalis. J Ultrasound Med 1987;6:679-84.

Concurrence of mouthing movement and rapid eye movement/non–rapid eye movement phases with advance in gestation of the human fetus

Naoki Horimoto, MD, Takashi Koyanagi, MD, Shin Nagata, MD, Hiromasa Nakahara, MD, and Hitoo Nakano, MD

Fukuoka, Japan

To evaluate whether mouthing movement in the human fetus is related to advance in gestational age and whether such movement is concurrent with rapid eye movement or non–rapid eye movement phases, 26 fetuses from 28 to 41 weeks' gestation were observed for 60 continuous minutes with real-time ultrasonography. It was evident that regular mouthing movement every 300 to 600 msec concurred with the non–rapid eye movement period, from 35 weeks' gestation to term. Random mouthing movements that occurred at a wide range of intervals from 300 msec to approximately 525 seconds were observed predominantly during the rapid eye movement period and were unrelated to advance in gestational age. These observations were discussed in relation to biologic implications. (Am J Obstet Gynecol 1989;161:344-51.)

Key words: Eye movement, mouthing movement, concurrent development, human fetus, real-time ultrasonography

Behavioral states of human neonates have been classified by means of various combinations of biophysical parameters, including electroencephalographic findings, heart rate change, the presence or absence of eye movement, regular or irregular respiratory movement, and whether the eye is closed or open.[1-6]

Regular mouthing movement was reported to occur only during quiet sleep of newborn human infants.[1, 2, 5, 7] Regular mouthing was evident even in the human fetus in utero, as observed with real-time ultrasonography.[8] However, with respect to developmental characteristics the biologic implications remain to be elucidated. The objective of this study was to elucidate the manifestation and ontogeny of various human functions, assessed in relation to correlative development of the central nervous system.

We studied the human fetus with real-time ultrasonography to determine whether mouthing movement is related to advance in gestational age and whether mouthing movement is concurrent with rapid eye movement and non–rapid eye movement phases.

Material and methods

Fetal population. This study was conducted on 26 fetuses from 28 to 41 weeks of gestation without mal-

From the Department of Gynecology and Obstetrics, Faculty of Medicine, Kyushu University.
Supported by a grant-in-aid for scientific research (No. 63480369), and a grant for maternal and child health research from the Ministry of Health and Welfare of Japan.
Presented in part at the Third International Symposium, "The Fetus as a Patient," Matsue, Japan, July 20-23, 1987.
Received for publication March 27, 1988; revised September 3, 1988; accepted February 15, 1989.
Reprint requests: Takashi Koyanagi, MD, Department of Gynecology and Obstetrics, Faculty of Medicine, Kyushu University 60, Maidashi 3-1-1, Higashi-ku, Fukuoka 812, Japan.

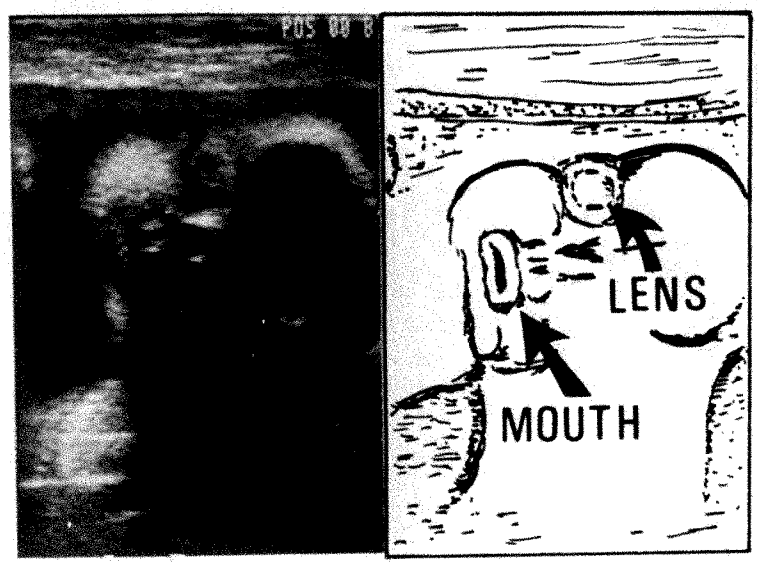


Fig. 1. Real-time ultrasonographic image and illustration used for simultaneous evaluation of eye and mouthing movements obtained from fetus at 35 weeks' gestation.

formation or intrauterine growth retardation. Each fetus was examined only once (Table I).

All mothers were cared for in the Maternity Care Unit of Kyushu University Hospital and gave informed consent to participate in this study. The period of gestation was calculated from the date of the last menstrual period and was confirmed with serial ultrasonographic measurements of crown-rump length before 11 weeks of gestation and by biparietal diameter at ≥12 weeks' gestation.

Data acquisition and processing. The ultrasonographic equipment used was an ALOKA SSD-280 model with a linear array transducer of 3.5 MHz. With the mother placed in the semi-Fowler position, the transducer was moved to obtain a simultaneous observation of the fetal eye, mouth, and jaw in one image (Fig. 1). The ultrasonographic image was continuously taken for approximately 60 minutes and simultaneously recorded on videotape with a Hitachi VT-Z70 videotape recorder in real-time fashion. When the videotape was replayed all instances of both eye and mouthing movements were marked by means of two specially devised switches that were operated independently by two different examiners. These switches were linked to a microcomputer system (NEC:PC-9801VM2). The two types of original data thus obtained were stored simultaneously on a floppy disk in an actual time sequence from the start of the recording (Fig. 2).

The eye movement from one position to another of the pair of dotted echoes, originating from both near and far margins of the lens, was defined as one unit of movement. The frequency of eye movement was calculated for every given one-minute epoch during the observation period. Rapid eye movement and non–rapid eye movement periods were regarded as those sustaining the presence and absence of movement, respectively.[9]

Table I. Population studied

Gestational age (wk)	*No. of fetuses*
28	1
29	2
30	1
31	1
32	3
33	3
34	4
35	2
36	1
37	2
38	2
39	2
40	0
41	2
TOTAL	26

We defined one unit of mouthing movement as that action in which the mouth opened and returned to a closed position. The interval between two consecutive occurrences of mouthing movement was used as a parameter for subsequent analysis.

The χ^2 test was applied for analysis of fetuses divided into two groups: the gestational age group and the fetal state group. After examination of the data, age-related changes in mouthing movements of all the fetuses were retrospectively arranged into three age-groups: 5 fetuses from 28 to 31 weeks' gestation, 10 from 32 to 34 weeks' gestation, and 11 from 35 to 41 weeks' gestation. In the last group the fetal states were stratified into two

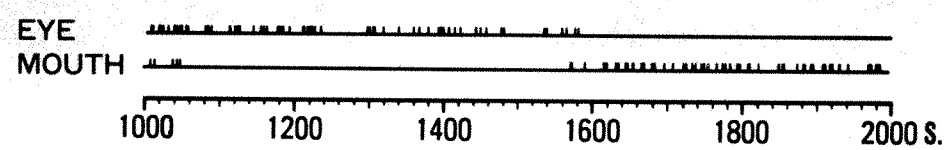


Fig. 2. Original data for all incidences of eye *(upper)* and mouthing *(lower)* movements recorded according to elapsed time (0 to 3600 seconds). *Vertical lines* indicate one unit of movement.

Table II. Analysis of variance obtained from data measured on 200 regular pulse signals of 500 and 1000 msec

	500 msec		*1000 msec*	
Examiners	*Mean value*	*Sum of squares*	*Mean value*	*Sum of squares*
A	503	217,800	1,007	336,200
B	502	304,200	1,006	273,800
C	498	168,200	1,001	288,800
D	505	273,800	996	352,800
E	496	245,000	997	320,000
F value	2.26		2.00	
F test ($p < 0.05$)	NS		NS	

A, B, C, D, E, represent findings obtained by five different medical examiners.

subgroups according to rapid eye movement and non–rapid eye movement periods.[9]

Accuracy of measurements in experimental system. The overall accuracy of measurements between two consecutive occurrences in a simulation experiment, from pushing the recording switch, through the analog/digital converter (MF-7016, Toitu, Ltd.) and onto storing of the information on floppy disk was evaluated with regular pulse signals created by an electric generator (FG-121B, NF Corp.). Using 200 sequential events corresponding to pulse signals at fixed rates of 500 and 1000 msec, five different examiners measured the time interval between two successive events. There were no significant interexaminer differences as determined by analysis of variance (Table II).

Results

Changes in fetal mouthing movement in relation to advance in gestational age. Fig. 3 shows chronologic changes in incidences, by percent, of the interval between two consecutive mouthing movements. The pattern of distribution was fairly uniform in all five fetuses from 28 to 31 weeks of gestation. Seven of the 10 fetuses between 32 and 34 weeks' gestation demonstrated a high percentage of incidences when the interval between mouthing movements was concentrated at a range between approximately 300 and 600 msec. The remaining three fetuses still showed a pattern of distribution similar to that found between 28 and 31 weeks' gestation (uniform). From 35 weeks of gestation to term, all 11 fetuses showed a pattern of distribution with a mound-shaped high percentage of incidence and low incidence at the interval range of <600 msec and >600 msec, respectively. Considering these chronologic changes in distribution of mouthing movement pattern, an intergroup comparison was restricted to two groups, from 28 to 31 weeks and from 35 to 41 weeks of gestation.

Fig. 4 shows the cumulative incidences of mouthing movement interval corresponding to each fetus (i.e., 5 at 28 to 31 and 11 at 35 to 41 weeks' gestation). In all fetuses before 32 weeks' gestation, the cumulative incidences rose in a slow, almost linear fashion (termed pattern I); these incidences started with intervals of 300 to 400 msec and reached a saturation point of 100% at intervals of 165 to 390 seconds. In 8 of 11 fetuses at ≥35 weeks of gestation, there was a rapid rise in cumulative incidences when the interval between mouthing movements ranged from approximately 300 to 600 msec. At the movement interval of 600 msec, the percentage of cumulative incidence ranged from 49% to 80% in these eight fetuses. This rapid rise was followed by a nearly linear slow rise as the length of the interval increased (termed pattern II). The remaining three fetuses showed pattern I activity, with cumulative incidences rising in a slow, almost linear fashion. The cumulative incidences reached 100% between about 170 and 760 seconds in fetuses at ≥35 weeks of gestation.

There was a statistical difference ($p < 0.05$) between gestational ages of 28 to 31 weeks/pattern I (5/5, 100%) and gestational ages of 35 to 41 weeks/pattern I (3/11, 27%), using the χ^2 test (Table III).

Changes in fetal mouthing movement in relation to the rapid eye movement and non–rapid eye movement periods. In 11 fetuses at ≥35 weeks' gestation, all

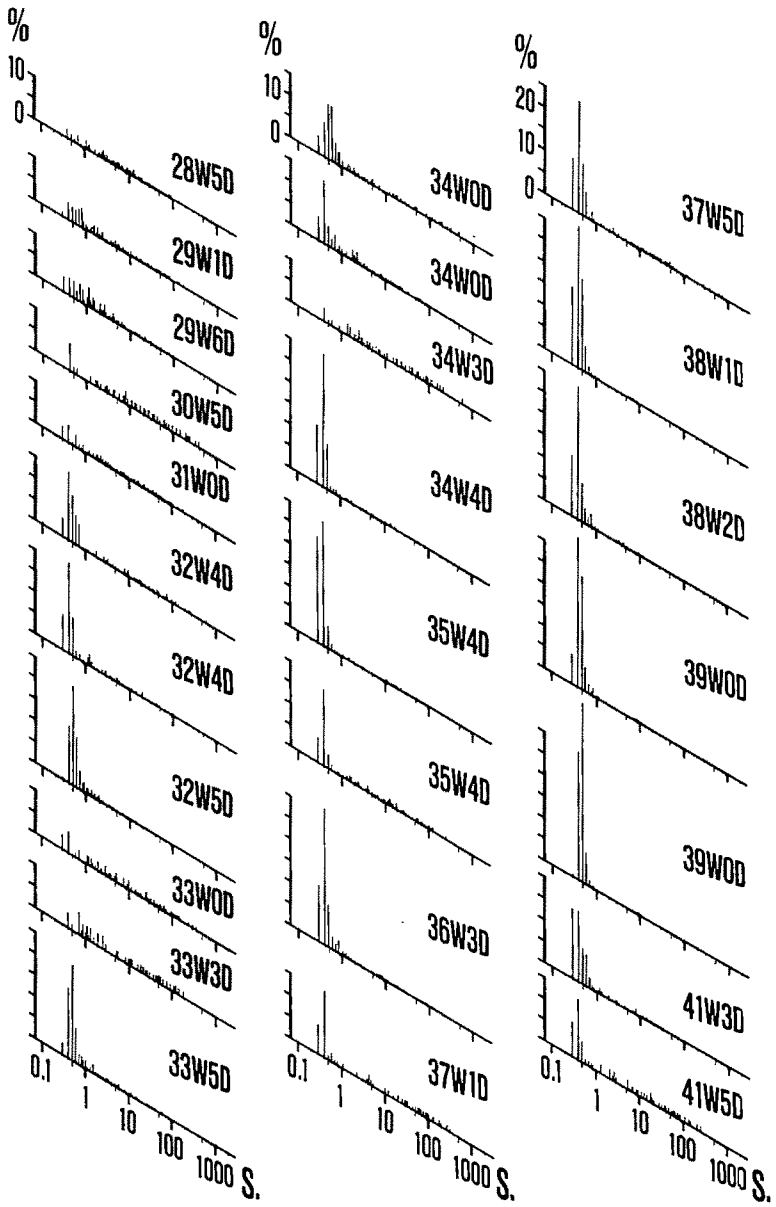


Fig. 3. Chronologic changes in interval between two consecutive mouthing movements in 26 fetuses from 28 weeks 5 days to 41 weeks 5 days of gestation. Vertical axis shows percentage of incidence at given movement interval. Horizontal axis indicates interval in seconds between two consecutive mouthing movements.

mouthing movements recorded over the entire observation period were divided into two groups according to the rapid eye movement and non–rapid eye movement periods (Fig. 5). Movements during the rapid eye movement period showed only a uniform distribution. The pattern of distribution during the non–rapid eye movement period is characterized by a mound shape, created by a high percentage of incidences at short intervals followed by low incidence with longer interval ranges.

Fig. 6 shows the cumulative incidence of the mouthing movement interval, by percent, for each case obtained and taken from the data shown in Fig. 5. During the non–rapid eye movement period, the cumulative incidence in all fetuses showed a rapid rise, ranging from an interval of approximately 300 to 600 msec followed by a nearly linear slow rise with an increase in the movement interval (pattern II). At an interval of 600 msec, the range of cumulative incidence was within 53% and 85%. During the rapid eye movement period, the cumulative incidence in all cases showed an almost linear slow rise (pattern I).

A statistical significance ($p < 0.005$) was found between rapid eye movement/pattern I (11/11, 100%)

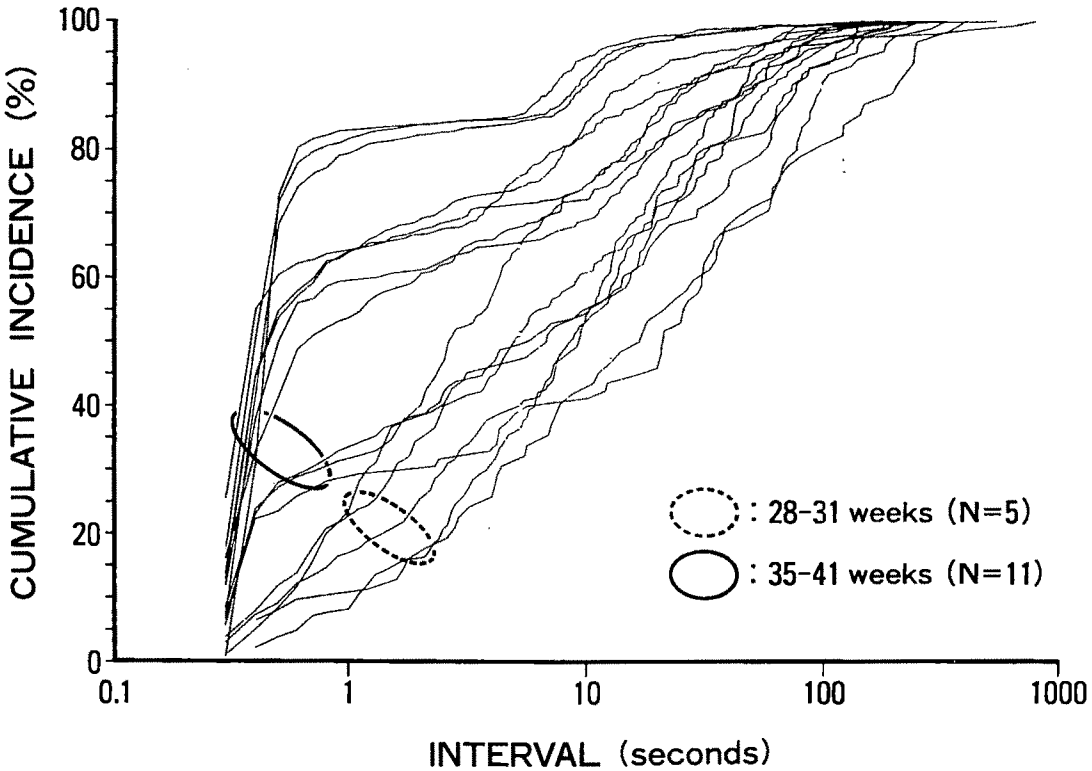


Fig. 4. Comparison of cumulative incidences of mouthing movement interval between two age groups: five fetuses at 28 to 31 weeks (encircled by *dotted line*) and 11 at 35 to 41 weeks of gestation (encircled by *solid line*). Vertical axis indicates cumulative incidence by percent. Horizontal axis shows interval in seconds between two consecutive mouthing movements.

Table III. Contingency table with respect to patterns of cumulative incidences of mouthing movement against gestational age groups

Gestational age groups	*Pattern I (No. of fetuses)*	*Pattern II (No. of fetuses)*	*Total*
28 to 31 wk	5	0	5
35 to 41 wk	3	8	11
TOTAL	8	8	16

Pattern I, Cumulative incidences rising in a slow, almost linear fashion. *Pattern II,* Rapid rise in cumulative incidences followed by a nearly linear slow rise.

Table IV. Contingency table with respect to patterns of cumulative incidences of mouthing movement against rapid eye movement and non–rapid eye movement periods

	Pattern I (No. of fetuses)	*Pattern II (No. of fetuses)*	*Total*
Rapid eye movement	11	0	11
Non–rapid eye movement	0	11	11
TOTAL	11	11	22

Data reflect incidences of 11 fetuses at ≥35 weeks' gestation.

Pattern I, Cumulative incidences rising in a slow, almost linear fashion. *Pattern II,* Rapid rise in cumulative incidences followed by a nearly linear slow rise.

and non–rapid eye movement/pattern I (0/11, 0%), with the χ^2 test (Table IV).

Comment

The history of studies of fetal behavioral development can be divided into two subgroups one the result of animal experiments[10, 11] and the other representing the observations of human neonates.[1, 2, 4-7] Both types of studies were extrapolated for interpretation of events occurring in the human fetus.[8, 12, 13] We used a different approach and focused directly on the human fetus in utero.[9, 14] Our objective was to evaluate the ontogeny of behavior in terms of how various fetal functions organize to become correlated and/or concurrent. We studied the concurrent development between mouthing movement and rapid eye movement/non–rapid eye movement periods with the advance in gestational age.

In all fetuses from 28 to 31 weeks of gestation, the pattern of percent-incidences of the interval between

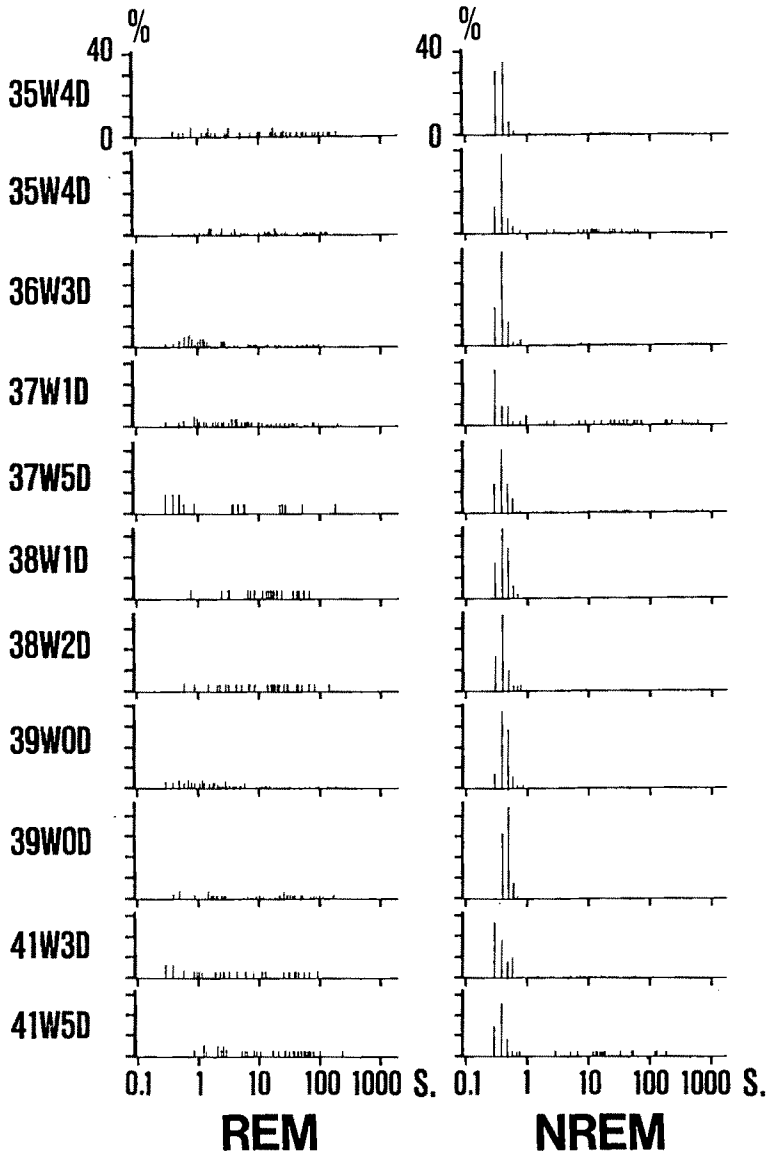


Fig. 5. Patterns of incidences of intervals of mouthing movement of fetuses from ≥35 weeks of gestation, divided into rapid eye movement and non–rapid eye movement periods (left and right columns, respectively). Data are arranged in same manner as in Fig. 3.

two consecutive mouthing movements showed a uniform distribution within the 60-minute observation period. This implies that mouthing movement occurs in a random manner during this stage of gestation. At ≥35 weeks' gestation during the rapid eye movement period, the incidence of mouthing movement was uniformly distributed and random occurrence of mouthing movement ensued. Conversely, during the non–rapid eye movement period a high incidence was noted at short intervals between movements, in which the mouthing movement was seen to repeat as a short-term, regular occurrence.

This regular mouthing concurred closely with the non–rapid eye movement period from 35 weeks of gestation to term, whereas the random mouthing movement was observed predominantly during the rapid eye movement period and was unrelated to the advance in gestational age. From 32 to 34 weeks of gestation, the patterns of the incidences of the mouthing movement intervals showed a transitional stage between 28 and 31 weeks and between 35 and 41 weeks of gestation. This corresponds to findings regarding age-related changes in the eye movement phase when rapid eye movement starts to consolidate at about 28 weeks and becomes fully manifest at 33 weeks' gestation. In addition, from 35 to 36 weeks of gestation to term, rapid eye movement and non–rapid eye movement periods occurred alternately with intervals of about 30 to 40

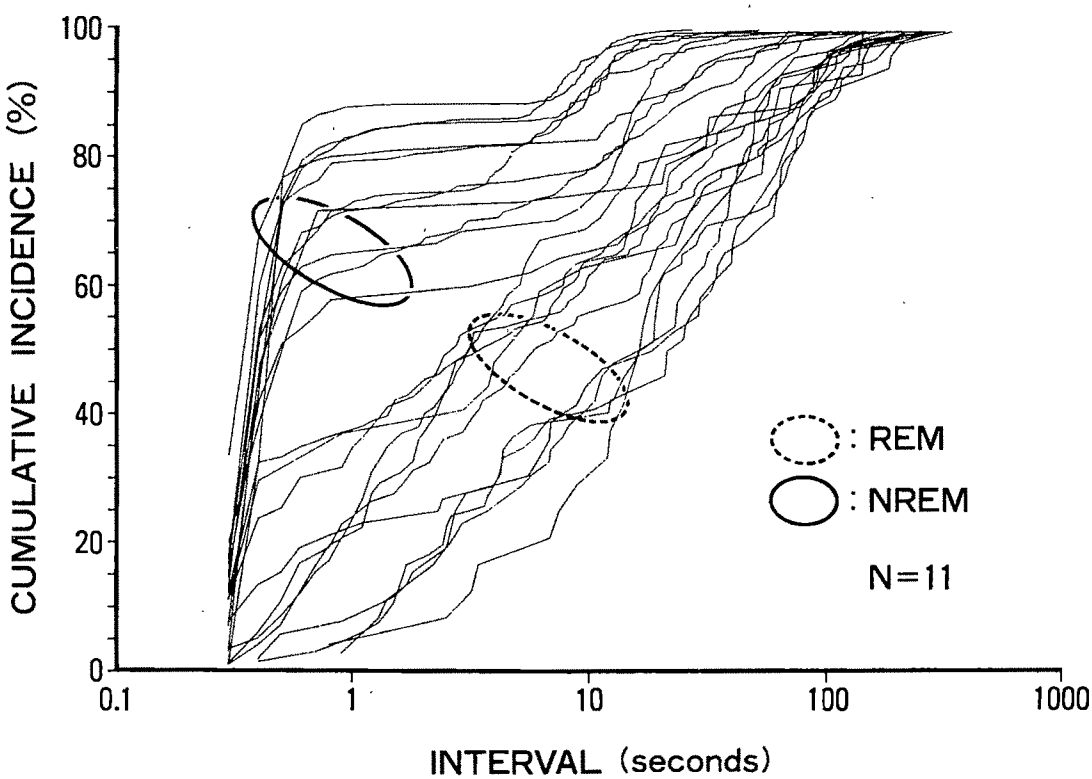


Fig. 6. Comparison of cumulative incidence of mouthing movement intervals between rapid eye movement (encircled by *dotted line*) and non–rapid eye movement (encircled by *solid line*) periods in 11 fetuses at 35 weeks of gestation to term. Data are displayed in fashion similar to that in Fig. 4.

and 20 to 30 minutes, respectively, both in the neonate[3, 6] and in the fetus.[7, 8, 13, 15]

Regular mouthing movement was observed only during non–rapid eye movement sleep in infants.[1, 2, 5, 7] In the infant delivered at or after 35 to 36 weeks of gestation, the non–rapid eye movement sleep may be an indication of functions of the thalamocortical pathway.[16] Thus the concurrence of regular mouthing with the non–rapid eye movement period suggests that the brain of a 35 to 36-week gestation fetus is functioning to the extent seen in the newborn.

In relation to behavioral states according to Prechtl's "state 1" in the neonate[5] and "state 1F" of Nijhuis et al.[8] in the fetus, these results should prove useful for evaluating brain stem function in the developing fetus.

We thank Dr. M. Inoue for data collection, M. Ohara for critical comments, L. Saza for helpful advice on the manuscript, M. Kurata for assistance with the computer analysis, and K. Akazawa for statistical analysis.

REFERENCES

1. DeHaan R, Patric J, Chess GF, Jaco NT. Definition of sleep state in the newborn infant by heart rate analysis. Am J Obstet Gynecol 1977;127:753.
2. Dreyfus-Brisac C. Ontogenesis of sleep in human prematures after 32 weeks of conceptional age. Dev Psychobiol 1970;3:91.
3. Junge HD. Behavioral states and state related heart rate and motor activity patterns in the newborn infant and the fetus antepartum—a comparative study. I. Technique, illustration of recordings, and general results. J Perinat Med 1979;7:85.
4. Parmelee AH Jr, Wenner WH, Akiyama Y, Schultz M, Stern E. Sleep states in premature infants. Dev Med Child Neurol 1967;9:70.
5. Prechtl HFR. The behavioural states of the newborn infant (a review). Brain Res 1974;76:185.
6. Roffwarg HP, Muzio JN, Dement WC. Ontogenetic development of the human sleep-dream cycle. Science 1966; 152:604.
7. Wolff PH. The serial organization of sucking in the young infant. Pediatrics 1986;42:943.
8. Nijhuis JG, Prechtl HFR, Martin CB Jr, Bots RSGM. Are there behavioural states in the human fetus? Early Hum Dev 1982;6:177.
9. Inoue M, Koyanagi T, Nakahara H, Hara K, Hori E, Nakano H. Functional development of human eye movement in utero assessed quantitatively with real-time ultrasound. Am J Obstet Gynecol 1986;155:170.
10. Dawes GS, Fox HE, Leduc BM, Liggins GC, Richards RT. Respiratory movements and rapid eye movement sleep in the foetal lamb. J Physiol 1972;220:119.
11. Ruckebusch Y, Gaujoux M, Eghbali B. Sleep cycles and kinesis in the foetal lamb. Electroencephalogr Clin Neurophysiol 1977;42:226.
12. Rosen MG, Dierker LJ Jr, Hertz RH, Sorokin Y, Timor-Tritsch I. Fetal behavioral states and fetal evaluation. Clin Obstet Gynecol 1979;22:605.

13. Timor-Tritsch IE, Dierker LJ, Hertz RH, Deagan NC, Rosen MG. Studies of antepartum behavioral state in the human fetus at term. AM J OBSTET GYNECOL 1978; 132:524.
14. Koyanagi T, Horimoto N, Hirose K, Nakano H. A multiple and comprehensive study of fetal behavior. In: Maeda K, ed. The fetus as a patient '87. Amsterdam: Excerpta Medica, 1987:71.
15. Visser GHA, Carse EA, Goodman JDS, Johnson P. A comparison of episodic heart-rate patterns in the fetus and newborn. Br J Obstet Gynaecol 1982;89:50.
16. Watanabe K, Iwase K. Spindle-like fast rhythms in the EEGs of low-birthweight infants. Dev Med Child Neurol 1972;14:373.

Fetal heart rate patterns in red blood cell isoimmunized pregnancies

K. H. Nicolaides, BSc, MD, G. Sadovsky, MD, and E. Cetin, MD
London, England

Fetal heart rate was monitored in 33 red blood cell isoimmunized patients at 32 to 36 weeks' gestation immediately before cordocentesis. The fetal heart rate patterns were analyzed according to 25 different classifications, and the values were related to the fetal hemoglobin concentration, oxygen tension, and oxygen content. Nonreactive, suboptimal, or pathologic fetal heart rate patterns were encountered more commonly in fetuses that were hypoxemic and anemic than in those that were normoxemic and nonanemic. However, the positive predictive value of these abnormal fetal heart rate patterns with respect to fetal anemia was 50% to 100%; the negative predictive value was 18% to 40%. (AM J OBSTET GYNECOL 1989;161:351-6.)

Key words: Fetal heart rate monitoring, cordocentesis, fetal hypoxemia, fetal anemia, red blood cell isoimmunization

In red blood cell isoimmunized pregnancies, abnormal fetal heart rate (FHR) patterns have been associated with poor perinatal outcome and with low hemoglobin concentration in samples obtained from the umbilical cord at birth.[1, 2] However, the validity of this association is questionable because of the temporal dissociation between measurement of these parameters.

This study describes the relationship between FHR patterns and hemoglobin concentration, oxygen tension, and oxygen content in blood samples obtained by cordocentesis from red blood cell isoimmunized pregnancies immediately after FHR monitoring at 32 to 36 weeks' gestation.

Patients and methods

FHR monitoring was performed immediately before cordocentesis in 33 red blood cell isoimmunized patients. The blood samples were analyzed for fetal hemoglobin concentration and blood gases. FHR monitoring was undertaken with patients in a semirecumbent position. Recordings were made at a paper speed of 1 cm/min (model 8041A, Hewlett Packard, Boblingen, West Germany). In all cases gestational age was determined by Naegele's rule and confirmed by ultrasonographic measurement of the fetal biparietal diameter at 16 to 20 weeks' gestation. None of the fetuses were hydropic at the time of cordocentesis.

Cordocentesis was performed as an outpatient procedure without maternal fasting or sedation. The umbilical cord vessel was identified as artery or vein by the intravascular injection of 200 to 400 μl of normal saline solution.[3] Fetal blood (200 μl) was collected into heparinized syringes and blood gas analysis was performed with a Radiometer ABL 30 blood gas analyzer (Copenhagen). For hemoglobin analysis, fetal blood samples (180 μl) were collected into 20 μl of isotonic edetic acid solution (0.5 mmol/L in 0.15 mmol/L sodium chloride); the hemoglobin concentation (grams per deciliter) was determined with a Coulter S Plus counter (Coulter Electronics Ltd, Luton, England). The fetal origin of blood was confirmed subsequently by Betke-Kleihauer testing. Oxygen content was calculated from

From the Harris Birthright Research Centre for Fetal Medicine, Department of Obstetrics and Gynaecology, King's College Hospital.
Received for publication October 23, 1988; revised January 28, 1989; accepted March 10, 1989.
Reprint requests: K. H. Nicolaides, MD, Harris Birthwright Research Centre for Fetal Medicine, Department of Obstetrics and Gynecology, King's College Hospital, Denmark Hill, London, England SE5 8RX.

Table I. Classification of patterns

Authors	Reactive or nonreactive				
	Accelerations			Observation time (min)	Extension time (min)
	No.	Amplitude (beats/min)	Duration (sec)		
(a) Rayburn et al. (1978)	1	—	—	20	0
(b) Amankwah et al. (1980)	1	10	10	60	0
(c) Mendenhall et al. (1980)	1	10	—	30	0
(d) Lee and Drukker (1979)	>2	10	—	20	20
(e) Platt et al. (1983)	2	15	15	20	20
(f) Schifrin (1977)	2	15	15	10	30
(g) Goldkrand and Benjamin (1984)	2	15	15	20	0
(h) Manning et al. (1979)	2	15	30	20	20
(i) Pratt et al. (1979)	2	15	30	30	0
(j) Koller and Curet (1978)	3	10	—	15	0
(k) Devoe (1980)	3	15	15	30	0
(l) Lauersen et al. (1983)	3	15	15	20	0
(m) O'Leary et al. (1980)	4	15	15	20	0
(n) Phelan and Lewis (1981)	4	15	15	20	20
(o) Brown and Patrick (1971)	5	15	15	20	100
(p) Evertson et al. (1979)	5	15	15	20	20
(q) Ingardia et al. (1980)	5	15	15	20	0

Scoring systems

Rochard et al.[1] Reactive, nonreactive, sinusoidal

Visser and Huisjes[8] Normal, suboptimal, decelerative, terminal

Kubli and Ruttgers[9] Normal, suspicious, pathologic

Score	0	1-2	3-6

Hammacher et al.[10] Normal, suspicious, prepathologic, pathologic

Score	0-2	3-4	5-7	8-18

Meyer-Menk et al.[11] Normal, prepathologic, pathologic

Score	8-10	5-7	0-4

Fisher et al.[12] Normal, suspicious, pathologic

Score	8-10	5-7	0-4

Krebs and Petres[13] Normal, abnormal

Score	9-12	0-8

Lyons et al.[14] Normal, prepathologic, pathologic

Score	8-10	5-7	0-4

the Po_2, hemoglobin concentration, and pH using the equations of Bohr et al.[4] and Hill.[5]

In normal fetuses the hemoglobin concentration increases and the oxygen tension decreases with gestation.[6, 7] Furthermore, the oxygen content for umbilical venous blood (mean ± SD, 6.6 ± 0.6 mmol/L) is higher than that in the umbilical artery (mean ± SD, 5.8 ± 1.1 mmol/L).[7] Therefore, in each red blood cell isoimmunized pregnancy, the fetal hemoglobin concentration was subtracted from the normal mean for gestation to calculate the degree of anemia. The oxygen tension and oxygen content were expressed as the number of standard deviations by which the observed values differed from the corresponding normal means for gestation for the appropriate vessel sampled.

FHR analysis. The FHR traces were examined by two observers who were unaware of the indications for fetal blood sampling or of the blood gas and hemoglobin results. The traces were divided into recordings of 1-minute intervals; each interval was examined for FHR baseline, oscillation amplitude, and oscillation frequency. The amplitude and duration of each increase (acceleration) and decrease (deceleration) from the baseline were also recorded. FHR tracings were classified as *reactive* or *nonreactive*, on the basis of the presence or absence of FHR accelerations according to 17 different definitions. Thus a reactive trace was one in which there were 1 to 5 accelerations of 10 to 15 beats/min, lasting for 15 to 30 seconds during a period of 10 to 60 minutes (Table I). Tracings also were classified as *normal, suboptimal,* or *pathologic* on the basis of baseline, baseline variability (oscillation amplitude and oscillation frequency) and the presence or absence of accelerations or decelerations, according to eight quantitative or descriptive scoring systems.[1, 8-14] Each system has different criteria of normality and abnormality. For example, Fischer et al.[12] define a normal baseline variability by the presence of an oscillation frequency of at least 6 cycles/min (cpm), whereas Hammacher et al.[10] accept 2 cpm as normal. Similarly, Lyons et al.[14] consider the absence of at least four accelerations of at least 15 beats/min during a 20-minute period of recording to be a pathologic feature; Kubli and Rutgers[9] ignore accelerations in their scoring system. In the statistical

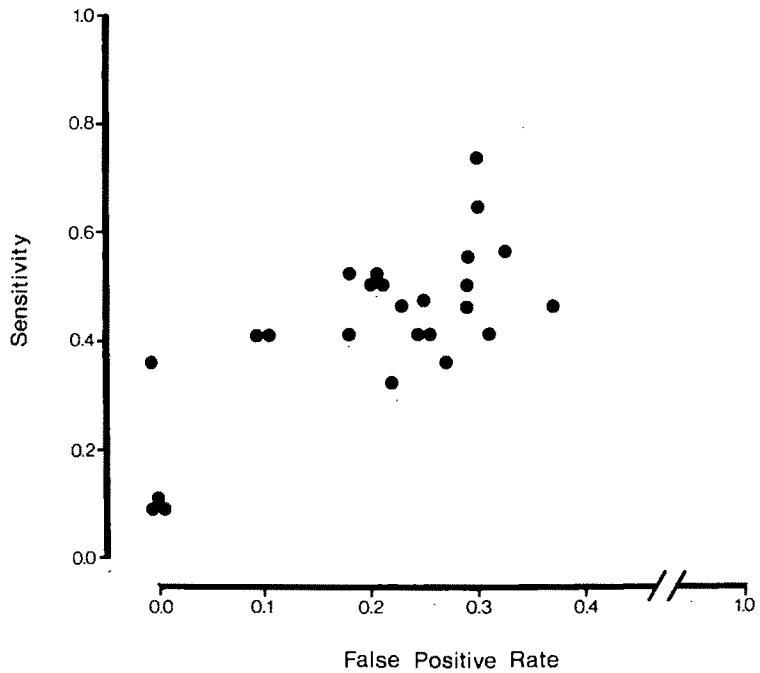


Fig. 1. Sensitivity and false-positive rates of 25 classifications of FHR patterns in predicting fetal oxygen content deficit in rhesus-isoimmunized pregnancies.

analysis of the data from the scoring systems, suspicious, suboptimal, prepathologic, and pathologic classifications were grouped together as abnormal.

Results

In the 33 red blood cell isoimmunized pregnancies, the ranges in fetal hemoglobin concentration, oxygen tension, and oxygen content were +1.7 to −10.1 gm/dl (+0.8 to −2.9 and +1.0 to −7.0 SD) from the respective normal means for gestation (Table II). The classification of the FHR pattern in each case as reactive or nonreactive and normal, suboptimal, or pathologic is shown in Table II.

Nonreactive traces were more commonly encountered in severely anemic and hypoxemic fetuses. However, there were large variations in the sensitivity and specificity of FHR patterns in the prediction of fetal oxygenation or hemoglobin concentration (Table III). Thus, when the criteria for reactivity are lenient (one acceleration of 10 beats/min, lasting for 10 seconds during 60 minutes of recording), only two extremely anemic (hemoglobin deficit, 10.1 gm/dl) fetuses had nonreactive FHR patterns (sensitivity, 10%; specificity, 100%). With use of more stringent criteria (a minimum of five accelerations during 20 minutes of recording), the FHR patterns were nonreactive in 24 of the 33 (73%) fetuses, and the sensitivity and specificity of predicting anemia were 68% and 18%, respectively. The sensitivity and specificity of most classifications improved when the predicted parameter was fetal Po_2 or oxygen content rather than hemoglobin concentration (Table III). A receiver-operating characteristic plot is shown in Fig. 1.

Pathologic FHR patterns were observed in 10 of the 11 fetuses with an oxygen content at least 4 SD below the normal mean. In two of these fetuses the hemoglobin was normal but the Po_2 was below the 2.5th percentile of the normal range.

Sinusoidal FHR pattern (frequency 3 cpm, amplitude 6 beats/min, duration, 100 of 120 minutes of recording) was observed in only one severely anemic fetus (case 1, Table II).[1, 2]

Comment

This study examines the relationship between fetal blood hemoglobin concentration, oxygen tension, or oxygen content and FHR patterns in the undisturbed fetus from red blood cell isoimmunized pregnancies. The minimum gestational age of 32 weeks was selected because both normal and pathologic FHR patterns exhibit a gestation-related "maturation."[15, 16] The majority of published classifications of normality or abnormality are based on data derived from pregnancies of at least 32 weeks' gestation.

Previous reports of FHR patterns in red blood cell isoimmunized pregnancies have focused on the association between fetal compromise and sinusoidal FHR patterns. Thus Rochard et al.[1] evaluated antenatal FHR in 50 red blood cell isoimmunized pregnancies and reported the presence of sinusoidal FHR patterns in 20

Table II. FHR patterns in 33 read blood cell isoimmunized pregnancies according to 25 classifications

Case No.	*Gestation (wk)*	*Hemoglobin (gm/dl)*	*PO_2 (SD)*	*Oxygen content (SD)*	*Reference letter**									
					a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	*j*
1	33	−10.1	−1.7	−4	A	A	A	A	A	A	A	A	A	A
2	36	−10.1	−0.1	−7	A	A	A	A	A	A	A	A	A	A
3	34	−7.7	−0.2	−6	N	N	N	A	A	A	A	A	A	A
4	33	−7.5	+0.5	−5	N	N	N	N	N	N	N	N	N	N
5	33	−7.1	+0.2	−5	N	N	N	N	N	N	N	N	A	N
6	32	−7.0	+0.1	−5	N	N	N	A	A	A	A	A	A	A
7	34	−6.4	+0.2	−4	N	N	N	N	N	N	N	N	N	N
8	33	−6.2	−0.3	−6	N	N	N	N	N	N	N	N	N	N
9	34	−4.9	−0.4	−3	N	N	N	N	N	N	N	N	N	N
10	34	−4.6	+0.5	−3	N	N	N	A	A	A	A	A	N	N
11	33	−4.3	−1.0	−4	N	N	N	N	N	N	N	N	A	A
12	33	−3.6	−0.4	−3	N	N	N	N	N	N	N	N	N	N
13	35	−3.2	+0.1	−2	N	N	N	N	N	N	N	N	N	N
14	36	−3.1	+0.1	−1	N	N	N	N	N	N	N	N	N	N
15	35	−3.0	−0.2	−2	N	N	N	N	N	N	N	N	N	N
16	35	−2.9	+0.6	−1	N	N	N	N	N	N	A	A	A	N
17	35	−2.9	−1.0	−4	N	N	N	A	A	A	A	A	A	A
18	35	−2.9	−0.6	−3	N	N	N	A	A	A	A	A	A	A
19	32	−2.6	+0.6	−1	N	N	N	N	N	N	N	N	N	N
20	32	−2.6	+0.2	−2	N	N	N	N	N	N	N	N	N	N
21	34	−2.5	+0.6	−1	N	N	N	N	N	N	N	N	N	A
22	32	−2.3	+0.8	−1	N	N	N	N	N	N	N	N	N	N
23	33	−1.9	+0.4	−1	N	N	N	N	A	A	A	A	A	A
24	33	−1.7	−1.2	−2	N	N	N	N	N	N	N	N	N	N
25	37	−1.1	−2.3	−4	N	N	N	N	A	A	A	A	A	A
26	34	−0.9	0	0	N	N	N	N	N	N	N	N	N	A
27	33	−0.8	−2.9	−4	N	N	N	A	A	A	A	A	A	A
28	33	−0.7	−0.2	0	N	N	N	N	N	N	N	N	N	A
29	35	−0.1	+0.1	+1	N	N	N	N	N	N	N	N	N	N
30	33	+0.7	−0.5	0	N	N	N	N	N	N	N	N	N	N
31	36	+1.4	+0.6	−2	N	N	N	N	N	N	N	N	N	A
32	32	+1.5	−0.9	0	N	N	N	N	N	N	A	A	A	A
33	35	+1.7	−2.1	−2	N	N	N	N	N	N	A	N	A	A

A, Abnormal/pathologic; *N*, normal/reactive. For references 9 through 14, the numbers adjacent to *A* or *N* are the actual values according to the scoring systems.

*See Table I.

Table III. Efficacy of FHR patterns in predicting an abnormal (>2 SD below normal mean) fetal hemoglobin, oxygen tension, or oxygen content

FHR classification by letter or reference number*	*Hemoglobin*				*Oxygen tension*				*Oxygen content*			
	Sens.	*Spec.*	*PPV*	*NPV*	*Sens*	*Spec.*	*PPV*	*NPV*	*Sens.*	*Spec.*	*PPV*	*NPV*
a	10	100	100	35	0	93	0	90	9	100	100	36
b	10	100	100	35	0	93	0	90	9	100	100	36
c	10	100	100	35	0	93	0	90	9	100	100	36
d	32	91	88	40	33	77	13	92	36	100	100	44
e	32	72	70	35	67	73	20	96	41	91	90	44
f	32	72	70	35	67	73	20	96	41	91	90	44
g	36	55	62	33	100	67	23	100	46	73	77	40
h	36	64	67	33	67	67	17	95	41	73	75	38
i	41	55	64	32	100	63	21	100	50	73	79	42
j	36	27	50	18	100	57	19	100	46	46	63	29
k	36	64	75	33	67	67	17	95	41	64	69	35
l	36	36	67	35	100	53	18	100	55	55	71	38

Sens., Sensitivity, *Spec.*, specificity, *PPV*, Positive predictive value; *NPV*, negative predictive value.

Reference letter or number*														
k	*l*	*m*	*n*	*o*	*p*	*q*	*1*	*8*	*9*	*10*	*11*	*12*	*13*	*14*
A	A	A	A	A	A	A	A	A	A3	A5	A3	A5	A6	A3
A	A	A	A	A	A	A	A	A	A3	A3	A7	A6	A7	A7
A	A	N	A	A	A	A	N	N	A3	N1	A7	A7	N9	A7
N	N	N	N	—	—	A	N	A	A3	A3	N9	N8	N10	A7
N	N	A	N	—	A	A	A	A	A3	N1	N9	N8	N9	N8
A	A	A	A	A	—	A	A	N	A3	A6	A5	A5	N6	A5
N	N	N	N	N	N	N	A	A	A3	A5	A6	A6	N9	A6
N	N	N	N	N	N	N	N	N	N0	N1	N9	N8	N10	N9
N	N	N	N	A	A	A	N	A	A3	A3	A7	N8	A6	A7
N	A	N	A	N	N	A	N	N	N0	N0	N10	N9	N11	N9
A	A	A	A	A	A	A	A	A	A2	A3	A7	A7	A7	A7
N	N	N	N	N	N	N	N	N	N0	N0	N10	N10	N12	N10
N	N	N	A	N	N	A	N	N	A1	A3	N9	N8	N10	N9
N	N	N	N	N	N	A	N	N	N0	N0	N10	N10	N12	N10
N	N	N	N	N	N	N	N	N	N0	N0	N10	N10	N12	N10
A	A	N	A	N	A	A	N	N	A1	N0	N9	N8	N10	N8
A	A	A	A	A	A	A	N	A	A2	N1	N9	N8	N10	N8
A	A	A	A	—	A	A	N	A	A3	N6	A5	N5	A6	A5
N	N	N	N	N	N	N	N	A	N2	N4	N6	N7	N9	A7
N	N	A	A	N	N	A	N	N	N1	N0	N10	N10	N12	N9
N	N	N	N	N	N	N	N	N	N0	N0	N10	N10	N11	N9
N	N	N	N	N	N	N	N	N	N0	N0	N9	N9	N11	N9
A	A	A	A	A	—	A	N	N	A2	N1	A7	N8	N10	A7
N	N	N	N	N	N	N	N	N	N0	N0	N10	N10	N12	N10
A	A	A	A	A	A	A	A	A	A5	A5	A4	A5	A6	A4
N	A	N	A	N	N	A	N	A	A3	A4	A6	A6	A8	A6
A	A	A	A	A	A	A	A	A	A1	N1	N6	N6	A8	N6
A	A	A	A	A	A	A	N	A	A3	A4	A7	A7	A8	A7
N	N	N	N	N	N	A	N	N	A2	N0	N9	N8	N9	N9
N	N	N	N	N	N	N	N	N	N0	N0	N10	N9	N11	N10
N	N	N	A	A	A	A	N	N	A1	N1	N8	N8	N10	N8
N	A	A	A	N	N	A	N	N	N0	N0	N10	N9	N11	N8
N	A	N	A	N	N	A	N	N	N0	N1	N9	N9	N11	N8

FHR classification by letter or reference number*	*Hemoglobin*				*Oxygen tension*				*Oxygen content*			
	Sens.	*Spec.*	*PPV*	*NPV*	*Sens.*	*Spec.*	*PPV*	*NPV*	*Sens.*	*Spec.*	*PPV*	*NPV*
m	36	55	62	30	67	60	14	95	50	73	79	33
n	50	27	58	21	100	50	17	100	56	46	67	33
o	37	55	58	33	67	63	17	94	47	73	75	44
p	45	60	69	35	67	57	14	94	50	64	71	41
q	68	18	61	29	100	33	13	100	73	37	70	40
l	27	82	75	36	67	80	25	9	36	100	100	44
8	46	64	71	25	67	60	14	95	50	73	79	42
9	59	36	65	31	67	40	11	92	64	46	70	39
10	36	73	73	36	37	67	9	91	41	82	82	41
11	37	64	73	33	33	63	8	91	41	73	75	38
12	27	73	67	33	33	73	11	92	32	82	78	38
13	27	55	55	27	67	70	18	95	36	73	73	36
14	45	64	71	37	33	57	7	90	46	64	71	37

cases; 18 of the fetuses were hydropic and 10 died in utero or during the neonatal period.[1] Similarly, in a review of 42 cases of red blood cell isoimmunized pregnancies at 33 to 38 weeks' gestation, sinusoidal and/or decelerative FHR patterns were observed within 5 days of delivery in 12 cases; the umbilical cord hemoglobin concentration at birth was 3 to 12.3 gm/dl. In contrast, the FHR patterns were normal in 30 cases with cord hemoglobin concentrations of 9.6 to 20.9 gm/dl. It was concluded that FHR monitoring is an excellent tool for detecting fetal anemia.

Our study suggests that a sinusoidal FHR is observed only in association with extreme fetal compromise, since it was present in one of the two most anemic and hypoxemic fetus (hemoglobin concentration deficit, 10.1 gm/dl; oxygen content, 5 SD below the normal mean). Lesser degrees of anemia were associated with decreased FHR reactivity and baseline variability as well as the development of decelerations. Thus nonreactive, suboptimal, or pathologic FHR patterns were more commonly observed in the anemic (hemoglobin deficit >2 gm/dl) than nonanemic fetuses (Table II). However, there is a large overlap in values. Therefore in the clinical management of red blood cell isoimmunized pregnancies examination of FHR patterns cannot predict accurately the degree of fetal anemia. As expected, there was a trend of increased sensitivity with reduced specificity as the criteria for normality of FHR became more stringent (Table III, Fig. 1).

The observation that pathologic FHR patterns were more closely related to fetal oxygenation than to hemoglobin concentration (Table III) is consistent with the physiology of the fetal cardiovascular system. The medulla oblongata, which controls cardiac activity through the autonomic nervous system, is influenced by afferents from peripheral chemoreceptors and by the direct action of carbon dioxide and hydrogen ions. The association between pathologic FHR patterns and low fetal blood oxygen content is presumably the result of tissue hypoxia in the medulla oblongata, whereas that between pathologic FHR patterns and low Po_2 is the result of stimulation of peripheral chemoreceptors.

This study establishes that in the management of red blood cell isoimmunized pregnancies, the present criteria of normality or abnormality of FHR patterns do not allow for an accurate prediction of the degree of fetal anemia. However, fetal anemia is associated with pathologic FHR patterns. The association is more likely to be mediated by the accompanying disturbance in fetal oxygenation than by the hemoglobin deficit.

REFERENCES

1. Rochard F, Schifrin BS, Goupil F, Legrand H, Blottiere J, Sureau C. Nonstressed fetal heart rate monitoring in the antepartum period. Am J Obstet Gynecol 1976; 126:699.
2. Visser GHA. Antepartum sinusoidal and decelerative heart rate patterns in Rh disease. Am J Obstet Gynecol 1982;143:538.
3. Nicolaides KH, Soothill PW, Rodeck CH, Campbell S. Ultrasound-guided sampling of the umbilical cord and placental blood to assess fetal wellbeing. Lancet 1986; 1:1065.
4. Bohr C, Hasselbach KA, Krogh A. Uber einen in biologischer Beziehung wichtigen Einfluss, den die Kohlensaurespannung des Blutes auf dessen sauerstoffbinding ubt. Skand Arch Physiol 1904;16:402.
5. Hill AV. The possible effects of the aggregation of the molecules of haemoglobin on its dissociation curve. J Physiol 1910;40:1244.
6. Nicolaides KH, Soothill PW, Clewell WH, Rodeck CH, Mibashan RS, Campbell S. Fetal haemoglobin measurement in the assessment of red cell isoimmunization. Lancet 1988;1:1073.
7. Soothill PW, Nicolaides KH, Rodeck CH, Campbell S. Effect of gestational age on fetal and intervillous blood gas and acid-base values in human pregnancy. Fetal Ther 1986;1:168.
8. Visser GHA, Huisjes HJ. Diagnostic value of the unstressed antepartum cardiotocogram. Br J Obstet Gynaecol 1977;84:321.
9. Kubli F, Ruttgers H. Semiquantitative evaluation of antepartum fetal heart rate. Int J Gynecol Obstet 1972; 10:180.
10. Hammacher K, Brur. Del Re R, Gaudenz R, De Gzandi P, Richter R. Kardiotokographischer Nachweis einer fetalen gefahrdung mit einem CTG-score. Gynakol Rundsch 1974;14:61.
11. Meyer-Menk W, Ruttgers H, Boos R, Wurth G, Adis B, Kubli F. A proposal for a new method of CTG-evaluation. In: Abstracts of the Fifth European Congress of Perinatal Medicine. Uppsala: Almquist and Wiksell, 1976:138.
12. Fisher WM, Stude I, Brandt H. Ein Vorschlag Zur Beurteilung des antepartualen Kardiotokogramms. Z Geburtshilfe Perinatol 1976;180:117.
13. Krebs HB, Petres RE. Clinical application of a scoring system for evaluation of antepartum fetal heart rate monitoring. Am J Obstet Gynecol 1978;130:765.
14. Lyons ER, Bylsma-Howell M, Shamsi S, Towell ME. A scoring system for nonstressed antepartum fetal heart rate monitoring. Am J Obstet Gynecol 1979;133:242.
15. Smith CV, Phelan JP, Paul RH. A prospective analysis of the influence of gestational age on the baseline fetal heart rate and reactivity in a low risk population. Am J Obstet Gynecol 1985;153:780.
16. Nicolaides KH, Sadovsky G, Visser GHA. Heart rate patterns in normoxemic, hypoxemic, and anemic second-trimester fetuses. Am J Obstet Gynecol 1989;160:1034.

A complete list of references is available from the authors on request.

Plasma atrial natriuretic peptide in human fetus: Response to intravascular blood transfusion

Marios Z. Panos, MD, Kypros H. Nicolaides, MD, John V. Anderson, MD, Demetrios L. Economides, MD, Lesley Rees, MD, and Roger Williams, MD

London, England

To investigate whether atrial natriuretic peptide is present in the human fetal circulation and changes in response to fetal blood volume expansion, the concentration of plasma atrial natriuretic peptide was measured by radioimmunoassay in samples obtained by cordocentesis. Twenty-four patients referred for intravascular blood transfusion because of red cell isoimmunization at 21 to 35 weeks' gestation were studied. Plasma atrial natriuretic peptide concentrations in fetal blood (median, 8.0 pmol/L; range, 1 to 27.3) were significantly higher than those of 25 young 18- to 32-year-old adult control subjects (median, 4.5 pmol/L; range, 1 to 11.3, $p < 0.002$ but similar to those found in the umbilical cord blood of 10 normal neonates immediately after delivery (median, 7.35 pmol/L; range, 2.7 to 15.5). In seven patients in whom fetal and maternal plasma atrial natriuretic peptide was measured simultaneously, all fetal concentrations were higher ($p < 0.01$). The concentration of fetal atrial natriuretic peptide before and immediately after blood transfusion ($n = 12$) rose significantly ($p < 0.05$), and the rise correlated positively with the transfusion rate ($p < 0.05$). We conclude that atrial natriuretic peptide is present in plasma of the human fetus as early as 21 weeks' gestation and that its concentration increases promptly in response to vascular volume expansion. These findings suggest that atrial natriuretic peptide may play a role in fetal volume homeostasis. (Am J Obstet Gynecol 1989;161:357-61.)

Key words: Atrial natriuretic peptide, fetal blood, volume expansion, cordocentesis

Atrial natriuretic peptide is a natriuretic and diuretic substance that suppresses the activity of the renin-aldosterone system and has a vasodilator action.[1] It is secreted by the cardiac atria in response to rises in atrial pressure through atrial wall stretch and thus appears to have a role in blood volume regulation.[1] In adults, plasma concentration of atrial natriuretic peptide has been shown to rise in response to central volume expansion as induced by intravenous infusion of saline sodium, postural change, and headout water immersion.[1,2] Whether physiologic levels of atrial natriuretic peptide affect volume homeostasis has been debated, although recent data suggest that atrial natriuretic peptide affects short-term regulation of sodium excretion.[3] It is not known whether atrial natriuretic peptide is involved in blood volume homeostasis in intrauterine life. The aim of the present study was to examine whether atrial natriuretic peptide is present in the circulation of the human fetus, and if so, whether its circulating levels change in response to fetal blood volume expansion.

From the Liver Unit and Harris Birthright Research Centre for Fetal Medicine, King's College Hospital School of Medicine and Dentistry, and the Department of Chemical Endocrinology, St. Bartholomew's Hospital.

Received for publication October 2, 1988; revised February 16, 1989; accepted February 24, 1989.

Reprint requests: Marios Z. Panos, MD, Liver Unit, King's College Hospital, School of Medicine and Dentistry, Denmark Hill, London SE 5, England.

Patients and methods

Twenty-four normotensive pregnant women (21 to 35 weeks' gestation), ages 18 to 39 years, referred for intravascular blood transfusion because of severe red cell isoimmunization, were studied. None of the fetuses was hydropic, as judged by ultrasound examination. Cordocentesis was performed as an outpatient procedure without maternal fasting, sedation, or fetal paralysis.[4] Pure fetal blood (3 ml) was aspirated from the umbilical vein into heparinized syringes for fetal hemoglobin, blood gases, and atrial natriuretic peptide measurement just before intravascular blood transfusion. Packed blood compatible with that of the mother (taken from the donor 24 to 36 hours earlier) was infused into the fetal circulation.[5] At the end of the transfusion, the needle was flushed with 1 ml of normal saline solution, and after 1 minute to allow for equilibration, an additional 1 ml sample was aspirated and discarded, and 0.2 ml was obtained for hemoglobin measurement. In 12 patients an additional 2.2 ml of fetal blood was aspirated for atrial natriuretic peptide assay. The fetal heart rate was monitored throughout the transfusion, and none of the procedures was complicated by fetal bradycardia. Transfusions were timed. In seven patients maternal blood was taken from an

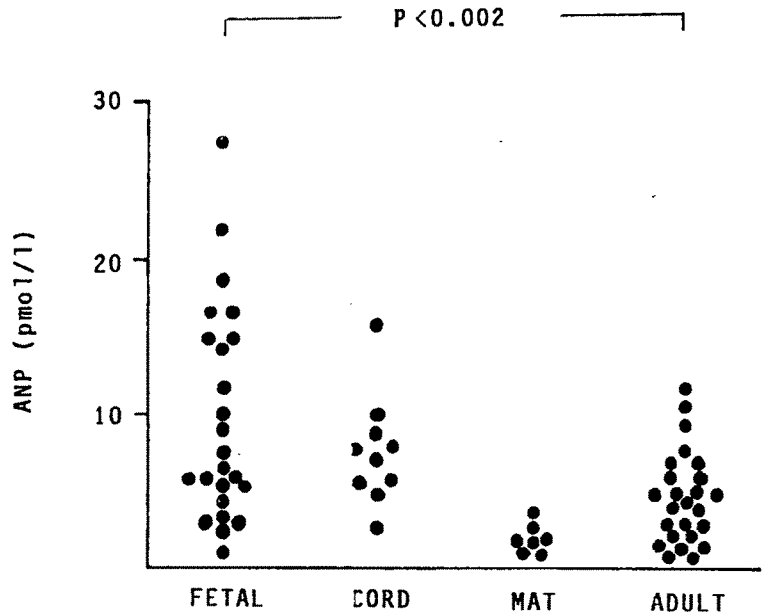


Fig. 1. Plasma atrial natriuretic peptide *(ANP)* concentration in fetal umbilical vein at 21 to 35 weeks' gestation *(FETAL)*, umbilical cord vein at full-term delivery of normal neonates *(CORD)*, maternal plasma *(MAT)*, and normal 18- to 32-year-old adults *(ADULT)*.

antecubital vein before and after fetal blood transfusion.

For comparison, blood was taken for atrial natriuretic peptide assay from the umbilical cord vein at birth of 10 neonates of normal full-term pregnancies within 5 minutes of delivery of the placenta. Plasma atrial natriuretic peptide concentration was also measured in 25 nonpregnant adult volunteers, ages 18 to 32 years. Venous blood samples were taken from an antecubital vein at 9:00 AM, while sitting (30 minutes) after an overnight fast. Plasma from donor blood was separated just before transfusion and assayed for atrial natriuretic peptide.

All samples were collected into chilled heparinized tubes and centrifuged at 4° C for 10 minutes within 10 minutes of collection. Plasma was stored at −20° C until assayed. Fetal hemoglobin was measured on a Coulter Counter S-Plus system (Coulter Electronics Ltd., Luton, United Kingdom) and blood oxygen tension (Po_2) on Radiometer ABL 30 (Radiometer, Copenhagen, Denmark).

Atrial natriuretic peptide immunoreactivity in plasma was measured after plasma extraction by means of a modification of a previously described radioimmunoassay procedure.[6] A new rabbit atrial natriuretic peptide antiserum was used (YB8) that increased the assay sensitivity to 0.3 fmol/tube, which allowed smaller samples to be processed. This modified assay procedure detects amounts of atrial natriuretic peptide immunoreactivity in plasma extracts not significantly different from values measured with an older, less-sensitive antiserum (Y2) in identical samples. Antiserum YB8 was used at a titer of 1 in 6400, at which 50% binding occurred. Otherwise the assay conditions were identical to those described earlier for antiserum Y2[6] except that a 10-point standard curve of 0.25 to 25 fmol of synthetic human α atrial natriuretic peptide was used. Human plasma extracts caused displacement of binding in the YB8 assay in parallel with that caused by pure synthetic α human atrial natriuretic peptide. Fragment testing revealed antiserum YB8 to bind to the central part of the atrial natriuretic peptide molecule with negligible binding to carboxy- and aminoterminal atrial natriuretic peptide fragments. For adult samples, 3 ml of plasma was extracted on Sep-Pak C-18 cartridges (Millipore, Bedford, Mass.). The retained peptides were eluted with 2 ml of 60% acetonitrile containing 0.1% trifluoracetic acid, lyophilized in 1.2 ml of radioimmunoassay buffer. Aliquots (200 μl) were assayed in duplicate. One milliliter fetal plasma samples were treated similarly but reconstituted in 900 μl of buffer. Three hundred microliter aliquots were assayed in duplicate and 100 μl aliquots in singlet. Assay sensitivity was equivalent to 0.3 pmol/L in adult plasma and 1 pmol/L in smaller-volume fetal samples. Intra-assay variation was 9% and interassay variation was 11%. Mean ± SEM recovery of atrial natriuretic peptide immunoreactivity from the Sep-Pak extraction step was 79% ± 2% for the adult plasma samples and 74% ± 4% for the smaller fetal samples. Results are presented as measured without correction for extraction recovery.

The volume of blood neccesary for transfusion was

calculated from the expected fetoplacental blood volume (adjusted for gestation) and the pretransfusion and donor hemoglobin concentrations.[7] The fetoplacental blood volume after each transfusion was calculated from the change in fetal hemoglobin concentration and the volume and hemoglobin concentration of the transfused blood.[8] Rate of transfusion was calculated from the duration and volume of blood transfused. Since fetal hemoglobin and umbilical venous Po_2 change with gestation, these were expressed as a difference from the normal means for gestation, δ hemoglobin gm/dl and δ Po_2 mm Hg, respectively.[7, 9]

Statistical analysis was by the Wilcoxon two-sample rank sum test and the Wilcoxon signed rank test for paired values as appropriate. Tests of correlation were by the Spearman rank test. The level of statistical significance was taken as $p \leq 0.05$. The study was performed with approval from the King's College Hospital Ethical Committee.

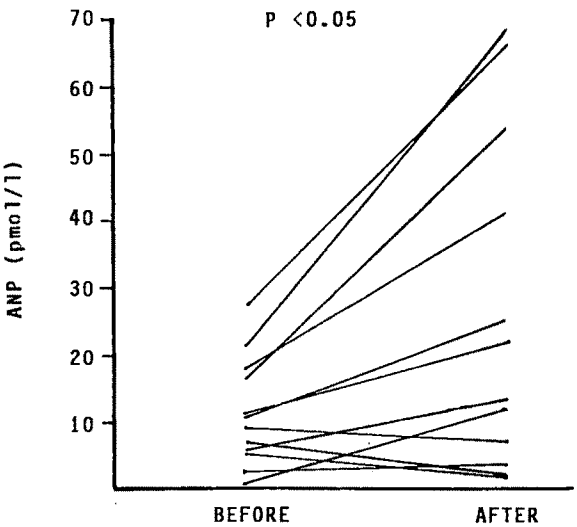


Fig. 2. Plasma atrial natriuretic peptide *(ANP)* concentrations before and immediately after intravascular blood transfusion in 12 fetuses with red cell isoimmunization.

Results

Intravascular blood transfusion was completed in all cases, and there were no complications. Basal atrial natriuretic peptide concentration was significantly higher in the fetus (median, 8.0 pmol/L; range, 1 to 27.3) than in 18- to 32-year-old nonpregnant adults (median, 4.5 pmol/L; range, 1 to 11.3, $p < 0.002$) and not significantly different from umbilical cord blood values after delivery (median, 7.35 pmol/L; range, 2.7 to 15.5) (Fig. 1). There was no correlation between length of gestation and basal fetal concentration of atrial natriuretic peptide. In the seven patients in whom paired fetal and maternal blood samples were taken, fetal plasma atrial natriuretic peptide concentrations (median, 16.5; range 5.8 to 27.3) were higher than the corresponding maternal atrial natriuretic peptide concentrations in all cases (median, 1.5 pmol/L; range, 1 to 3.3, $p < 0.01$). There was no correlation between basal δ Po_2 or δ hemoglobin (median, 2.4 mm Hg; range, −24.4 to 11.37 and median −3.2 gm/dl; range, −7.88 to 0.8 gm/dl, respectively), and basal atrial natriuretic peptide concentration, which suggests that oxygen tension or the degree of anemia did not influence basal fetal atrial natriuretic peptide levels.

Atrial natriuretic peptide was not detectable in donor blood. Fetal transfusions lasted between 3 and 16 minutes (median, 7.45), and the rate of transfusion was between 4.26 and 18.57 ml/min (median, 11.65). Volume of blood transfused expressed as a ratio of the estimated fetoplacental volume was 0.29 to 1.37 (median, 0.76). There was a significant increase in fetal atrial natriuretic peptide concentrations after transfusion ($p < 0.05$) (Fig. 2.), with a positive correlation between the rate of transfusion and change in atrial natriuretic peptide concentration ($p < 0.05$). There was no correlation either between the volume of blood transfused or the rate of expansion of the estimated fetoplacental blood volume and the change in fetal atrial natriuretic peptide concentration. There was no significant difference in the plasma atrial natriuretic peptide concentration of the paired maternal samples taken before and after intravascular blood transfusion ($n = 7$).

Comment

A previous study[10] reported that atrial natriuretic peptide was detectable in human umbilical cord blood. Our study concurs with this report and goes further to show that atrial natriuretic peptide is present in fetal plasma in considerable concentrations as early as 21 weeks' gestation. The demonstration of an increase in fetal atrial natriuretic peptide concentration after fetal blood volume expansion suggests that the mechanisms governing its release in the fetus are similar to those operating in adults.[1] Whether the observed rise in atrial natriuretic peptide after intravascular blood transfusion was enough to cause a natriuresis or an increase in venous capacitance in the fetus is not shown by the present study and will require further investigation. However, in the ovine fetus intravascular infusion of saline solution increases glomerular filtration rate and decreases fractional sodium reabsorption, findings consistent with previous reports of renal actions of atrial natriuretic peptide in adults.[1, 11, 12] Studies of fetal volume homeostasis in animals have demonstrated that changes in maternal hydration cause short-term changes in fetal blood volume through transplacental water exchange, with corresponding changes in fetal arginine-vasopressin and urine and lung fluid

flows.[13, 14] Collectively, these findings allow us to postulate that fetal atrial natriuretic peptide may act in a manner complementary to that of fluid-conserving hormones to protect the fetal blood volume from overexpansion.

Concentrations of atrial natriuretic peptide in the fetus were higher than in maternal plasma and adult control subjects, ages 18 to 32 years, but similar to those found in the umbilical cord blood of normal babies immediately after delivery. It is possible that the similarity between fetal umbilical vein concentrations of atrial natriuretic peptide and full-term umbilical vein concentrations after delivery may be fortuitous because of the longer gestation in the latter. Even though no hydrops was noted on ultrasound examination, it is possible that some fetuses may have been on the verge of "high output" heart failure, and this might account for the relatively high plasma concentrations of atrial natriuretic peptide in fetuses. The present results are consistent with the recent finding of a greater than twofold concentration of atrial natriuretic peptide in the plasma of healthy ovine fetuses compared with that of maternal plasma.[11] Messenger ribonucleic acid coding for the atrial natriuretic peptide prohormone is now known to be present in increased concentration in fetal cardiac ventricles compared with ventricles of normal adults,[15] and it is possible that release of atrial natriuretic peptide from the fetal ventricle contributes to the higher plasma concentrations of atrial natriuretic peptide found in the fetus.

The higher circulating levels of atrial natriuretic peptide in the fetus could be attributed either to an increase in secretion or reduced breakdown. The latter seems unlikely, since the ovine fetus has been shown to have a rapid clearance rate of plasma atrial natriuretic peptide.[12] On the other hand, hemodynamic and endocrine conditions in the fetus favor increased secretion of atrial natriuretic peptide: fetal cardiac output is proportionately increased compared with that of adults,[16] and increased plasma concentrations of glucocorticoids, progesterone, and aldosterone are known to be present in the fetal circulation. All these factors are known to potentiate atrial natriuretic peptide secretion.[1]

The significant rise in fetal plasma atrial natriuretic peptide concentration after transfusion did not correlate with volume of blood transfused or estimated fetoplacental volume expansion. This finding contrasts with animal data from a study of healthy ovine fetuses. Ross et al.[11] showed a positive correlation between volume of saline solution infused into the ovine fetal circulation and increase in atrial natriuretic peptide. Circulating atrial natriuretic peptide began to rise 10 to 20 minutes after the start of the infusion and reached a maximum at 40 minutes, a delay similar to that observed in human adults.[2] It is possible that the discrepancy between our findings in the human fetus and those in the ovine fetus[11] is the result of the timing of fetal blood samples after transfusion, which, for ethical reasons, were taken immediately after completion of transfusion when maximal levels of atrial natriuretic peptide may not have been reached. The positive correlation between the rate of infusion and rise in atrial natriuretic peptide is in agreement with findings of previous studies on the effects of saline solution infusion given at varying rates in young adults.[2] The increase in fetal atrial natriuretic peptide concentration after transfusion despite high basal levels demonstrates that the fetus retains significant atrial natriuretic peptide reserves.

In conclusion, atrial natriuretic peptide is present in the human fetal circulation as early as 21 weeks' gestation, and its concentration rises in response to blood volume expansion. These findings support the hypothesis that plasma release of atrial natriuretic peptide in the fetus may play a role in fetal blood volume homeostasis.

REFERENCES

1. Goetz KL. Physiology and pathophysiology of atrial peptides. Am J Physiol 1988;254: E1-5.
2. Anderson JV, Donckier J, McKenna WJ, Bloom SR. The plasma release of atrial natriuretic peptide in man. Clin Sci 1986;71:151-5.
3. Anderson JV, Donckier J, Payne NN, et al. Atrial natruretic peptide: evidence of action as a natriuretic hormone at physiological plasma concentrations in man. Clin Sci 1987;72:305-12.
4. Nicolaides KH, Rodeck CH, Soothill PW, Campbell S. Ultrasound guided sampling of umbilical cord to assess fetal well being. Lancet 1986;2:1065-7.
5. Nicolaides KH, Soothill PW, Rodeck CH, Clewell W. Rhesus disease: intravascular blood transfusion by cordocentesis. Fetal Ther 1986;1:185-92.
6. Anderson JV, Christofides ND, Vinas P, Varndell IM, Polak JM, Bloom SR. Radioimmunoassay of alpha rat atrial natriuretic peptide. Neuropeptides 1986;7:159-73.
7. Nicolaides KH, Clewell W, Rodeck CH. Measurement of human fetoplacental blood volume in erythroblastosis fetalis. Am J Obstet Gynecol 1987;157:50-3.
8. Nicolaides KH, Soothill PW, Clewell WH, Rodeck CH, Mibashan RS, Campbell S. Fetal haemoglobin measurement in the assessment of red cell iso-immunisation. Lancet 1988;1:1073-5.
9. Soothill PW, Nicolaides KH, Rodeck CH, Campbell S. The effect of gestational age on fetal and intervillous blood gas and acid-base values in human pregnancy. Fetal Ther 1986;1:166-73.
10. Yamaji T, Hirai N, Ishibashi M, et al. Atrial natriuretic peptide in umbilical cord blood: evidence for a circulating hormone in human fetus. J Clin Endocrinol Metab 1986;63:1414-7.
11. Ross MG, Ervin MG, Lam RW, Castro L, Leake RD, Fisher DA. Plasma atrial natriuretic peptide response to volume expansion in the ovine fetus. Am J Obstet Gynecol 1987;157:1292-7.
12. Ervin MG, Ross MG, Lam RW, et al. Atrial natriuretic peptide: ovine fetal cardiovascular responses and arginine-vasopressin secretion. Clin Res 1987;35:233A.

13. Hurley JK, Kirkpatrick SE, Pitlick PT, Friedeman WF, Mendoza SA. Renal responses of the fetal lamb to fetal or maternal volume expansion. Circ Res 1977;40:557-60.
14. Ross MG, Leake RD, Ervin MG, Fisher DA. Fetal lung fluid response to maternal hyperosmolality. Pediatr Pulmonol 1986;250:E253-8.
15. Tsuchimochi H, Kurimoto F, Ieki K, et al. Atrial natriuretic peptide distribution in fetal and failed adult human hearts. Circulation 1988;78:920-7.
16. Walker AM. Physiological control of the fetal cardiovascular system. In: Beard RW, Nathaniels ZPW, eds. Fetal physiology and medicine. London: Butterworths, 1984: 287-316.

Fetal cardiac output in the isoimmunized pregnancy: A pulsed Doppler-echocardiographic study of patients undergoing intravascular intrauterine transfusion

Joshua A. Copel, MD,* Peter A. Grannum, MD, Jacqueline J. Green, MS, RDMS, Kathy Belanger, PhD, Nagui Hanna, MD, C. Carl Jaffe, MD, John C. Hobbins, MD, and Charles S. Kleinman, MD**

New Haven, Connecticut

The mechanism of development of hydrops fetalis in severe isoimmunization has been subject to speculation. We performed pulsed Doppler assessment of cardiac output in 13 severely isoimmunized fetuses before and after intravascular transfusion and compared the results with 37 control fetuses between 20 and 34 weeks' gestation. The cardiac index of the anemic fetuses was significantly greater than that of the control group. A significant (right ventricle, $p < 0.01$; left ventricle, $p < 0.02$) increase in indexed output was noted from both ventricles and in the combined ventricular output (mean ± SEM 644 ± 35.3 ml/kg/min in control fetuses versus 879 ± 86.0 ml/kg/min in anemic fetuses, $p < 0.006$). An increase in cardiac output was also noted when anemic fetuses were compared with gestational age–specific norms. We conclude that severely anemic fetuses of isoimmunized pregnancies tend to have significantly higher cardiac output than do unaffected fetuses and that this high output state may play a part in the development of hydrops fetalis. (Am J Obstet Gynecol 1989;161:361-5.)

Key words: Prenatal diagnosis, fetal therapy, isoimmunization, Doppler ultrasound, cardiac output, intrauterine transfusion

The mechanism for the development of hydrops fetalis in the anemic, isoimmunized fetus remains the subject of speculation. Among the mechanisms proposed are anemia with secondary cardiac pump failure and increased venous pressures, hypoproteinemia with low colloid osmotic pressure, portal hypertension caused by extramedullary hematopoiesis, and high output cardiac failure.

From the Yale Fetal Cardiovascular Center, Departments of Obstetrics and Gynecology, Pediatric Cardiology, and Diagnostic Imaging, Yale University School of Medicine.
Received for publication August 7, 1988; revised January 20, 1989; accepted March 3, 1989.
Reprint requests: Joshua A. Copel, MD, Department of Obstetrics and Gynecology, Yale University School of Medicine, 333 Cedar St., P.O. Box 3333, New Haven, CT 06510.
**Dr. Copel was Kennedy-Dannreuther Foundation Fellow of the American Gynecologic and Obstetric Society.*
***Dr. Kleinman is the recipient of Clinical Research Grant No. 6-225 from the March of Dimes Birth Defects Foundation, Mamaroneck, N.Y.*

Although the studies of Phibbs et al.[1] suggest that the blood volume of erythroblastotic infants at birth is not generally elevated, careful study of the cardiac output of severely anemic fetuses has not been carried out. We have performed a series of intravascular fetal transfusions on severely isoimmunized fetuses.[2,3] On the basis of previous observations that support the concept that nonimmune hydrops represents congestive heart failure in the fetus and that cardiac Doppler waveform changes could be observed in fetuses with hydrops caused by tachyarrhythmias,[4,5] we have pursued further investigation of the mechanism of hydrops fetalis using pulsed Doppler flow analysis. The present study examines the cardiac output, as measured by pulsed Doppler ultrasound studies, of fetuses with severe anemia caused by isoimmunization.

Material and methods

Thirty-five nonanemic fetal patients underwent a single examination at the time of fetal echocardiography

Table I. Clinical comparison of control and isoimmunized groups

	Control (N = 37)	*Isoimmunized (N = 11)*	*p*
Gestational age (wk)			NS*
20-23.9	9	2	
24-27.9	13	5	
28-31.9	13	4	
32-35.9	6		
36 and above	3		
Estimated fetal weight (gm)			NS*
below 500	4		
500-999	11	4	
1000-1499	10	5	
1500-1999	4	1	
2000-2499	4	1	
2500 and above	2		

*χ^2.

for routine indications between 19 and 34 weeks' gestation (mean, 28.2 weeks; SD, 5.0). These fetuses made up the control group. All had normal anatomic studies and uncomplicated pregnancies. The control and isoimmunized groups were similar in relevant demographic characteristics (Table I). For both control and isoimmunized groups, all studies were carried out by one of the authors (J. A. C.) after informed consent was obtained for participation in this study, which was performed in accordance with a protocol approved by the institutional Human Investigation Committee.

The anemic study group was drawn from 37 patients with isoimmunization caused by rhesus or other antibodies who were referred to the Yale Perinatal Unit for intrauterine intravascular transfusion. Thirteen fetuses were examined before any intrauterine treatment. The transfusion procedures and patient population have been described separately.[2, 3] Pulsed Doppler echocardiography was performed within 24 hours before and 12 hours after transfusion.

The initial examination consisted of a survey of fetal anatomy, biometry, and evaluation for the presence or absence of hydrops (defined for this study as at least two of the following: ascites, pleural or pericardial effusions, or skin edema). Fetal weight was estimated on the basis of biparietal diameter and abdominal circumference.[6] If fetal ascites was present, fetal weight was estimated by using a mean for gestational age by last menstrual period, which was confirmed by biometric measurements, including consideration of the biparietal diameter and multiple long bones. All of the anemic fetuses had accurate menstrual dating as confirmed by these sonographic criteria. Patients were studied in the Sims' position, with right hip elevation used for those who reported discomfort in the supine position.

Complete fetal echocardiographic examination was undertaken with the use of standard tomographic planes. Anatomy was normal in all studied patients. All studies were recorded on videotape and analyzed separately. Measurement of the atrioventricular valve diameters was accomplished from recordings of a four-chamber view of the heart in real time, with the ultrasound beam as close as possible to perpendicular to the interventricular septum. Structural recordings were made by means of a 5 MHz-phased array sector transducers (model HP 77020A, Hewlett-Packard, Andover, Mass.). Videotaped real-time images were played back at slow speed and frozen at maximal valve leaflet separation and then measured at a point just apical to valve insertion into the annulus, where the leaflets could be seen extending parallel to each other. At least four measurements were obtained for each valve during fetal apnea.

Table II. Transatrioventricular valve flow*

	Control	*Isoimmunized*	*p*
Tricuspid valve	361 (20.8)	489 (49.8)	<0.01
Mitral valve	284 (17.8)	390 (43.7)	<0.02

*Values are milliliters per kilogram per minute (± SEM).

Pulsed Doppler information was obtained with an apical four-chamber view of the fetal heart, with the ultrasound beam as close as possible to parallel to the plane of the interventricular septum. Doppler interrogation of the atrioventricular valves was carried out just below the valve annulus. Angle correction was applied assuming that inflow was parallel to the ventricular septum. Instruments used were specifically calibrated by the manufacturers to comply with current Food and Drug Administration acoustic output limitations with I(SPTA) below 94 mW/cm² (Model HP 77020A, Hewlett-Packard; ATL UltraMark 4, Advanced Technology Laboratories, Bothell, Wash.).

Analysis of Doppler waveforms was accomplished from videotaped waveforms digitized on a VAX workstation (Digital Equipment Corp., Maynard Mass.) by means of software developed by one of the authors (C. C. J.). Mean flow velocity across the atrioventricular valves was calculated by digitizing the maximal envelope of the waveform and applying a correction factor of 0.7.[7] All waveforms used had minimal spectral broadening (plug flow). Stroke volume was calculated by multiplying mean velocity by valve area (derived from the two-dimensional image, assuming a circular valve orifice). Cardiac output was calculated by multiplying stroke volume times fetal heart rate.

Group means were compared by the *t* test. For multiple comparisons, analysis of variance was used.

Results

Thirty-seven studies were carried out on 18 isoimmunized fetuses (range, 1 to 5 studies per patient) between 21 and 35 weeks' gestation (mean, 29.1 ± SD

Table III. Components of cardiac output and index calculations in control and isoimmunized groups*

	Control (N = 35)	*Isoimmunized (N = 11)*	*p*
Tricuspid			
Heart rate	138 (1.69)	143 (4.22)	NS
Diameter (mm)	8.5 (0.32)	8.9 (0.57)	NS
Output (ml/min)	402 (36.3)	502 (72.4)	NS
Index (ml/min/kg)	361 (20.8)	489 (49.8)	< 0.01
Mitral			
Heart rate	140 (1.86)	143 (4.22)	NS
Diameter	7.8 (0.29)	8.4 (0.45)	NS
Output	302 (24.2)	382 (41.0)	NS
Index	284 (17.8)	390 (43.7)	< 0.02
Combined output (index)	644 (35.5)	879 (86.0)	< 0.006

*Values given are mean (± SEM)

Table IV. Stroke volumes and ventricular outputs of control and isoimmunized fetuses matched for gestational age*

	Control	*Isoimmunized*	*p*
RV stroke volume	104 (5.6)	186 (24.4)	< 0.0001
LV stroke volume	104 (4.7)	229 (67.3)	< 0.002
Combined stroke volume	103 (4.6)	203 (40.1)	< 0.0001
RV output	105 (5.7)	192 (26.9)	< 0.0001
LV output	104 (4.9)	234 (74.2)	< 0.003
Combined output	104 (4.9)	208 (44.0)	< 0.0002

RV, Right ventricular; *LV*, left ventricular.
*Values are percent predicted, mean (± SEM).

3.7). Thirteen studies were performed before the first intravascular transfusion of that pregnancy; the remainder were performed on patients undergoing second or subsequent transfusions. Of the 13 patients, two were studied before initial transfusions; their results were markedly higher than any other patients in either group (combined ventricular outputs of 1391 and 2380 ml/min/kg). The studies of these patients were performed at relatively high incident angles (40 and 60 degrees), introducing potential error into the flow estimate. To avoid any skew in the data resulting from exaggeration of the isoimmunized group results by these two patients, they have been removed from comparisons with the control group. Therefore the remaining analysis involves a comparison of 11 patients studied before initial intravascular transfusion.

Transatrioventricular valve flow from each ventricle and combined ventricular output are shown in Table II. Each ventricle's output when indexed to estimated fetal weight was significantly higher than that of the control group. The combined indexed ventricular output of the isoimmunized group was similarly significantly elevated.

Because several factors contribute to the final measurement of flow, we examined these in the two groups. As already noted, the gestational ages and estimated fetal weights were similar in the two groups. Table III shows the valve diameters, mean velocities, and heart rates of the two groups. The two groups did not differ significantly in any of these variables, although there was a tendency, albeit not at a level of statistical significance, for each of the measured factors except for estimated weight to be higher in the anemic state.

To provide another means of comparison of the two groups independent of fetal weight, which might have been inaccurately estimated in the hydropic fetuses, the cardiac output of our normal population was expressed relative to gestational age. The stroke volume (milliliters) and ventricular output (milliliters per minute) of the anemic fetuses could then be compared with age-matched control fetuses. Table IV shows the differences between the two groups expressed as a percentage of predicted in the control group. The anemic fetuses had significantly greater stroke volumes and ventricular outputs than those predicted for gestational age.

Comparison of the right and left ventricular outputs before and after transfusion failed to show any significant changes. Comparison of the indexed flow was similar. However, one patient was notable for an increase of approximately 50% in the flow. This patient, who is reported on in greater detail elsewhere,[3] had a bradycardic, terminally hydropic fetus, whose cardiac output was 444 ml/min/kg (1.5 SD below the mean for isoimmunized fetuses and the lowest of any fetus in the group). Fetal activity improved after transfusion, but 2 weeks later porencephaly and diffuse cortical atrophy

were noted. No further transfusions were requested by the parents.

Comparison of cardiac output to fetal hematocrit failed to demonstrate any significant correlation between the hematocrit and either ventricle's flow or the combined ventricular output.

The right and left ventricular outputs were compared with each other. In the control group the right to left ventricular output ratio was 1.33, which is similar to values reported by others.[8-11] In the anemic group the ratio was 1.31.

Comment

Fetal hydrops has been postulated to occur as the result of a number of influences. Anemia leading to congestive heart failure and increased venous pressure,[1] low plasma oncotic pressure,[1] or anemia and hypoproteinemia complicated by hypoxia[12] have been implicated as etiologic agents. To date only one case report has appeared comparing cardiac output in anemic fetuses to normal fetuses.[13] That study showed no difference between a control population and a severely anemic fetus (hematocrit, 7.5%).

In this study we found significant elevations in the right and left ventricular output indexes and the combined ventricular output index of anemic fetuses compared with a control group. These differences could not be explained on the basis of greater heart rates, larger valve diameters, or smaller fetuses in the anemic group.

The fetal hematocrit level was not significantly correlated with cardiac output indexes of either ventricle or the combined ventricular index. The absence of such a finding might be postulated to relate to different stages of disease. Earlier stages might elevate output, with a depression in output at a later stage. This speculation is supported by the depressed output seen in the most severely ill fetus as previously mentioned.

The relationship between fetal anemia and increased cardiac output is uncertain in origin. Fumia et al.[14] found no change in combined ventricular output in sheep fetuses made acutely anemic by exchange transfusion. The mechanism for our findings is unlikely to be hypoxia. Although an increase in lactate concentrations have been reported in anemic fetuses,[15] this was most remarkable in the umbilical artery when the hemoglobin level was <4 gm/dl (hematocrit, 12%). Only three of 11 fetuses in the present study had hematocrit values below 12%. Tachycardia might have been anticipated in the presence of significant tissue hypoxia, but no relationship with fetal hematocrit was found ($r = 0.12$, $p = 0.69$ for right heart rate; $r = 0.43$, $p = 0.14$ for left heart rate [different heart rates result from beat-to-beat measurement of Doppler cycles]).

The performance of Doppler-based estimations of absolute flow is potentially influenced by a number of sources of error. In the present study these include angle estimation, waveform digitization, atrioventricular valve measurement, and estimation of fetal weight. The incident angles were minimized for all fetuses scanned and were similar in the anemic and control groups. Small errors would have been unlikely to systematically elevate the measurements in the anemic group only. Similar considerations apply to the digitization and valve measurement components of the cardiac output calculation.

A remaining concern is the effect of including hydropic fetuses. Because standard formulas for the estimation of fetal weight are based on biparietal diameter and abdominal circumference,[6] ascites would exaggerate fetal weight. We chose to use a mean for gestational age, as determined by menstrual history and sonography, in such situations. To compare the anemic and control groups independent of estimated fetal weight, formulas expressing stroke volumes and ventricular outputs as a function of gestational age were derived for the control group. The anemic fetuses retained their significant differences from the control fetuses. Because these two measurements were calculated independent of estimated fetal weight, inaccuracies in estimated fetal weight from inclusion of hydropic fetuses seem not to be the sole explanation for the differences found between the two groups. All the estimated fetal weights of the control group fell within 2 SD of the mean for gestational age, averaging 0.5 SD below the mean, thereby making elevated weight estimates and consequently lower cardiac indexes in control subjects unlikely to be the explanation for the differences seen.

We conclude that severely isoimmunized, anemic fetuses tend to have higher cardiac output than do control fetuses whether expressed as a function of estimated fetal weight or gestational age. The increase is shared proportionately by both ventricles. Some affected fetuses will have values in the normal range. Although the relationships described are significant, wide variations exist, making predictions of severity of the disease in the nonhydropic fetus unlikely to be accurate. Although there is no significant relationship between hematocrit values and cardiac output, extremely compromised fetuses may demonstrate diminished cardiac function as a terminal finding.

Further areas for investigation will include diastolic function of the ventricle, ventricular contraction characteristics, and longitudinal evaluation of the fetus over a course of intrauterine therapy. Finally, the basic cause of hydrops remains subject to speculation. Cardiac function must still be included as a potential part of

the explanation for why hydrops develops in the severely anemic fetus.

We gratefully acknowledge the patient care provided by the Yale Obstetrics and Gynecology housestaff and the nursing care provided by Mitzi Barger, RN, and Nancy Nickless, RN.

REFERENCES

1. Phibbs RH, Johnson P, Tooley WH. Cardiorespiratory status of erythroblastotic newborn infants: II. Blood volume, hematocrit and serum albumin concentration in relation to hydrops fetalis. Pediatrics 1974;53:13-23.
2. Grannum PA, Copel JA, Plaxe S, et al. Intrauterine intravascular transfusion for severe erythroblastosis fetalis. N Engl J Med 1986;314:1431-4.
3. Grannum PA, Copel JA, Moya F, et al. The reversal of hydrops fetalis by intravascular intrauterine transfusion in severe isoimmune fetal anemia. AM J OBSTET GYNECOL 1988;158:914-9.
4. Kleinman CS, Donnerstein RL, DeVore GR, et al. Fetal echocardiography for evaluation of in utero congestive heart failure: a technique for the study of non-immune fetal hydrops. N Engl J Med 1982;306:568-75.
5. Kleinman CS, Weinstein EM, Copel JA. Pulsed Doppler analysis of human fetal blood flow. Clin Diagn Ultrasound 1986;17:173-85.
6. Shepard MJ, Richards VA, Berkowitz RL, et al. An evaluation of two equations for predicting fetal weight by ultrasound. AM J OBSTET GYNECOL 1982;142:47-54.
7. Hatle L, Angelsen B. Doppler ultrasound in cardiology. 2nd ed. Philadelphia: Lea & Febiger, 1985:324.
8. Kenny JF, Plappert T, Doubilet P, et al. Changes in intracardiac blood flow velocities and right and left ventricular stroke volumes with gestational age in the normal human fetus: a prospective Doppler echocardiographic study. Circulation 1986;74:1208-16.
9. Reed KL, Meijboom EJ, Sahn DJ, et al. Cardiac Doppler flow velocities in human fetuses. Circulation 1986;73:41-6.
10. Kenny JF, Plappert T, Doubilet P, et al. Effects of heart rate on ventricular size, stroke volume, and output in the normal human fetus: a prospective Doppler echocardiographic study. Circulation 1987;76:52-8.
11. De Smedt MCH, Visser GHA, Meijboom EJ. Fetal cardiac output estimated by Doppler echocardiography during mid- and late gestation. Am J Cardiol 1987;60:338-42.
12. Nicolaides KH, Warenski JC, Rodeck CH. The relationship of fetal plasma protein concentration and hemoglobin level to the development of hydrops in rhesus isoimmunization. AM J OBSTET GYNECOL 1985;152:341-4.
13. Barss VA, Doubilet PM, St. John-Sutton M, et al. Cardiac output in a fetus with erythroblastosis fetalis: assessment using pulsed Doppler ultrasound. Obstet Gynecol 1987;70:442-4.
14. Fumia FD, Edelstone DI, Holzman IR. Blood flow and oxygen delivery to fetal organs as functions of fetal hematocrit. AM J OBSTET GYNECOL 1984;150:274-82.
15. Soothill PW, Nicolaides KH, Rodeck CH, et al. Relationship of fetal hemoglobin and oxygen content to lactate concentration in Rh isoimmunized pregnancies. Obstet Gynecol 1987;69:268-71.

Twin-to-twin transfusion syndrome: What are appropriate diagnostic criteria?

Fiona H. Danskin, MB, ChB,* and James P. Neilson, MD
Glasgow, Scotland

One hundred seventy-eight consecutive twin pregnancies were studied to reevaluate the standard diagnostic criteria for chronic twin-to-twin transfusion syndrome of an intertwin hemoglobin difference >5 gm/dl and a birth weight difference >20%. Hemoglobin differences > 5 gm/dl were found in six pregnancies with monochorionic placentas but also in seven with dichorionic placentas. Birth weight differences >20% occurred no more commonly in monochorionic than in dichorionic pregnancies. Of the four pregnancies with a coexisting hemoglobin difference >5 gm/dl and birth weight difference >20%, only one had a monochorionic placenta and therefore likelihood of vascular anastomoses. Diagnosis of twin-to-twin transfusion syndrome cannot be definitively established by current standard diagnostic criteria. (AM J OBSTET GYNECOL 1989;161:365-9.)

Key words: Twin pregnancy, twin-to-twin transfusion syndrome, diagnosis

From the Department of Midwifery, University of Glasgow, the Queen Mother's Hospital.
Received for publication August 7, 1988; revised December 8, 1988; accepted February 24, 1989.
Reprints not available.
**Dr. Danskin holds the Cruden Medical Research Scholarship administered by the Scottish Hospital Endowments Research Trust.*

Twin-to-twin transfusion syndrome is an important complication of twin pregnancy,[1, 2] resulting from shunting of blood from one twin to the other through placental vascular anastomoses. Injection studies of twin placentas have shown that such anastomoses are almost universally present in monochorionic placentas

Table I. Zygosity studies in 178 consecutive twin pregnancies

Monochorionic placenta	51	70 Monozygotic
Blood group studies	19	
	35	87 Dizygotic
Discordant sex	52	
Intrauterine death	4	21 Unknown
Technical failure	17	

but so extremely rare in dichorionic placentas[1, 3] that the possibility of the syndrome occurring with this type of placenta can be disregarded. Both acute and chronic forms of the twin-to-twin transfusion syndrome have been described.[4, 5] The acute form has been attributed to rapid transfer of blood through superficial artery-to-artery or vein-to-vein anastomoses during labor and delivery, resulting in a hypovolemic, anemic donor twin and a hypervolemic, plethoric recipient twin of similar birth weight.[4, 5] The chronic syndrome results from gradual antepartum transfusion through deep artery-to-vein anastomoses, rendering the donor twin not only hypovolemic and anemic but also growth retarded, whereas the hypervolemic, plethoric recipient is usually larger.[6-8] These features may be accompanied by oligohydramnios in the sac of the donor and polyhydramnios in that of the recipient and may be identifiable before birth by ultrasonography.[9, 10]

Twin-to-twin transfusion syndrome is usually diagnosed or confirmed after birth by the discovery of a hemoglobin difference >5 gm/dl between twins[2] and a birth weight difference >20%, providing evidence of the chronic form of the syndrome.[4] We have reevaluated these standard and widely accepted diagnostic criteria as part of an ongoing intensive study of twin pregnancy. Despite an exhaustive MEDLINE literature search, we have not been able to identify any other such reevaluation.

Material and methods

One hundred seventy-eight consecutive twin pregnancies delivering in The Queen Mother's Hospital, Glasgow, (a tertiary referral center) between August 1984 and February 1988 were studied. Of the mothers, 160 had attended the hospital for antenatal care, mostly at a special twin pregnancy antenatal clinic, and the other 18 were transferred from other hospitals, usually in established or incipient preterm labor. Venous blood samples were usually obtained from neonates by pediatricians on the first day of life for hematologic indexes, including hemoglobin concentration. In some cases in which both babies were well, at term, and appropriate for gestational age, the pediatric staff considered venesection unnecessary on clinical grounds. All birth weights were recorded, and the intertwin birth weight difference was calculated as a percentage of the weight of the larger twin.

Zygosity was determined in 88% of pregnancies (Table I) by inspection of the placentas (a monochorionic placenta indicating a monozygotic pregnancy), sex discordance (indicating a dizygotic pregnancy), or when necessary by comparing red blood cell antigens of the ABO, rhesus, MNS, Kell, Duffy, Kidd, Lutheran, and Wright systems.[11] In all 21 cases in which zygosity was not established, the placenta was dichorionic and the twins were of like sex; in four there had been intrauterine death of one twin, and in the remainder blood was not sent for grouping.

Total perinatally related wastage, an index that includes late abortions, stillbirths, early and late neonatal deaths, and perinatally related infant deaths,[12] is shown in Table II. In all three monochorionic monozygotic pregnancies in which one or both fetuses were stillborn, features were compatible with, although not diagnostic of, twin-to-twin transfusion syndrome. In one preterm labor occurred at 34 weeks, and the leading twin died of asphyxia after abdominal dystocia caused by ascites; the second twin weighed 530 gm less and had a hemoglobin value at birth of 11 gm/dl. In another pregnancy acute polyhydramnios occurred in the amniotic sac of the first twin, which resulted in abruptio placentae and intrauterine death. The second twin, who survived, was very pale at birth and weighed 27% less than the first. In a third pregnancy acute polyhydramnios developed at 32 weeks, which was more marked in the amniotic sac of the first twin. Labor supervened within 24 hours and stillborn female infants were born; the first twin weighed 1.71 kg and appeared very plethoric with congested organs at postmortem examination, and the second twin weighed only 1.46 kg and appeared very pale. Hemoglobin values were not obtained in the latter two pregnancies.

Results

The hemoglobin concentrations of venous blood samples withdrawn from both babies on the first day of life were obtained in 118 of the 178 pregnancies. Both hemoglobin values were known in 35 of the 51 monochorionic pregnancies, and in six there was an intertwin difference >5 gm/dl. However, there were two pregnancies in the monozygotic dichorionic group, one in the dizygotic group, and four in the zygosity unknown group, all of which had dichorionic placentas, in which hemoglobin differences >5 gm/dl were also found. Oligohydramnios occurred in the amniotic sac of one twin in only two of these 13 pregnancies, both

Table II. Outcome in 178 consecutive twin pregnancies

Zygosity/placentation	*No. of babies*	*Alive*	*Late abortions*	*Stillbirths*	*Fetus papyraceus*	*Early NND*	*PRID**	*TPRW/1000†*
Monozygotic monochorionic	102	93	1	4	1	3‡	0	64.3
Monozygotic dichorionic	38	38	0	0	0	0	0	
Dizygotic	174	164	0	1	1	8§‖	0	57.5
Not known	42	32	1	1	4	2	2	238.1

NND,Neonatal deaths; *PRID*, perinatally related infant deaths; *TPRW*, total perinatally related wastage.

*Perinatally related infant deaths are all infant deaths (between 1 month and 1 year of life) from or partly from a primary perinatal complication or its treatment.[12]

†Total perinatally related wastage includes all late abortions, except terminations of pregnancy for fetal abnormalities, all perinatal deaths, all late neonatal deaths (during the second, third, and fourth weeks of life), and all perinatally related infant deaths.[12]

‡One exomphalos.

§One anencephalic.

‖One cyanotic congenital heart disease.

of which had dichorionic placentas. None of the twenty-six babies in this group required treatment for anemia or polycythemia in the neonatal period.

Birth weight differences, which were expressed as a percentage of the weight of the larger twin, were calculated for all pregnancies. Excluding those cases in which there was a fetal abnormality or a fetus papyraceus, a birth weight difference >20% was present in 17.4% of pregnancies. The distribution of these according to zygosity is shown in Table III. Oligohydramnios was again an infrequent occurrence in this group and was found in the sac of one twin in only four pregnancies. Three of these had a dichorionic placenta and one had a monochorionic placenta.

In only four pregnancies did a hemoglobin difference >5 gm/dl and a birth weight difference >20% coexist, and none of these four pregnancies showed evidence of oligohydramnios or polyhydramnios in either amniotic sac. Only one of these pregnancies was associated with a monochorionic placenta, and in it alone did the heavier twin have the higher hemoglobin value (Table IV).

Table III. Birth weight difference >20%

Zygosity/placentation	*N*	*Birth weight difference >20%*	*%*
Monozygotic monochorionic	51	8	15.7
Monozygotic dichorionic	19	4	21.1
Dizygotic	87	14	16.0
Not known	21	5	23.8
	178	31	17.4

Comment

Since the recognition of the twin-to-twin transfusion syndrome by Herlitz[13] in 1941, much interest has been focused on this rare complication of twin pregnancy. Initial publications were single case reports of the syndrome, whereas larger studies later attempted to define the incidence, pathogenesis, and associated morbidity and mortality.[2, 4] It was suggested that when a large volume of blood is transferred antepartum, one or both twins may die in utero.[2] Since many early studies only considered those pregnancies resulting in the live birth of both babies, they probably underestimated the true incidence of the syndrome. In our study all three monochorionic pregnancies resulting in the stillbirth of one or both babies demonstrated features compatible with the twin-to-twin transfusion syndrome.

However, the purpose of our study was neither to determine the significance of the twin-to-twin transfusion syndrome in contributing to perinatal mortality and morbidity nor to define its incidence (we did not have hemoglobin values from all sets of twins). Rather, we aimed to reevaluate the standard diagnostic criteria for the twin-to-twin transfusion syndrome—a matter not only of clinical importance but also vital for further perinatal research.

Indeed, our study was generated by conflicting reports of fetal velocity waveforms of the umbilical artery as assessed by the new technology of Doppler ultrasonography on twin pregnancies believed complicated by the twin-to-twin transfusion syndrome. Concordant,[14] discordant,[15] and variable[16] patterns have been described, and these discrepancies may have stemmed from diagnostic confusion.

Twin-to-twin transfusion syndrome has traditionally been diagnosed after birth by finding a hemoglobin difference >5 gm/dl—a criterion introduced by Rausen et al.[2] in 1965. They had found such a hemoglobin difference in 19 of 130 monochorionic pregnancies, whereas in 43 dizygotic pregnancies no difference >3.3

Table IV. Birth weight difference >20% and hemoglobin difference >5 gm/dl

Zygosity/placentation	Birth weight			Hemoglobin		
	Twin 1 (kg)	Twin 2 (kg)	Difference (%)	Twin 1 (gm/dl)	Twin 2 (gm/dl)	Difference (gm/dl)
Monozygotic monochorionic	2.85	2.18	23.51	24.0	16.0	8.0
Monozygotic dichorionic	2.39	1.49	37.66	14.9	27.4	12.5
Dizygotic dichorionic	1.47	2.41	39.00	22.9	17.0	5.9
Dizygotic dichorionic	3.16	2.25	28.80	13.7	25.0	11.3

gm/dl was found. Therefore they selected a hemoglobin difference > 5 gm/dl as a suitable cutoff value, and this has been accepted by authors of subsequent publications as definitive evidence of the twin-to-twin transfusion syndrome. However, our study showed that although a hemoglobin difference > 5 gm/dl existed in six monochorionic pregnancies, it also occurred in seven dichorionic pregnancies; in fact the largest hemoglobin differences (12.5 and 11.3 gm/dl) occurred in the latter group. Because placental anastomoses are extremely rare in fused dichorionic placentas,[3] it is unlikely that the twin-to-twin transfusion syndrome occurred in these seven cases. Thus a difference in hemoglobin concentration is not in itself sufficient to establish a diagnosis of twin-to-twin transfusion syndrome.

Tan et al.[4] studied 482 twin pairs in an attempt to differentiate the acute and chronic forms of the syndrome. They diagnosed the syndrome in 35 sets of twins on finding obvious plethora in one twin and pallor in the other, along with a hemoglobin difference >5 gm/dl. They divided the twins into two groups according to whether the birth weight difference between each pair was greater or less than 20% of the weight of the heavier twin. They found that in those twins who had a large weight difference, the twin with the lower hemoglobin was invariably lighter, whereas in those twins with a small weight difference, the twin with the lower hemoglobin value was heavier. They concluded that birth weight difference provides a simple yet reliable yardstick for differentiation of twin pregnancies with hemoglobin differences into acute and chronic forms. This additional criterion of birth weight difference >20% has been widely adopted.

However, our study showed that a birth weight difference >20% occurred no more commonly in monochorionic pregnancies than in dichorionic pregnancies. In addition, of the four pregnancies in which a hemoglobin difference >5 gm/dl and a birth weight difference >20% coexisted, only one had a monochorionic placenta. In this pregnancy the heavier twin had the higher hemoglobin value, whereas in the three dichorionic pregnancies the lighter twin had the higher hemoglobin value. These presumably represent growth retardation in association with polycythemia, a well-recognized combination. Because Tan et al. used a hemoglobin difference >5 gm/dl as the sole diagnostic criterion for the twin-to-twin transfusion syndrome and did not take into account chorionicity of the placenta, it is likely that their subgroup included a similar proportion of polycythemic growth retarded babies from dichorionic pregnancies.

In summary, the standard diagnostic criteria used in clinical obstetrics and perinatal research do not permit a definitive diagnosis of the twin-to-twin transfusion syndrome. Differences in hemoglobin and birth weight must be interpreted in the light of knowledge of placental morphology and whether the heavier or lighter twin had the higher hemoglobin value. Further studies are still required to elucidate this enigmatic condition.

We gratefully acknowledge the contributions of Dr. A. A. M. Gibson, consultant pathologist, and the Regional Blood Transfusion Service, Law Hospital, in establishing zygosity, and the helpful criticisms of this manuscript by Professor C. R. Whitfield and Dr. B. Holland.

REFERENCES

1. Benirschke K. Twin placenta in perinatal mortality. NY State J Med 1961;61:1499-508.
2. Rausen AR, Seki M, Strauss L. Twin transfusion syndrome. J Pediatr 1965;66:613-28.
3. Robertson EG, Neer KJ. Placental injection studies in twin gestation. Am J Obstet Gynecol 1983;147:170-4.
4. Tan KL, Tan R, Tan SH, Tan AM. The twin transfusion syndrome. Clin Pediatr 1979;18:111-4.
5. Klebe JG, Ingomar CJ. The fetoplacental circulation during parturition illustrated by the interfetal transfusion syndrome. Pediatrics 1972;49:112-6.
6. Becker AH, Glass H. Twin to twin transfusion syndrome. Am J Dis Child 1963;106:624-9.
7. Corney G, Aherne W. The placental transfusion syndrome in monozygotic twins. Arch Dis Child 1965;40:264-70.
8. Naeye RL. Human intrauterine parabiotic syndrome and its complications. N Engl J Med 1963;268:804-8.
9. Brennan JN, Dirnan RV, Rosen MG, Bellon EM. Fetofetal transfusion syndrome: prenatal ultrasonographic diagnosis. Radiology1982;143:535-6.
10. Wittman BK, Baldwin VJ, Nichol B. Antenatal diagnosis of twin transfusion syndrome by ultrasound. Obstet Gynecol 1981;58:123-7.
11. Wilson RS. Blood typing and twin zygosity. Hum Hered 1970;20:30-56.

12. Whitfield CR, Smith NC, Cockburn F, Gibson AAM. Perinatally related wastage—a proposed classification of primary obstetric factors. Br J Obstet Gynaecol 1986;93:694-703.
13. Herlitz G. Zur Kennitis der anamischen und polyzytamischen Zutstande bei Neugeborenen, sowie des Icterus gravis neonatorum. Acta Paediatr (Uppsala) 1941;29:211.
14. Giles WB, Trudinger BJ, Cook CM. Fetal umbilical artery flow velocity-time waveforms in twin pregnancies. Br J Obstet Gynaecol 1985;92:490-7.
15. Farmakides G, Schulman H, Saldana LR, Bracero LA, Fletcher A, Rochelson B. Surveillance of twin pregnancy with umbilical arterial velocimetry. Am J Obstet Gynecol 1985;153:789-92.
16. Erskine RLA, Ritchie JWK, Murnaghan GA. Antenatal diagnosis of placental anastomoses in a twin pregnancy using Doppler ultrasound. Br J Obstet Gynaecol 1986; 93:955-9.

The prevalence and biologic significance of lupus anticoagulant and anticardiolipin antibodies in a general obstetric population

C. J. Lockwood, MD, R. Romero, MD, R. F. Feinberg, MD, PhD, L. P. Clyne, FIMLS, MS, B. Coster, BS, and J. C. Hobbins, MD

New Haven, Connecticut

Circulating antibodies to negatively-charged phospholipids have been implicated in the genesis of adverse pregnancy outcomes. However, it has yet to be established that these antibodies are causative or that they are invariably associated with untoward perinatal outcomes. To address this issue, the prevalence of lupus anticoagulant and anticardiolipin antibodies was recorded in a low-risk obstetric population, and the outcomes of untreated pregnancies were evaluated. Two of 737 patients (0.27%) had lupus anticoagulant documented by a prolonged activated partial thromboplastin time that did not correct with mixing studies. In comparison, greatly elevated concentrations of immunoglobulin M–anticardiolipin antibodies or immunoglobulin G–anticardiolipin antibodies were identified in 16/737 (2.2%) patients by means of an enzyme-linked immunosorbent assay. Spontaneous abortions occurred in both lupus anticoagulant–positive patients, suggesting that the activated partial thromboplastin time used was a relatively insensitive but specific marker for antiphospholipid antibody–associated adverse pregnancy outcomes. In contrast, although 12 of 16 anticardiolipin antibodies–positive pregnancies were complicated by perinatal loss, preterm delivery, or fetal growth retardation, four patients had uncomplicated pregnancies. Moreover, the distribution of anticardiolipin antibodies concentrations in these four patients was not clustered among the lowest anticardiolipin antibodies values, and anticardiolipin antibodies concentrations correlated weakly with adverse outcomes. These findings suggest that antiphospholipid antibodies are related to adverse pregnancy outcomes in a complex fashion and that therapy is not always required for acceptable outcomes in patients without other risk factors. (Am J Obstet Gynecol 1989;161:369-73.)

Key words: Lupus anticoagulant, anticardiolipin antibodies, activated partial thromboplastin time

Circulating lupus anticoagulants are either identical to or closely associated with immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies to negatively-charged phospholipids.[1] A laboratory characteristic of lupus anticoagulant is the inhibition of the activated partial thromboplastin time and its derivative assays.[1] This in vitro laboratory phenomenom is a consequence of antibody binding to negatively-charged phospholipid surfaces on which factors Xa and Va convert prothrombin to thrombin.[1] Paradoxically, in vivo lupus anticoagulant and related antiphospholipid antibodies promote thrombosis[1] via a myriad of potential prothrombotic mechanisms.[2-6]

Reportedly 94% of pregnancies occurring in patients with lupus anticoagulant who do not receive appropriate treatment result in spontaneous abortion or stillbirth.[7] A 13% to 40% prevalence of antibodies to the divalent phospholipid, cardiolipin, has been noted in patients evaluated for a history of "idiopathic" recur-

From the Departments of Obstetrics and Gynecology and Medicine, Yale University School of Medicine.
Received for publication July 9, 1988; revised September 18, 1988; accepted February 24, 1989.
Reprint requests: Charles J. Lockwood, MD, Director of Perinatal Research, St. Margaret's Hospital, Tufts University, School of Medicine, 90 Cushing Ave., Boston, MA 02125.

Table I. Pregnancy outcomes in patients with anticardiolipin antibodies

Patient	*ACA level (SD)*	*Outcome*	*GA delivers/loss (wk)*
IgM cardiolipin-specific antibodies			
1	2.32	PTD/IUGR	33
2	2.32	IUGR	40
3	2.40*	SAB	16-21
4	2.45	TAGA	40
5	2.70	TAGA	40
6	2.80	PTD/IUFD	25
7	3.80	PTD/NND	25
IgG cardiolipin-specific antibodies			
1	2.38	TAGA	40
2	2.40	SAB	<13
3	2.45	TAGA	38
4	2.60	SAB	<13
5	2.70	SAB	<13
6	2.70	PTD/NND	23
7	2.90	PTD	25
8	3.10	PTD	30
9	3.20	PTD/IUGR	33
10	3.40*	SAB	18
11	3.45	TAGA	40
12	3.70	IUGR	40

GA, Gestational age; *SAB*, fetal loss at <20 weeks; *IUFD*, fetal loss at >20 weeks; *IUGR*, birth weight <tenth percentile at any gestational age; *TAGA*, birth weight appropriate for gestational age (>tenth percentile) and delivered at term; *PTD*, delivery at <37 weeks; *NND*, neonatal death.

* Lupus anticoagulant–positive.

rent abortion.[8, 9] These observations lend credence to the hypothesis that antiphospholipid antibodies are directly responsible for the associated pregnancy wastage. Intervillous thrombosis,[7] spiral artery vasculopathy,[10] and immune-mediated trophoblast damage[11] are the postulated pathologic mechanisms. The presumed link between adverse pregnancy outcomes and antiphospholipid antibodies is so convincing that aggressive treatment has been advocated in "at risk" patients despite a lack of prospective, double-blind, placebo-controlled trials.[7]

This study was designed to establish the prevalence and biologic significance of lupus anticoagulant and antiphospholipid antibodies in a low-risk obstetric population. A prevalence of antiphospholipid antibodies in a low-risk obstetric population similar to that in populations with recurrent pregnancy wastage would be evidence against the pathogenicity of these antibodies. Normal outcomes in untreated, low-risk patients with the antibodies would also contradict a pathogenic role, as would a lack of correlation between antiphospholipid antibody concentration and adverse pregnancy outcomes.

Material and methods

Patient population. The study population was derived from the Yale–New Haven Hospital obstetric clinic. The investigation was approved by the Yale Human Investigation Committee and informed consent was obtained. Patients with a history of three consecutive spontaneous losses or stillbirths were excluded, and a total of 737 sequential eligible patients were enrolled during a 9-month study period. One additional citrated plasma and serum sample was obtained from each study patient undergoing phlebotomy during routine obstetric care. The samples were placed on ice, centrifuged at 5000 rpm for 10 minutes, and the supernatant frozen in multiple aliquots at −70° C for 4 to 8 months. Prior studies have demonstrated that lupus anticoagulant effect is preserved for prolonged periods after similar storage.[12]

Identification of lupus anticoagulant effect. The presence of lupus anticoagulant was assessed by activated partial thromboplastin time. The partial thromboplastin reagent consisted of cardiolipin- and phosphotidylserine-rich mixed-brain phospholipid. Colloidal silica was used as the activator, and an automated method was used (MLA-Electra 600, Medical Laboratory Automation, Inc., Mount Vernon, N.Y.). The activated partial thromboplastin time recorded was the average of duplicated determinations. Pooled plasma from nonpregnant patients was used with each run as a control. Mixing studies were performed on prolonged samples with the use of a separate aliquot of the patient's citrated plasma and an equal quantity of control plasma.

Detection of antiphospholipid antibodies: Anticardiolipin antibodies enzyme-linked immunosorbent assay. Anticardiolipin antibodies were isolated by means of an enzyme-linked immunoabsorbent assay that was developed by Cowchock et al.[8] Microtiter plates (Fisher Scientific Co., Springfield, N.J.) were coated with 1 μg of bovine cardiolipin in ethanol (Sigma Chemical, St. Louis, Mo.), air evaporated in a cold room overnight, and blocked with fetal calf serum. Uncoated plates were similarly blocked. The wells were then successively incubated with test or control serum, goat antihuman γ- or μ-chain IgG (Sigma), and alkaline phosphatase conjugated–rabbit antigoat γ-chain IgG (Sigma). The plates were washed with a Titertek Washer (Flow Laboratories, McLean, Va.) with phosphate-buffered saline solution between incubations. The substrate 4 *p*-nitrophenylphosphate was then added for 1 hour in a dark room, and the subsequent colorimetric reaction was read at 405 nm with Multiskan Plus microtiter plate reader (Flow Laboratories).

A standard linear curve was then generated on log-logistic paper of the Δ optical density 405 nm readings from serial dilutions of positive control serum containing known concentrations of IgM or IgG anticardiolipin antibodies. Dilutions of both control sera and antibody reagents were chosen to yield a maximal absorbance of 0.8 at 405 nm. Average IgM or IgG anti-

cardiolipin antibody units were assigned to each patient's duplicated sample by plotting against the standard curve. All elevated samples were repeated, and only those samples found elevated in two separate assays were considered significant.

Since polyclonal elevations of IgM and IgG may cause false-positive readings as a result of nonspecific binding, only specific binding to cardiolipin was considered diagnostic. This was calculated by subtracting the total IgM or IgG unit values obtained in the cardiolipin-coated plates from the nonspecific IgM or IgG unit values obtained in the uncoated plates.

Data collection and analysis. All assays were performed by one investigator (C. L.) before the review of patient records. Demographic and outcome variables were collected after delivery. Nine subjects were lost to follow-up, and 22 patients had therapeutic abortions. Final outcome data were available for the remaining 706 patients.

Statistical analysis of the data was done by means of the Student *t* test, Mann-Whitney test, χ^2, Fisher's exact test, Z test for proportions, and multiple logistic regression.

Results

Population demographics on enrollment. The median gestational age at sampling was 16 weeks. The mean maternal age was 22.7 years (95% confidence interval 22.3, 23.1). Median gravidity was three and median parity was one. The racial distribution was 63% black, 18.5% white, and 18.5% Hispanic.

Outcomes in patients with lupus anticoagulant. The mean activated partial thromboplastin time of the study patients was 30.6 seconds (95% confidence interval 30.3, 30.9) and was not different from the nonpregnant control value of 30.5 seconds (95% confidence interval 30.4, 30.6). The activated partial thromboplastin time displayed a slight inverse correlation with gestational age at sampling ($R = -0.24$; $p < 0.0001$).

An activated partial thromboplastin time >2.3 standard deviations above the total population mean was considered abnormal (equivalent to 1.3 multiples of the mean, or 40 seconds). This excluded 99% of the unaffected population. Two patients demonstrated an activated partial thromboplastin time >40 seconds, which did not correct on mixing studies, thus giving a lupus anticoagulant prevalence of 0.27%. Both of these patients had midsecond-trimester losses and false-positive syphilitic serologic studies (case 1 activated partial thromboplastin time = 45.0/30.2 seconds; case 2 activated partial thromboplastin time = 42.6/28.0 seconds). After correcting for gestational age at sampling, the activated partial thromboplastin time did not correlate with birth weight or gestational age at delivery.

Outcomes in patients with anticardiolipin antibodies. The distribution of IgM and IgG anticardiolipin antibody concentrations was evaluated in a population subset with normal outcomes (nongrowth retarded, term, live births). This distribution was positively skewed. However, the natural log transformation of the data was normally distributed. A concentration greater than 2.3 SD above the transformed mean was chosen as a cutoff value, since this excluded 99% of unaffected patients.

IgM and IgG anticardiolipin antibody concentrations did not correlate with the activated partial thromboplastin time or gestational age at sampling. There were significant differences between mean activated partial thromboplastin time in the anticardiolipin antibody–positive (33.0 seconds, 95% confidence interval 30.73, 35.29) versus the anticardiolipin antibody–negative (30.5 seconds, 95% confidence interval 30.26, 30.86) populations ($p < 0.015$). With a 2.3 SD cutoff value, seven patients had elevated concentrations of cardiolipin-specific IgM, and 12 patients had elevated concentrations of cardiolipin-specific IgG. The anticardiolipin antibody concentrations in SD above the mean and the pregnancy outcomes are presented in Table I.

A total of 16 patients had either IgM or IgG anticardiolipin antibodies, resulting in a 2.2% prevalence of anticardiolipin antibodies in this low-risk population. There were no differences in maternal age or parity between the affected and unaffected populations. However, patients with elevated anticardiolipin antibodies levels were more likely to have had a prior spontaneous abortion or preterm delivery ($p < 0.0001$). The anticardiolipin antibody–positive population had significantly different pregnancy outcomes compared with the unaffected population (Tables II and III). Patients with anticardiolipin antibodies were noted to deliver at earlier gestational ages and have lower mean

Table II. Comparisons of pregnancy outcomes in patients with IgM or IgG cardiolipin-specific antibodies compared with the unaffected population

Variable	*ACA negative mean (95% CI)*	*ACA positive mean (95% CI)*	*p value*
Birth weight (gm)			
All births	3174	1801	0.0038*
	(3159, 3273)	(976, 2625)	0.0000†
Live births	3216	2360	0.0280*
	(3159, 3276)	(1627, 3093)	0.0000†
Gestational age at delivery (wk)			
All births	37.3	27.5	0.0039*
	(36.6, 37.9)	(21.4, 33.5)	0.0000†
Live births	39.3	34.6	0.0484*
	(39.1, 39.5)	(30.0, 39.2)	0.0000†

* By *t* test.
†Mann-Whitney two-sample test.

Table III. Comparisons of pregnancy outcomes in patients with IgM or IgG cardiolipin-specific antibodies compared with the unaffected population

Variable	*ACA negative* N	(%)	*ACA positive* N	(%)	*p value*
LB	655/718	(91%)	10/16	(62%)	0.0001*
SAB	36/718	(5%)	4/16	(25%)	0.0001*
IUFD	4/718	(1%)	2/16	(12%)	NS
NND	5/718	(1%)	2/16	(12%)	NS
LBW	43/360	(11%)	6/10	(60%)	0.0001†
False (+) RPR	5/460	(1%)	5/7	(71%)	0.0000†
Cigarette smoking	157/548	(29%)	6/14	(43%)	NS
Racial composition					
Black	453/720	(63%)	8/16	(50%)	NS
White	133/720	(18%)	3/16	(18%)	NS
Hispanic	131/720	(18%)	5/16	(31%)	NS

RPR, Rapid plasma reagin test; *LB*, live births; *NND*, neonatal death; *SAB*, spontaneous abortion; *IUFD*, intrauterine fetal death; *LBW*, intrauterine growth retardation or preterm delivery.

*χ^2.

†Fisher's exact test.

birth weights compared with the unaffected population. The anticardiolipin antibody–positive population had a fivefold relative risk of having a spontaneous abortion or low birth weight infant compared with the anticardiolipin antibody–negative population. Patients with anticardiolipin antibodies were more frequently Hispanic and smokers, although these differences did not attain statistical significance.

Ten patients in the study population had a false-positive rapid plasma reagin test; five were positive for anticardiolipin antibodies. Three of the five anticardiolipin antibody–negative patients with a false-positive rapid plasma reagin test had greatly elevated (>3 SD) nonspecific IgM binding, which suggested polyclonal IgM elevations. Two of these three patients were human immunodeficiency virus positive.

Multiple stepwise regression analysis demonstrated a weak inverse correlation between IgM anticardiolipin antibody concentrations and birth weight ($R = -0.26$, $R^2 = 0.07$, $p = 0.025$). No statistically significant correlations were demonstrated for IgG anticardiolipin antibody concentrations and birth weight or for IgM or IgG anticardiolipin antibody concentrations and gestational age at delivery.

Comment

The prevalence of lupus anticoagulant as ascertained by activated partial thromboplastin time was 0.27% in this low-risk obstetric population. The two affected pregnancies resulted in mid-gestational losses. However, the activated partial thromboplastin time was able to detect only two of 16 (12.5%) patients with antiphospholipid antibodies identified by an anticardiolipin antibodies enzyme-linked immunosorbent assay. We conclude that the particular activated partial thromboplastin time used in this low-risk population has low sensitivity but high specificity for the prediction of adverse pregnancy outcomes in patients who also had antiphospholipid antibodies.

Prior studies have demonstrated that the activated partial thromboplastin time will identify 80% to 90% of patients harboring lupus anticoagulant detectable by more elaborate coagulation assays.[13] The relative insensitivity of the activated partial thromboplastin time used in this study is unlikely to have resulted from antibody absorption by platelet phospholipid, since centrifugation had rendered the samples platelet poor. The use of mixed-brain phospholipid as the partial thromboplastin may have reduced efficiency, since it has been shown to be less sensitive than synthetic preparations.[13] Alternatively, functional coagulation assays that detect the indirect effect of antiphospholipid antibodies on in vitro prothrombin complex formation may simply be less sensitive than direct assays for the presence of antiphospholipid antibodies. A number of investigators have noted this relative insensitivity.[8, 11, 14]

Both patients with lupus anticoagulant had either IgM or IgG anticardiolipin antibodies; however, concentrations of anticardiolipin antibodies did not correlate with activated partial thromboplastin time in the 16 patients with elevated concentrations of anticardiolipin antibodies or with concentrations in the general population. This is in contrast to other investigators who have demonstrated a significant correlation ($r = 0.702$) between the kaolin partial thromboplastin time and IgG anticardiolipin antibody activity.[15] The lack of correlation between anticardiolipin antibody concentration or immunoglobulin class and activated partial thromboplastin time prolongation suggests that the insensitivity of the coagulation assay is not simply a quantitative- or immunoglobulin class–dependent phenomenon.

The prevalence of elevated concentrations of cardiolipin-specific IgM or IgG was 2.2% in this low-risk obstetric population. This frequency is comparable with the 2.1% (2/94) prevalence of anticardiolipin antibodies found by Petri et al.[14] in a sample low-risk, pregnant population derived by use of a 97.5% confidence interval cutoff in a positively skewed distribution. The sensitivity of the anticardiolipin antibody enzyme-linked immunosorbent assay in identifying the presence of any antiphospholipid antibody production has been reported at >80%.[8] Nonetheless, the failure to evaluate other potential phospholipid epitopes may have resulted in a modest underestimation of the prev-

alence of antiphospholipid antibody–mediated pregnancy wastage in this study. Furthermore, the relatively late median date of sampling may have caused the exclusion of a number of patients with spontaneous abortions and therefore underestimated the prevalence of anticardiolipin antibodies.

Although 12 of 16 patients (75%) with anticardiolipin antibodies had adverse perinatal outcomes, including spontaneous abortions, stillbirths, preterm delivery, or growth retardation, these adverse outcomes were not invariable. Moreover, the concentration of the antibody was only weakly correlated with adverse outcomes. The distribution of anticardiolipin antibody concentrations in the four patients with normal pregnancy outcomes was not clustered among the lowest concentrations, suggesting that a higher cutoff value would not have eliminated these patients but would have lowered sensitivity for the prediction of adverse pregnancy outcomes. Furthermore, the cutoff value used in this study after log transformation of the data corresponded to 6 SD above the mean of the original nongaussian distribution. This is a far-higher cutoff value than has been used by a number of other investigators[8, 9, 11, 14] and is comparable with the optimal cutoff value suggested by Harris et al.[16] Thus adverse pregnancy outcomes do not appear to be an invariable consequence of antiphospholipid antibodies. This suggests that therapy is not always required to achieve normal outcomes.

The complex relationship between anticardiolipin antibodies and both adverse pregnancy outcomes and activated partial thromboplastin time inhibition demonstrated in this study may be a consequence of a number of biologic modifiers. Heterogeneity in antibody effect may result from individual variations in antibody affinity and avidity or from variations in the stimulus for anticardiolipin antibodies production (e.g., anticardiolipin antibodies produced in response to syphilis or viral infections are not associated with thrombosis). A second modifier of the biologic effect of antiphospholipid antibodies is host resistance. Patients with greater endothelial surface concentrations of antithrombin III, protein C and S, or prostacyclin synthetase may be less susceptible to the potential adverse consequences of antiphospholipid antibodies. Variation in membrane phospholipid content caused by dietary or genetic factors may also modify host resistance to antibody effect.

We acknowledge the assistance of Ms. Kathleen Costigan in the statistical analysis of the data and the advice and technical assistance of Dr. Susan Cowchock and Ms. Denise Ruch in establishing the anticardiolipin antibody enzyme-linked immunosorbent assay.

REFERENCES

1. Shapiro S, Thiagarajan P. Lupus anticoagulants. Prog Hemost Thromb 1982;6:263-85.
2. DeCastellarnau C, Vila L, Sancho M, et al. Lupus anticoagulant, recurrent abortion and prostacyclin production by cultured smooth muscle cells. Lancet 1983; 2:1137-8.
3. Carreras L, Machin S, Deman R, et al. Arterial thrombosis, intrauterine death and "lupus" anticoagulant: detection of immunoglobulin interfering with prostacyclin formation. Lancet 1981;1:244-6.
4. Cariou R, Tobelem G, Soria C, et al. Inhibition of protein C activation by endothelial cells in the presence or lupus anticoagulant [Letter]. N Engl J Med 1986;314:1193-4.
5. Cosgriff T, Martin B. Low functional and high antigenic antithrombin III level in a patient with lupus anticoagulant and recurrent thrombosis. Arthritis Rheum 1981; 24:94-6.
6. Sanfelippo M, Drayna C: Prekallikrein inhibition associated with the lupus anticoagulant. Am J Clin Pathol 1981;77:275-9.
7. Lubbe W, Liggins G. Lupus anticoagulant and pregnancy. Am J Obstet Gynecol 1985;153:322-7.
8. Cowchock S, Smith J, Gocial B. Antibodies to phospholipids and nuclear antigens in patients with repeated abortions. Am J Obstet Gynecol 1986;155:1002-10.
9. Unander A, Norberg R, Hahn L, Arfors L. Anticardiolipin antibodies and complement in ninety-nine women with habitual abortion. Am J Obstet Gynecol 1987; 156:114-9.
10. DeWolf F, Carreras L, Moerman P, et al. Decidual vasculopathy and extensive placental infarction in a patient with repeated thromboembolic accidents, recurrent fetal loss, and a lupus anticoagulant. Am J Obstet Gynecol 1982;142:829-34.
11. Lockshin M, Druzin M, Goei S, et al. Antibody to cardiolipin as a predictor of fetal distress or death in pregnant patients with systemic lupus erythematosus. N Engl J Med 1985;313:152-6.
12. Tate G. Detection of lupus anticoagulants in stored serum. Lancet 1984;2:587.
13. Green D, Hougie C, Kazmier F, et al. Report of the Working Party on Acquired Inhibitors of Coagulation: studies of the "lupus" anticoagulant. Thromb Haemost 1983; 49:144-6.
14. Petri M, Rheinschmidt M, Whiting-O'Keefe Q, et al. The frequency of lupus anticoagulant in systemic lupus erythematosus. Ann Intern Med 1987;106:524-31.
15. Harris E, Loizou S, Englert H, et al. Anticardiolipin antibodies and lupus anticoagulant. Lancet 1984;2:1099.
16. Harris E, Chan J, Asherson R, et al. Thrombosis, recurrent fetal loss, and thrombocytopenia. Arch Intern Med 1986;146:2153-6.

Fetal blood gas analysis from cordocentesis for abnormal fetal heart rate patterns

Dinesh M. Shah, MD, and Frank H. Boehm, MD
Nashville, Tennessee

We present three cases with abnormal fetal heart rate patterns and cordocentesis umbilical venous blood gas analyses. Conflicting heart rate patterns and cordocentesis and postnatal blood gas analyses prompt questions with regard to abnormal fetal heart rate pattern interpretation and suggest that cordocentesis blood gas analysis aids perinatal management only as a reflection of fetal status at sampling time. (AM J OBSTET GYNECOL 1989;161:374-6.)

Key words: Cordocentesis, fetal blood gas analysis, fetal heart rate patterns

Fetal blood sampling through ultrasonography-guided needle puncture of umbilical cord was suggested by Hobbins et al.[1] for prenatal assessment of fetal hypoxia. Nicolaides et al.[2] reported diagnosis of severe hypoxic acidosis by fetal blood sampling in a case of symmetrical intrauterine growth retardation.

We used fetal blood sampling and subsequent blood gas analysis for in utero assessment of fetal status in three cases, which provided interesting results used in clinical management. Dilemmas that arose from conflicting fetal heart rate tracings, cordocentesis-obtained umbilical venous blood gas analysis, and postnatal cord gas analysis are presented.

Case 1

B. H., a 31-year-old gravida 2, para 0-0-1-0 woman at 24 weeks' gestation, transferred to Vanderbilt University Medical Center because of polyhydramnios and uterine irritability and was placed on oral tocolysis. External fetal heart rate monitoring revealed a basal rate of 140 to 150 beats/min and a flat baseline with frequent deep variable decelerations. Ultrasonography revealed polyhydramnios and double bubble sign consistent with diagnosis of duodenal atresia. The patient underwent fetal blood sampling to rule out chromosomal abnormality.

She was delivered of an infant 5 days after cordocentesis as a result of active preterm labor. The neonate weighed 630 gm, had respiratory distress, required duodenostomy, and was discharged on day 116 after birth. A representative monitor tracing and all results are presented in Fig. 1.

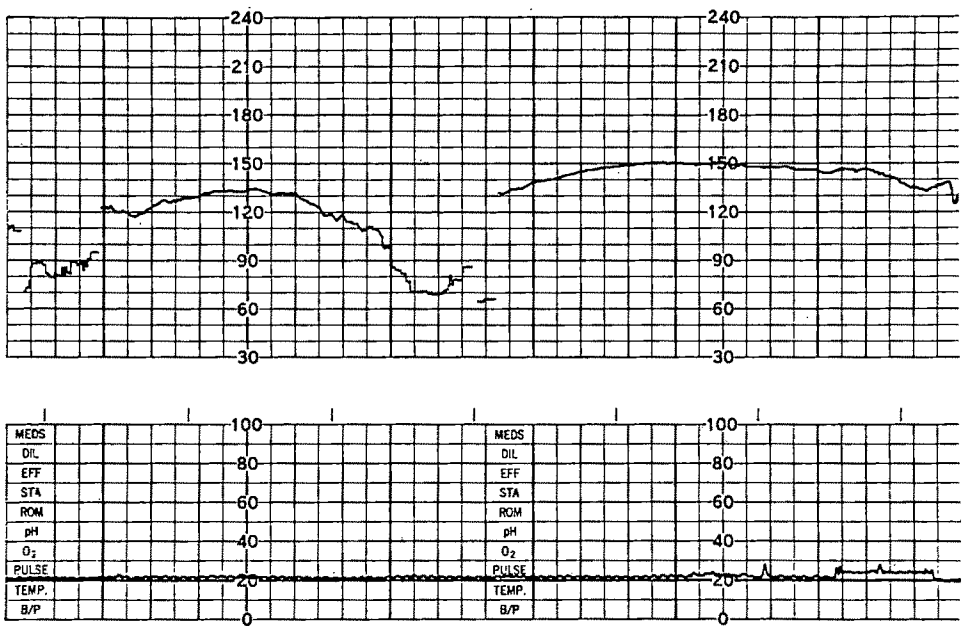
From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Vanderbilt University School of Medicine.
Received for publication October 27, 1988; revised February 14, 1989; accepted March 13, 1989.
Reprint requests: Dinesh M. Shah, MD, C-2213 M.C.N., Vanderbilt University, 21st and Garland, Nashville, TN 37232.

Case 2

J. B., an 18-year-old primigravid woman at 27 weeks' gestation, transferred to Vanderbilt University Medical Center because of preterm labor and was placed on intravenous and later oral tocolysis. Ultrasonography revealed biometry consistent with menstrual dates, a slightly increased amniotic fluid level. There were no notable anomalies. Biophysical profile revealed a score of 8 of 8. She had occasional spontaneous late decelerations, and persistence of variable decelerations was noted on day 5 with baseline rate in the 140s. Increased frequency of late decelerations with baseline rate in the 150s was noted on day 9. The patient was counseled and offered fetal blood sampling through cordocentesis. Fetal oxygenation and acid base status were within acceptable limits. While awaiting karyotype results, 24 hours after cordocentesis the patient had an acute drop in fetal heart rate to 60 beats/minuteand underwent emergency cesarean section. The newborn weighed 1120 gm with Apgar scores of 1 and 2 at 1 and 5 minutes, had respiratory distress, and was discharged to a level I center on day 39 of life. A representative monitor tracing and all results are presented in Fig. 2.

Case 3

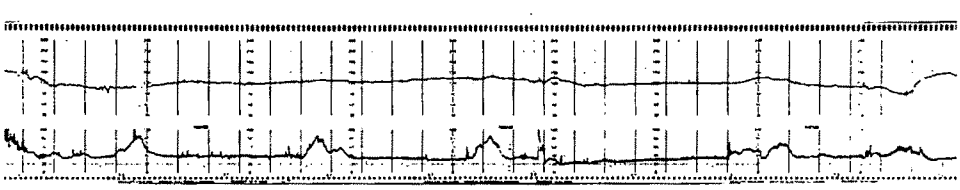
T. A., a 22-year-old obese woman, was first seen at 29 weeks' gestation by ultrasonography with severe oligohydramnios detected 2 weeks before transfer. Vanderbilt University Medical Center ultrasonography revealed biometry consistent with 27 weeks' gestation, and there were no obvious anomalies. However, examination was limited as a result of the patient's obesity and oligohydramnios. Both kidneys and a small fluid-filled bladder were visualized. Fetal heart rate tracing revealed flat baseline with 145 beats/min and spontaneous frequent variable and late decelerations. Biophysical profile revealed a score of 0 of 8. The patient was counseled with regard to cordocentesis and the rationale for obtainment of fetal karyotype and blood gases.



PRENATAL ABG: pH 7.39, pCO_2 49 mmHg, pO_2 17 mmHg, base excess -5.8
POSTNATAL ABG: pH 7.39, pCO_2 44 mmHg, pO_2 16 mmHg, base excess -13.5

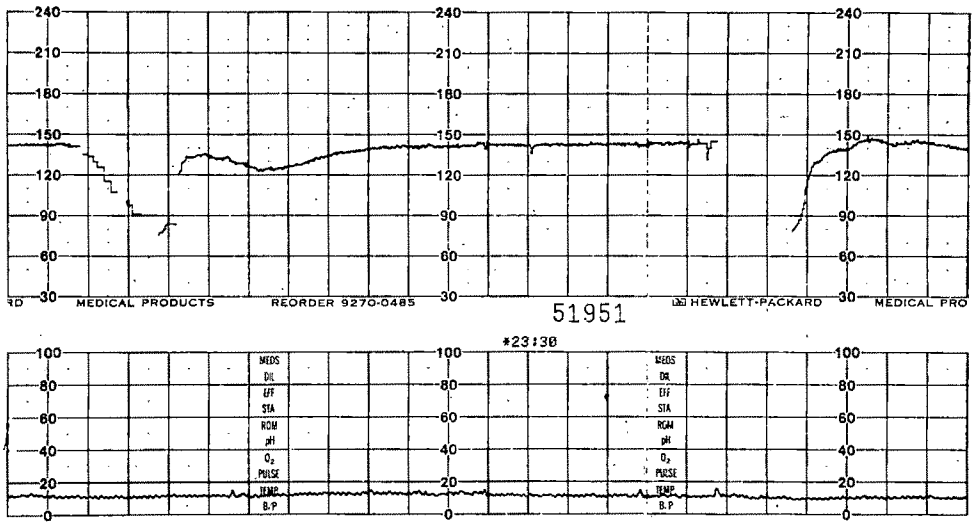
Fig. 1. Representative monitor tracing and fetal blood gas analysis results on fetus with duodenal atresia and normal male karyotype.

CASE 2



PRENATAL ABG: pH 7.25, pCO_2 48 mmHg, pO_2 29.9 mmHg, base excess -6.3
POSTNATAL ABG: pH 6.79, pCO_2 109 mmHg, pO_2 24 mmHg, base excess -19.6

Fig. 2. Representative monitor tracing and fetal blood gas analysis results on fetus with normal female karyotype that had bradycardia.



PRENATAL ABG: pH 7.11, pCO_2 73.6 mmHg, pO_2 8.5 mmHg, base excess -8.1
POSTNATAL ABG: pH 7.04, pCO_2 78 mmHg, pO_2 8 mmHg, base excess -9.4

Fig. 3. Representative monitor tracing and fetal blood gas analysis results on fetus with oligohydramnios and normal male karyotype.

She was delivered by immediate cesarean section of a 980 gm male with Apgar scores of 3 and 7 at 1 and 5 minutes, intrauterine growth retardation, and hyaline membrane disease. The infant was transferred to a level I center on day 57 after birth. A representative monitor tracing and all results are presented in Fig. 3.

Comment

Electronic fetal heart rate monitoring has been very useful in evaluation of fetal status. The clinical situations described herein characterize circumstances in which the obtainment of fetal blood gas information by cordocentesis may assist in management.

In Case 1, information with regard to normal fetal karyotype and blood gases through cordocentesis provided reassurance to the patient and assisted in expectant management. Abnormal postnatal gases in Case 2 are partially explained by the sustained fetal bradycardia that resulted in marked carbon dioxide retention. Differences between prenatal and postnatal gas analyses in Case 3 are easily explained because the prenatal sample was umbilical venous blood and the postnatal sample was umbilical arterial blood.

Our cases differ from previous reports in that gestational ages are significantly earlier and therefore the need for expectant management is even more critical if justified by blood gas information. In addition, each case had persistently abnormal fetal heart rate tracings that heightened the need for fetal blood gas information. Case 1 illustrates a dilemma in "accurate" interpretation of fetal heart tracing. Case 2 emphasizes that fetal blood gas analysis with cordocentesis sample reflects fetal status at sampling time only. In Case 3 blood gas results indicate fetal distress, which precipitates immediate cesarean section.

REFERENCES

1. Hobbins JC, Grannum PA, Romero R, Reece EA, Mahoney MJ. Percutaneous umbilical blood sampling. AM J OBSTET GYNECOL 1985;152:1-6.
2. Nicolaides KH, Soothill PW, Rodeck CH, Campbell S. Ultrasound-guided sampling of umbilical cord and placental blood to assess fetal well-being. Lancet 1986;1:1065-7.

Breakthrough bleeding can be frightening.

*"You know, I was scared, I mean I didn't want those weird things happening to my body."**

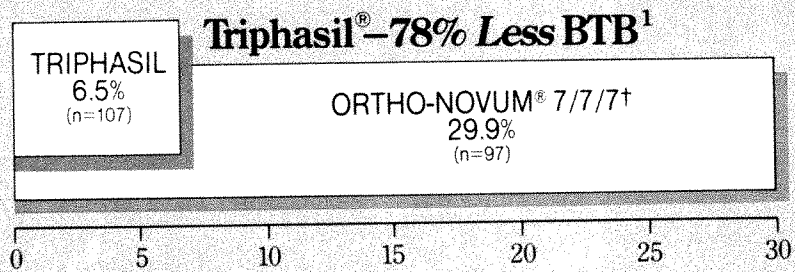


*Direct quote from OC user, data on file.

While the other triphasics have been minimizing the issue of breakthrough bleeding...

Wyeth has been minimizing breakthrough bleeding.*

In Cycle 1...the most critical cycle for compliance



In a separate study of over 4,300 women,[2] in over 70,000 cycles with TRIPHASIL, the total incidence of BTB was only 4.5%

TRIPHASIL –the only OC that needs no backup contraception††...from day 1

Triphasil®

Levonorgestrel and ethinyl estradiol tablets–Triphasic regimen
21- and 28-day regimens

MINIMIZES BTB... FROM DAY 1

* Serious as well as minor adverse reactions have been reported following the use of all oral contraceptives. See prescribing information.

† Norethindrone/ethinyl estradiol triphasic regimen, Ortho Pharmaceutical Corporation.

†† When used as directed.

1. Schilling LH, Bolding OT, Chenault CB, et al: *Am J Obstet Gynecol*, to be published. (Results of a randomized, four-cycle, multicenter U.S. comparison in 313 subjects: Triphasil® vs norethindrone triphasic OCs).
2. Woutersz TB, Korba VD: *Int J Fertil* 1988; 33:406-410.

See brief summary of prescribing information on adjacent page.

© 1989, Wyeth-Ayerst Laboratories.

IN BRIEF:
TRIPHASIL®—6 brown tablets containing 0.050 mg levonorgestrel with 0.030 mg ethinyl estradiol; 5 white tablets containing 0.075 mg levonorgestrel with 0.040 mg ethinyl estradiol; 10 light-yellow tablets containing 0.125 mg levonorgestrel with 0.030 mg ethinyl estradiol (7 light-green tablets containing inert ingredients are included in the 28-day regimen)—Triphasic regimen.

Indications and Usage—TRIPHASIL® is indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OC's) as a method of contraception.

Contraindications—OC's should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5). 8. Benign or malignant liver tumor which developed during use of OC's or other estrogen-containing products.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems*—An increased risk of thromboembolic and thrombotic disease associated with use of OC's is well established. Three principal studies in Great Britain and 3 in the U.S. have demonstrated increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OC's are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION (MI)—An increased risk of MI associated with the use of OC's has been reported, confirming a previously suspected association. These studies, conducted in the UK, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclamptic toxemia) the higher the risk of developing MI, regardless of whether the patient was an OC user or not. OC's, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to MI) are about twice as likely to have a fatal MI as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, amount of smoking is also an important factor. In determining importance of these relative risks, however, baseline rates for various age groups must be given serious consideration. Importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; quite likely the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE—In an analysis of data derived from several national adverse-reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to dose of estrogen in OC's. Preparations containing 100 mcg or more of estrogen were associated with higher risk of thromboembolism than those containing 50-80 mcg. Their analysis did suggest, however, that quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the U.S.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the UK estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OC's according to age, smoking habits, and duration of use. Overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), risk being concentrated in older women, in those with long duration of use and in cigarette smokers. It was not possible, however, to examine interrelationships of age, smoking, and duration of use, nor to compare effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess magnitude of relative risk for this younger group. Available data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates of risk of death for each method include combined risk of contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OC's) plus risk attributable to pregnancy or abortion in event of method failure. This latter risk varies with effectiveness of method. The study concluded that mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OC's in women over 40 who smoke. Lowest mortality is associated with condom or diaphragm backed up by early abortion. Risk of thromboembolic and thrombotic disease associated with OC's increases with age after about 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of pre-eclamptic toxemia, and especially cigarette smoking. Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A 4- to 6-fold increased risk of postsurgery thromboembolic complications has been reported in OC users. If feasible, OC's should be discontinued at least 4 weeks before surgery of a type associated with increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Britain involving cerebrovascular disease and another in the U.S. concerning MI suggest an increased risk of these conditions in users of OC's persists after discontinuation of the OC's. In the British study, risk of cerebrovascular disease remained elevated in former OC users for at least 6 years after discontinuation. In the U.S. study, increased risk of MI persisted for at least 9 years in women 40 to 49 years old who had used OC's for 5 or more years. Findings in both studies require confirmation since they are inconsistent with other published information.

2. *Ocular Lesions*—There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with use of OC's. Discontinue OC's if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. *Carcinoma*—Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in OC's, have been noted to increase incidence of mammary nodules, benign and malignant, in dogs. In humans, 3 case-control studies have reported an increased risk of endometrial carcinoma associated with prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OC's. Of cases found in women without predisposing risk factors (e.g., irregular bleeding at the time OC's were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These are no longer marketed. No evidence has been reported suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only OC's. Several studies have found no increase in breast cancer in women taking OC's or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women on OC's, found an excess risk in subgroups of OC users with documented benign breast disease. Reduced occurrence of benign breast tumors in users of OC's has been well documented. In summary, there is at present no confirmed evidence from human studies of increased risk of cancer associated with OC's. Close clinical surveillance of all women on OC's is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or abnormal mammograms should be monitored with particular care if they elect to use OC's.

4. *Hepatic Tumors*—Benign hepatic adenomas have been found to be associated with use of OC's. One study showed that OC's with high hormonal potency were associated with higher risk than lower potency OC's. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users. Two studies relate risk with duration of use of OC's, the risk being much greater after 4 or more years' use. While hepatic adenoma is rare, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women on OC's. Relationship of these drugs to this type of malignancy is not known.

5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring*—Use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have increased risk of developing in later life a form of vaginal or cervical cancer ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence now that OC's further enhance risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OC's. Furthermore, 30 to 90% of such exposed women have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with use of other estrogens, it cannot be presumed they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with use of sex hormones, including OC's, in pregnancy. One case-control study estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OC's, hormonal withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some exposures involved only a few days. Data suggest that risk of limb-reduction defects in exposed fetuses is somewhat less than 1 in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OC's. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OC's is unknown. It is recommended that, for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing OC's. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at time of first missed period, and further use of OC's should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and advisability of continuation of the pregnancy should be discussed. It is also recommended that women who discontinue OC's with intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this. The administration of progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease*—Studies report increased risk of surgically confirmed gallbladder disease in users of OC's and estrogens. In one study, increased risk appeared after 2 years' use and doubled after 4 or 5 years' use. In one of the other studies, increased risk was apparent between 6 and 12 months' use.

7. *Carbohydrate and Lipid Metabolic Effects*—Decrease in glucose tolerance has been observed in a significant percentage of patients on OC's. For this reason, prediabetic and diabetic patients should be carefully observed while on OC's. Increases in triglycerides and total phospholipids have been observed in patients on OC's. Three studies were performed with Triphasil and no significant alterations in lipid metabolism were noted except for a slight increase in triglyceride levels in 1 study. Clinical significance of these findings remains to be defined.

8. *Elevated Blood Pressure*—Increase in blood pressure has been reported in patients on OC's. In some women, hypertension may occur within a few months of beginning OC's. In the 1st year of use, prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. Prevalence in users increases, however, with longer exposure, and in the 5th year of use is 2½ to 3 times the reported prevalence in the 1st year. Age is also strongly correlated with development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure on OC's. Hypertension that develops as a result of taking OC's usually returns to normal after discontinuing the drug.

9. *Headache*—Onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of OC's and evaluation of the cause.

10. *Bleeding Irregularities*—Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing OC's. In breakthrough bleeding, as in all cases of irregular vaginal bleeding, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another OC may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase risk of thromboembolic disease. Women with past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuing OC's. Women with these preexisting problems should be advised of this possibility and encouraged to use other methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. *Ectopic Pregnancy*—Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. *Breast-feeding*—OC's given in the postpartum period may interfere with lactation and decrease quantity and quality of breast milk. Furthermore, a small fraction of the hormones in OC's has been identified in the milk of mothers on OC's; effects, if any, on the breast-fed child have not been determined. If feasible, defer OC's until infant has been weaned.

Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OC's. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Pap smear and relevant laboratory tests. As a general rule OC's should not be prescribed for longer than 1 year without another physical examination and Pap smear.

2. Under influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size.

3. Patients with history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while on OC's should stop OC's and use an alternate method to try to determine whether the symptom is drug-related.

4. OC's may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency.

5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while on OC's. If jaundice develops, OC's should be discontinued.

6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution.

7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. Clinical significance is undetermined.

8. Serum folate levels may be depressed by OC's. Since the pregnant woman is predisposed to development of folate deficiency and incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OC's, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency.

9. The pathologist should be advised of OC therapy when relevant specimens are submitted.

10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OC's:

a. Increased sulfobromophthalein retention.

b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.

c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered.

d. Decreased pregnanediol excretion.

e. Reduced response to metyrapone test.

Information for the Patient—See Patient Package Labeling.

Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Carcinogenesis—See Warnings section for information on carcinogenesis.

Pregnancy—Category X. See Contraindications, Warnings.

Nursing Mothers—See Warnings.

Adverse Reactions—An increased risk of these serious adverse reactions has been associated with use of OC's (see Warnings): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies. There is evidence of an association between the following conditions and use of OC's although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients on OC's and are believed to be drug-related. Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea, amenorrhea during and after treatment, temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes; tenderness, enlargement, and secretion; change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening), intolerance to contact lenses. The following adverse reactions have been reported in users of OC's, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, hemolytic uremic syndrome.

Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OC's by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Dosage and Administration—For maximum contraceptive effectiveness, Triphasil must be taken exactly as directed and at intervals not over 24 hours. (If Triphasil is first taken later than first day of first menstrual cycle of medication or postpartum, contraceptive reliance should not be placed on it until after the first 7 consecutive days of use. Possibility of ovulation and conception prior to initiation of medication should be considered.)

Any time patient misses 1 or 2 brown, white or light-yellow tablets, she should also use another contraceptive method until she has taken a tablet daily for 7 consecutive days.

For full details on dosage and administration see prescribing information in package insert.

Triphasil®

Levonorgestrel and ethinyl estradiol tablets—Triphasic regimen
21- and 28-day regimens

MINIMIZES BTB... FROM DAY 1

WYETH AYERST™

3421-3 12/1

©1989, Wyeth-Ayerst Laborator

KAISER PERMANENTE

OBSTETRICIAN-GYNECOLOGIST: BC/BE physician wanted full time or part time for satellite medical office practice in Gilroy, California, as part of The Permanente Medical Group, Inc., of San Jose, California. Excellent general Ob/Gyn outpatient skills required plus willingness to work with experienced Family Practice Physicians and Ob/Gyn nurse practitioners. Hospital call optional. Gilroy is located twenty minutes south of San Jose and shares excellent access to the Bay Area recreational activities. Salary is competitive and benefits include full coverage malpractice insurance.

Contact:
Jerome L. Nehemiah, M.D.
Chief
Department of OB/GYN
The Permanente Medical Group, Inc.
260 International Circle
San Jose, CA 95119

Or call: 408-972-6210 or 408-972-6113

MATERNAL-FETAL MEDICINE
(Board eligible or Certified)

600 Bed University-affilited Level III Hospital with 3,600 deliveries/year, 24-hour in-house Anesthesiology and Neonatology/large NICU, active maternal transfer service, perinatal outreach programs, and fully-approved OB/GYN residency program has immediate opening for a full-time position in Perinatology. Duties to include residency teaching, maternal transfer care, and private practice incentive. Competitive guaranteed salary plus incentive. Excellent public school system, housing, and Lake St. Clair in very close proximity.

CALL COLLECT: 313-343-3495
MINU KASHEF, M.D.
ST. JOHN HOSPITAL
DEPT. OB/GYN
DETROIT, MI 48236

PERINATOLOGIST
Denver, Colorado

Rose Medical Center, Denver, Colorado is seeking a second BE/BC perinatologist to work full-time in a 400-bed private community hospital adjacent to the University of Colorado Health Sciences Center. With 3,600 deliveries per year, and 27 University Residents rotating through the department annually, Rose is the major private teaching affiliate of the University.

Responsibilities include private consultation, physician, resident, and nursing education, clinical research, and a limited private practice in perinatology. This individual can expect frequent interaction with the Maternal/Fetal Medicine Division at the University of Colorado, and will be eligible for an appropriate clinical appointment at the University. All clinical responsibilities will be shared with the current full-time perinatologist at Rose.

A competitive salary guarantee is being offered, with excellent fringe benefits including malpractice insurance.

Qualified applicants, including individuals completing fellowship in June, 1989, should forward CV's to:

Robert E. Wall, M.D., Chairman
Department of Obstetrics & Gynecology
Rose Medical Center
4567 East Ninth Avenue
Denver, Colorado 80220
Telephone: (303) 320-2077

FELLOWSHIP
MATERNAL-FETAL MEDICINE
JULY, 1990

The Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, MacDonald Hospital for Women, University Hospitals of Cleveland, and Case Western Reserve University, has an opening at the instructor level beginning July 1, 1990. Applicants should desire a career in academic medicine and must be Board certified/eligible by the American Board of Obstetrics and Gynecology.

Fellows will be involved in teaching medical students and house officers. The fellowship program emphasizes clinical and basic research in clinical perinatology with a particular interest in ultrasound, Doppler and perinatal diagnosis. The individual will also be responsible for some general duties in obstetrics and gynecology.

Applicants should send a curriculum vitae to:

J.C. Veille, M.D.
Director, Division of Maternal-Fetal Medicine
MacDonald Hospital for Women, University Hospitals of Cleveland
2074 Abington Road
Cleveland, OH 44106
216/844-3338

The University is an equal opportunity/affirmative action employer.

DEPARTMENT OF OBSTETRICS AND GYNECOLOGY
MEDICAL COLLEGE OF GEORGIA

** FACULTY TENURE TRACK POSITIONS IN AN EXPANDING DEPARTMENT **

(1) Maternal-Fetal Medicine position – (1) Gynecologic Oncology Position – and (2) General Obstetrics and Gynecology positions.

— Duties: patient care – teaching – research.

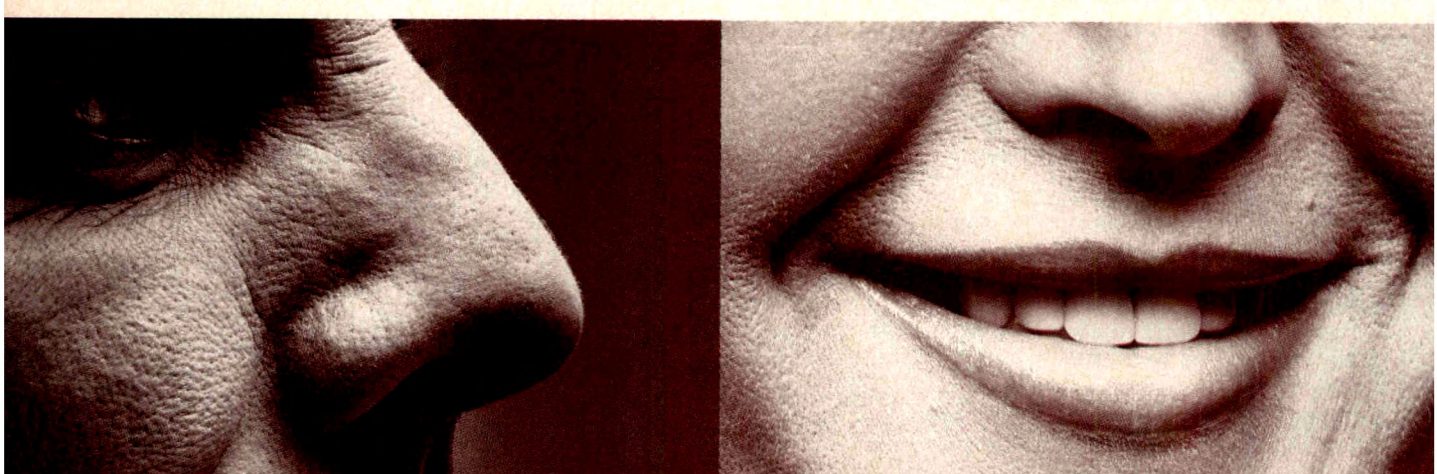
— Qualifications: Board pending or certified in Obstetrics and Gynecology and applicable sub-specialty.

— Rank and Salary: Open based on experience.

The Obstetrics and Gynecology Department at the Medical College of Georgia provides support and opportunity for academic advancement and professional growth. Located in Augusta, Georgia a progressive community offering a pleasant climate with an environment for quality family living. Area population 375,000.

Address inquiries with Curriculum vitae to: Donald M. Sherline, MD, Chairman, Department of Obstetrics and Gynecology, CK170, Medical College of Georgia, August, Ga. 30912.

Equal Opportunity/Affirmative Action Employer



Viable DNA are lookin

Recent studies[1,2] have shown that certain types of virulent DNA have been detected intact in the plume from CO-2 lasers.

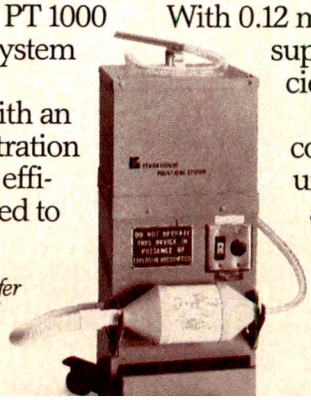
In other words, there are numerous cavities and areas where DNA can enter your body.

Now there's a safe and effective way to draw off the plume better than ever before:

The new POINT ONE™ PT 1000 Smoke Evacuation Filter System from Stackhouse.™

PT 1000 provides you with an unsurpassed final stage filtration to 0.01 micron at 99.998% efficiency. Penetration is limited to .002%.

0.01 micron filtration provides a safer environment for both the patient and the entire clinical staff.



With 0.12 micron particles, a superior 99.9995% efficiency is achieved.

A super ULPA filter, combined with a unique and highly advanced air flow design, help make it possible.

OB/GYN

Bay Shores Medical Group offers outstanding opportunities for board eligible/certified physicians to join rapidly expanding 80-physician multi-specialty group practice. Southern California community, close to ocean, easy access to city and recreational areas, excellent schools.

Please direct any inquiries and CV to:
Bradford B.Burnett, M.D.
Medical Director
Bay Shores Medical Group, Inc.
502 Torrance Boulevard
Redondo Beach, CA 90277
(213) 543-7247

Bay Shores Medical Group



or a few good openings.

Yet the PT 1000 makes less sound than most OR equipment. That's because a quiet tangential motor/blower unit is housed in a special sound suppression section.

And for added mobility and utility, just add the optional dolly with drawer to hold extra filters and accessories.

Most importantly, more than 6,000 Stackhouse units are presently in use worldwide—making it the system of choice among clinicians.

For more information about the system that filters out the little problems before they become big ones, call or write: Stackhouse Associates, Inc., 150 Sierra Street, El Segundo, California 90245. Telephone: (213) 322-6676 FAX: (213) 414-0153 Telex: 3719991

[1]Garden, J. et al (1988) Papillomavirus in the Vapor of Carbon Dioxide Laser—Treated Verrucae. *JAMA*, 259: 1199–1202.
[2]Walker, N. et al (1986) Possible Hazards from Irradiation with the Carbon Dioxide Laser. *Lasers in Surgery and Medicine* 6: 84–86.

(800) 325-0082

STACKHOUSE™

The Risks Are Too High To Settle For Less.™



PLEASE!

ASK ABOUT DRUG ABUSE,
BEFORE IT'S TOO LATE.

PARTNERSHIP FOR A DRUG-FREE AMERICA

Presence of autoantibodies in women with unexplained infertility

Pamela V. Taylor, PhD, James M. Campbell, MRCOG, and James S. Scott, MD
Leeds, England

Serum samples from 41 patients suffering from unexplained infertility and 351 normal pregnant women were assayed for a range of autoantibodies by means of immunofluorescence, counterimmunoelectrophoresis, double immunodiffusion, Western blots, and enzyme-linked immunosorbent assays. The prevalence of autoantibodies to smooth muscle, phospholipid, and nuclear antigens, the latter when detected by immunofluorescence, was elevated in women with infertility compared with normal pregnant women ($p < 0.001$, <0.001, and <0.05, respectively). Antiviral antibodies were not detected. The reason for the high level of autoreactivity in infertile women is unclear, but smooth muscle and antiphospholipid antibodies may actively interfere with the reproductive process. (Am J Obstet Gynecol 1989;161:377-9.)

Key words: Autoantibodies, phospholipid antibody, infertility

The incidence of infertile couples in the general population has been estimated at 10% to 15%. In a considerable proportion of these couples, no explanation is discovered for the condition using standard techniques of investigation. In recent years various types of reproductive failure have come to be considered as having an immune component. Immunologic effects are important at many levels in the reproductive process, including fertilization, implantation, and the development of the placenta.[1] A spectrum of antibodies may be present in women during their reproductive lives either related to underlying immune disorders or as a response to paternally-derived fetal antigens. Possible effects on potential development of the embryo range from prevention of fertilization,[2] an immunoregulatory function in maintenance of the fetoplacental unit,[3] or deleterious fetal and neonatal effects caused by transport of maternal IgG across the placenta.[4] Antiphospholipid antibodies[5] and antibodies to ribonucleoprotein antigens[6] have been particularly inculpated in fetal loss, and there is preliminary evidence that autoantibody profiles can be correlated with some types of reproductive failure.[7] We have tested women suffering from unexplained infertility for a range of autoantibodies to assess the pathogenic role in this disorder.

From the Department of Obstetrics and Gynaecology, University of Leeds.
Supported by a grant from Birthright.
Received for publication October 2, 1988; revised February 1, 1989; accepted February 17, 1989.
Reprint requests: P. V. Taylor, MD, Department of Obstetrics, D Floor, Clarendon Wing, Belmont Grove, Leeds LS2 9NS, England.

Patients

Serum samples were obtained from 41 infertile patients (26 with primary and 15 with secondary infertility) and 351 normal pregnant women, mostly in the first trimester. The infertile group was investigated by laparoscopy, which revealed bilateral tubal patency and no intrapelvic bar to pregnancy. Ovulation had been confirmed by luteal phase curettage, yielding secretory endometrium, biphasic basal body temperature charts, loss of ferning in the cervical mucus, and luteal phase progesterone levels, and no abnormality had been demonstrated on seminal analysis or postcoital tests.

Methods

Serum samples were stored at −70° C and antibodies to nuclear antigens and soluble ribonucleoproteins were assayed by immunofluorescence, counterimmunoelectrophoresis, double immunodiffusion, and Western blots as previously described.[8] Antiphospholipid antibodies were detected by prolongation of the activated partial thromboplastin time and enzyme-linked immunoassay[9] with bovine heart cardiolipin (Sigma Chemical Co., St. Louis, Mo.) as the antigen. Results were considered positive in the latter when they were greater than four times the standard deviation of a mean of 100 pooled control sera. This control pool and a positive serum were included in every microtiter plate.

Smooth muscle IgG, IgM, and IgA antibodies were detected by means of indirect immunofluorescence on rodent substrates.[10] Sera from 11 infertile patients were tested for antibodies to cytomegalovirus and herpes virus and toxoplasma. Results were analyzed by the χ^2 tests with Yates' correction.

Table I. Autoantibodies to smooth muscle and nuclear and phospholipid antigens in infertile women and normal pregnant women

	Smooth muscle antibody No. positive (%)	*Antinuclear antibody No. positive (%)*	*Antiphospholipid antibody No. positive (%)*
Infertile women (n = 41)	20* (49)	4 (10)	7‡ (17)
Normal pregnant women (n = 351)	58† (17)	11 (3)	5 (6) (of 80 tested)

*All IgG; five also IgM.
†All IgG; 12 also IgM.
‡All IgG.

Table II. Values for the activated partial thromboplastin times in 41 infertile women

Patient's time/control time (seconds)						
33/31*	33/33*	31/29	35/30	30/27	26/29	37/34
30/31*	30/32	28/27	31/30	30/27	26/27	24/27
31/30*	32/32	30/30	29/32	29/30	31/27	30/32
36/33*	24/27	31/31	30/30	35/32	31/28	36/34
25/28*	32/32	35/28	33/27	28/30	32/32	35/28
27/29*	29/31	31/30	32/31	31/29	31/31	

*Positive for IgG anticardiolipin antibody.

Results

Serologic findings with respect to smooth muscle antibody, antinuclear antibodies detected by immunofluorescence, and antiphospholipid antibodies are shown in Table I. The prevalence of these antibodies was elevated in women with infertility compared with normal pregnant women ($p < 0.001$, <0.05, and <0.001 for smooth muscle, and nuclear and phospholipid antibodies, respectively). Four of the 20 infertile women and eight of the 58 normal women who were positive for smooth muscle antibody had titers of one in 40, whereas the remainder were positive at one in 10. The seven infertile patients with IgG antiphospholipid antibody had values of 5, 8, 13, 8, 15, 7, and 7 times the standard deviation of the normal mean, respectively, for the enzyme-linked immunoassay. These values for the five normal pregnant women with IgG antiphospholipid antibody were 4, 7, 8, 9, and 12, respectively. None of the infertile patients had antibodies to ribonucleoprotein antigens, and none had prolongation of the activated partial thromboplastin time (Table II). No antiviral antibodies were detected; one patient had a positive titer for IgG but not IgM antibody to toxoplasma.

Comment

The elevated prevalences of these non-organ-specific antibodies in infertile women suggest a high level of autoreactivity in this group. Depression of immune reactivity in the control group is unlikely, since pregnancy per se seems to have no effect on the induction or suppression of antinuclear or smooth muscle antibodies[11] or antibodies to ribonucleoproteins.[8] The reported incidence of smooth muscle antibody in the normal population is in the range of 2% to 20%. Our findings of a 17% incidence associated with normal gestation agrees with those of Pietarinen et al.[12] When present in high titers, smooth muscle antibody is commonly associated with chronic active hepatitis. In low titers it is present in association with other hepatic disorders, malignancies, drug addiction, and viral infections, particularly infectious mononucleosis and cytomegalovirus. An increased prevalence of smooth muscle antibody has been reported in threatened and spontaneous abortion[12] and infertility.[13] The latter study included patients with various causes for their disorder, particularly anovulation. Persistent viral infection was suggested as a cause of both autoantibody production and infertility. We found no evidence for viral infection in the small number tested.

Prevalences of IgG antiphospholipid autoantibodies in an apparently healthy population have been reported between 1.25% and 2.25%, depending on the particular phospholipid antigen involved.[14] The presence of IgG antiphospholipid antibody has been correlated with both thrombosis and fetal death,[5] sometimes in the clinical setting of systemic lupus erythematosus but often in patients with no other abnormality. This has been designated the antiphospholipid syndrome, and our findings suggest that unexplained infertility in some patients may be associated with this syndrome. Women with unexplained infertility have a very similiar range and prevalence of autoantibody production to women suffering from spontaneous recurrent abortion.[15]

The stimulus to production of these autoantibodies

and whether they directly affect the reproductive process remain unclear. Smooth muscle antibody may reduce fertility by altering fallopian tubal function. The possibility that curettage performed as part of the infertility investigations is an etiologic factor in the production of the autoantibody requires assessment. The association between antiphospholipid antibodies and fetal loss seems to be a consequence of widespread abnormality of the uteroplacental vasculature with increased platelet aggregation, reduced blood flow, and placental infarction.[16] The mechanism of this is unclear, but the antibodies may bind to phospholipids in endothelial cell membranes and decrease prostacyclin production by blocking arachidonic acid release.[17] Such a mechanism could interefere with normal fertilization. Alternatively, if the effect is caused by the interaction at the placental site, identification of these autoantibodies in some infertile women suggests that there may be a group of such patients who suffer from undetected fetal loss rather than prevention of fertilization.

We thank Brenda Frater, Sandra Jackson, Alison Norman, and Geoffrey Tate for laboratory assistance.

REFERENCES

1. Billingham RE, Head JR. Current trends in reproductive immunology: an overview. J Rep Immunol 1981;3:253-65.
2. McLaren A. Immunological control of fertility in female mice. Nature 1964;201:582.
3. Chaowat G. The riddle of the fetal allograft. Am J Rep Immunol 1984;135D:301-51.
4. Scott JS, Maddison PJ, Taylor PV, et al. Connective tissue disease, antibodies to ribonucleoprotein and congenital heart block. N Engl J Med 1983;309:209-12.
5. Harris EN, Chan JKH, Asherson RA, et al. Thrombosis recurrent fetal loss and thrombocytopenia. Arch Intern Med 1986;146:2153-6.
6. Maddison PJ, Taylor PV, Tompkins DS, et al. False-positive VDRL and anti-Ro antibodies related to pregnancy outcome. Lancet 1984;1:108-9.
7. Taylor PV, Campbell JM, Jackson SM, Scott JS. The role of maternal autoimmunity in reproductive failure. Proceedings of the Third International Congress of Reproductive Immunology. J Rep Immunol 1986;(suppl):146.
8. Taylor PV, Taylor KF, Norman A, et al. Prevalence of maternal Ro (SS-A) and La (SS-B) autoantibodies in relation to congenital heart block. Br J Rheumatol 1988;27:128-32.
9. Gharavi AE, Harris EN, Asherson RA, Hughes GRV. Isotype distribution and phospholipid specificity in the antiphospholipid syndrome. Ann Rheum Dis 1987;46:1-6.
10. Bottazzo GF, Florin-Christensen A, Fairfax A, et al. Classification of smooth muscle autoantibodies detected by immunofluorescence. J Clin Pathol 1976;29:403-10.
11. Patton PE, Coulam CB, Bergstralh E. The prevalence of autoantibodies in pregnant and nonpregnant women. AM J OBSTET GYNECOL 1987;157:1345-50.
12. Pietarinen I, Hartikainen-Sorri AL, Makitaol R, et al. Smooth muscle antibodies in complicated early pregnancies. Am J Reprod Immunol 1983;3:43-5.
13. Wilson C, Elstein M, Eade OE, et al. Smooth muscle antibodies in infertility. Lancet 1975;2:1238-9.
14. El-Roeiy A, Gleicher N. Definition of normal autoantibody levels in an apparently healthy population. AM J OBSTET GYNECOL 1988;72:596-602.
15. Gleicher N, El-Roeiy A, Friberg J, Confino E. Autoantibodies in sera of patients with recurrent pregnancy loss and unexplained infertility. Presented at the Thirty-fourth Annual Meeting of the Society for Gynecologic Investigation, Atlanta, Georgia, March 18-21, 1987.
16. De Wolf F, Carreras LO, Moerman P. Decidua vasculopathy and extensive placental infarction in a patient with repeated thromboembolic accidents, recurrent fetal loss and a lupus anticoagulant. AM J OBSTET GYNECOL 1982;142:820-34.
17. Carreras LO, Vermylen JG. Lupus anticoagulant and thrombosis; possible role of inhibition of prostacyclin formation. Thromb Haemost 1982;48:38-40.

Successful twin pregnancy after embryo donation to a patient with XY gonadal dysgenesis

Mark V. Sauer, MD, Rogerio A. Lobo, MD, and Richard J. Paulson, MD
Los Angeles, California

A twin pregnancy was established in a patient with XY gonadal dysgenesis. The pregnancy was supported with exogenously administered hormones for the initial 100 days. The infants were delivered by emergency cesarean section at 35 weeks' gestation when severe preeclampsia developed in the mother. (AM J OBSTET GYNECOL 1989;161:380-1.)

Key words: Gonadal dysgenesis, donor embryo transfer

Successful pregnancies in functionally agonadal women who received donated embryos have been reported.[1] Whereas most women who received donated oocytes have premature ovarian failure and normal karyotypes, one case of a singleton pregnancy in an XY gonadal dysgenesis patient has been reported.[2] We report the first twin gestation in a patient with XY gonadal dysgenesis. This case further confirms the ability of some patients with XY genotypes to carry a pregnancy when provided donated embryos.

Case report

A 28-year-old gravida 0 woman was diagnosed as having XY gonadal dysgenesis at the age of 17 years when she was first seen with primary amenorrhea. Hormonal evaluation revealed hypergonadotropic hypogonadism, and the peripheral blood karyotype was 46,XY. Gonadectomy was performed and hormonal replacement therapy was instituted.

The patient desired a child and came to us at the University of Southern California In Vitro Fertilization Program for embryo donation. She was started on hormonal replacement that consisted of oral micronized estradiol and intramuscular progesterone as previously described[1] to simulate a normal menstrual cycle. An endometrial biopsy specimen obtained on day 26 of the simulated cycle was in phase. A nonanonymous donor volunteered to undergo ovarian stimulation with human menopausal gonadotropin and human chorionic gonadotropin that preceded transvaginal ultrasonography-directed oocyte aspiration. Seven oocytes were obtained, and after incubation with the sperm of the recipient's husband, four oocytes fertilized and cleaved. Four 4-cell embryos were then transferred to the recipient's uterus by the transcervical route with a standard, 3F embryo transfer catheter on simulated cycle day 18.

Nine days after transfer the patient's serum β-human chorionic gonadotropin was 16 mIU/ml. Vaginal ultrasonography subsequently demonstrated a twin pregnancy. The patient was maintained on exogenous hormone replacement for the first 100 days' gestation at which time serum progesterone levels were confirmed to be in the normal range and medications were discontinued. At 35 weeks' gestation pregnancy-induced hypertension worsened and required delivery by emergency cesarean section. A 2955 gm male and 1477 gm female were delivered. Both infants had Apgar scores of 7 at 1 minute and 7 at 5 minutes. Discordant growth was attributed to placental insufficiency in the female. The infants did well and respiratory support was not required. The patient recovered uneventfully.

Comment

Advances in reproductive medicine have made pregnancy attainable in many patients previously considered infertile. Women with gonadal dysgenesis may achieve pregnancy by receiving embryos that developed from donated oocytes. In these and other agonadal patients, the endometrial lining responds normally to exogenous hormone replacement and becomes indistinguishable by biopsy specimen from that of women who ovulate spontaneously. This endometrium is thus receptive to embryo implantation. As illustrated by this case and those reported by others,[1, 2] if implantation occurs pregnancy progresses uneventfully and is self-sufficient after an initial period of hormonal support.

Although optimism for patients with XY gonadal dysgenesis exists, not all women with an XY genotype have this prognosis. Others may have ambiguous genitalia or functional gonadal tissue and preclude pregnancy because production of mullerian inhibiting factor in utero induces the regression of female reproductive organs. Therefore, before embryo donation, documentation of a normal uterus on hysterosalpingography is required of all potential recipients. Finally,

From the Division of Reproductive Endocrinology, Department of Obstetrics and Gynecology, University of Southern California.
Received for publication February 14, 1989; accepted March 5, 1989.
Reprint requests: Mark V. Sauer, MD, Women's Hospital, Room L-946, 1240 N. Mission Road, Los Angeles, CA 90033.

an endometrial biopsy specimen is obtained to ensure a normal response to hormonal replacement.

REFERENCES

1. Rosenwaks Z. Donor eggs: their application in modern reproductive technologies. Fertil Steril 1987;47:895-909.
2. Frydman R, Parneix I, Fries N, Testart J, Raymond JP, Bouchard P. Pregnancy in a 46, XY patient. Fertil Steril 1988;50:813-4.

Ovarian hyperstimulation presenting as acute hydrothorax after in vitro fertilization

Charles R. Kingsland, MB, ChB,[a] John V. Collins, MD,[b] Botros Rizk, MB, ChB,[a] and Bridgett A. Mason, MB, BS[a]

London, England

Ovarian hyperstimulation that occurs with an isolated hydrothorax as the only symptom has previously been reported. We describe the second such case, the first to occur after in vitro fertilization and embryo transfer. (AM J OBSTET GYNECOL 1989;161:381-2.)

Key words: Ovarian hyperstimulation, isolated acute hydrothorax

Jewelewicz and Vande Wiele[1] first described a patient who had acute hydrothorax as the only symptom of ovarian hyperstimulation. In this article we report a similar case of acute hydrothorax that occurred after in vitro fertilization.

Case report

A 35-year-old woman was referred to the Bourn-Hallam Medical Centre for consideration of in vitro fertilization because of a 3-year history of primary unexplained subfertility. She had a history of recurrent chest infections but was otherwise in good health. Serum follicle-stimulating hormone concentration was 2.5 IU/L (normal range, 2.5 to 12, follicle stimulating hormone assay, AMERLEX, Amersham, England with standard second international reference preparation, 78/549) and the luteinizing hormone level was 5.3 IU/L (normal range, 5 to 20, luteinizing hormone assay, AMERLEX, Amersham, England, using standard first international reference preparation, 68/40) when taken on day 2 of the patient's cycle.

Ovarian stimulation was given in the form of clomiphene citrate (Serophene, Serono, Welwyn, England), 100 mg orally from day 2 to 6 of the menstrual cycle and human menopausal gonadotropin (Pergonal, Serono), 225 IU/L intramuscularly daily from the fourth to the ninth day of the cycle. Total urinary estrogen level rose to a maximum of 1221 nmol/24 hours. Five thousand international units of human chorionic gonadotropin (hCG, Profasi, Serono) was given intramuscularly on the tenth day of her cycle; 35 hours later ultrasonically directed oocyte recovery was performed.

Seven oocytes were retrieved. All seven were fertilized, and three were transferred via the transcervical route 72 hours after oocyte retrieval. Four embryos were frozen.

Seventy-two hours after embryo replacement, the patient complained of crampy abdominal pain. On examination, the abdomen was soft and nontender, with no evidence of ascites. An ultrasonogram showed both ovaries to be enlarged to a diameter of 5 cm. There was no evidence of free fluid. The abdominal discomfort resolved over the next 24 hours and the patient was reassured.

One week after embryo replacement, the patient was admitted to the hospital with an acute onset of dyspnea. A large right-sided hydrothorax was diagnosed clinically and was confirmed by a chest x-ray film. An ultrasonogram of the chest also showed a moderate right hydrothorax and a trace of fluid above the left hemidiaphragm. There was no ascites or evidence of pulmonary embolus. The hemoglobin value was 14.4 gm/dl, the white blood cell count was 20.2 × 10/L, and the platelets measured 761 × 10/L. Results of blood biochemistry and liver function tests were all within normal limits.

A total of 3½ L of straw-colored fluid was aspirated from the hydrothorax and had a protein count of 43 gm/L. No evidence of acid-fast bacilli was found in the

From the Bourn-Hallam Medical Centre[a] and the Brompton Hospital.[b]
Received for publication March 10, 1989; accepted March 17, 1989.
Reprint requests: C. R. Kingsland, MD, c/o 77 Hallam St., London, England W1N 5LR.

fluid with Ziehl-Neelsen staining. Cytologic and subsequent cultures of the fluid sputum and urine were negative. Results of fluorescence tests for antinuclear factor were also negative.

On the basis of these investigations, a diagnosis of hydrothorax caused by ovarian hyperstimulation was made. The patient made an uneventful recovery with no further treatment and was discharged 4 days after admission.

The patient returned to the Bourn-Hallam Centre two weeks after embryo replacement for review. She felt well and on examination had no evidence of recurrence of the effusion. A β-subunit hCG assay was performed, and the level was >25 IU/L, indicative of pregnancy. She returned home for antenatal care but miscarried at 8 weeks' gestation.

Comment

This is the second reported case of ovarian hyperstimulation that presents solely as acute hydrothorax, and is the first case to occur after in vitro fertilization. The severe form of ovarian hyperstimulation syndrome is characterized by gross ovarian enlargement, ascites, pleural effusion, and often thromboembolic phenomena that are potentially lethal conditions. In this case, although there was evidence of hemoconcentration, gross ovarian enlargement and ascites were not present either clinically or on ultrasonographic examination. Other investigations failed to reveal any other possible cause for the effusion.

Severe ovarian hyperstimulation occurs more commonly in patients who conceive after therapy. Some workers have quoted an incidence as high as 6%. However, with present methods of monitoring gonadotropin therapy, the incidence is now much lower.[2]

The pathogenesis of hyperstimulation is thought to be a transudation of fluid through the capillary basement membranes of the grossly enlarged ovaries. The absence of ascites and gross ovarian enlargement in this case leaves the mechanism by which the hydrothroax developed debatable.

REFERENCES

1. Jewelewicz R, Vande Wiele RL. Acute hydrothroax as the only symptom of ovarian hyperstimulation syndrome. AM J OBSTET GYNECOL 1975;121:1120.
2. Schenker JG, Weinstein D. Ovarian hyperstimulation syndrome: a current survey. Fertil Steril 1978;30:255-66.

Normal amniotic fluid volume changes throughout pregnancy

Robert A. Brace, PhD, and Edward J. Wolf, MD
La Jolla, California

The purpose of this study was to provide a quantitative characterization of the changes in amniotic fluid volume that occur throughout gestation. From 705 published amniotic volumes for normal pregnancies, we found that after log transformation, amniotic fluid volume had a uniform variability over 8 to 43 weeks' gestation. Thus the 95% confidence interval covered the range of 1/2.57 to 2.57 times the mean volume at any given gestational age. Contrary to expected trends, mean amniotic fluid volume did not change significantly between 22 and 39 weeks and averaged 777 ml, with the 95% confidence interval ranging from 302 to 1997 ml. The data are summarized in nomograms covering 8 to 43 weeks' gestation. (AM J OBSTET GYNECOL 1989;161:382-8.)

Key words: Amniotic fluid volume, gestational age

It is a well-recognized concept that maintenance of amniotic fluid volume within the normal range is important for promoting fetal well-being. This is because aberrations in volume either above or below normal are associated with an increased incidence of fetal and neonatal morbidity and mortality.[1, 2] A problem with this concept is that "normal" has not been defined; that is, although there have been several studies in which amniotic fluid volumes have been measured at various stages of gestation,[3-14] there has been neither an adequate characterization of the normal range of amniotic fluid volumes nor an adequate description of the variation in normal volumes with gestation. Thus the pur-

From the Division of Perinatal Medicine, Department of Reproductive Medicine, University of California, San Diego.
Supported in part by National Institutes of Health grant HD2372[4] from the National Institute of Child Health and Human Development.
Received for publication November 2, 1988; revised January 1 1989; accepted March 3, 1989.
Reprint requests: Robert A. Brace, PhD, Department of Reproductive Medicine T-002, University of California, San Diego, La Jolla, CA 92093.

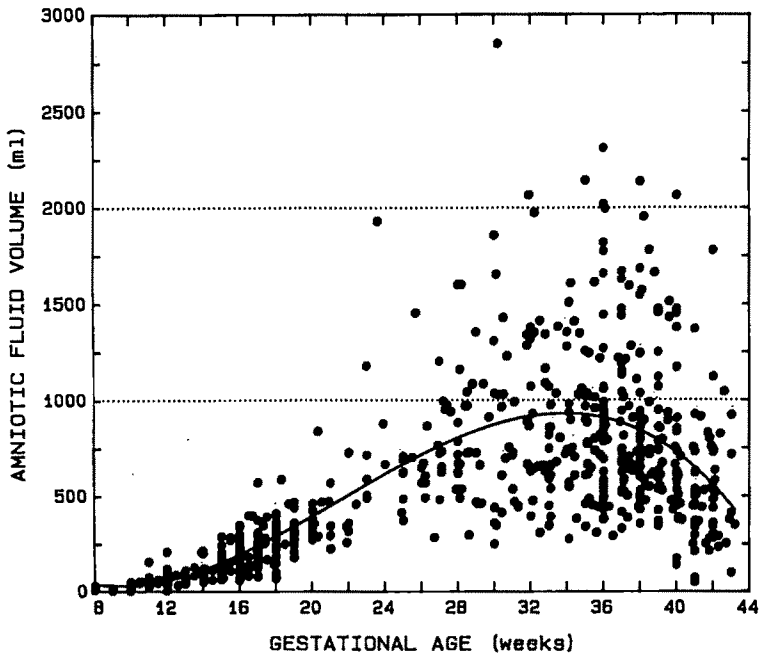


Fig. 1. Amniotic fluid volumes in human pregnancies with normal fetuses (n = 705). *Solid line* is polynomial regression line ($Y = 525.6 - 117.2X + 8.003X^2 - 0.1237X^3$, $R = 0.718$, $p < 10^{-6}$).

pose of the present study was to provide a quantitative characterization of the gestational changes in amniotic fluid volume that occur with normal pregnancies.

Methods

Paired data on amniotic fluid volume and fetal gestational age were collected from 12 previously published studies.[3-14] In these studies amniotic fluid volumes were measured either at the time of hysterotomy by direct collection,[3, 5, 6, 9, 11-14] or in continuing pregnancies by means of indicator dilution techniques.[1-8, 10, 13] For the present study, data were excluded if fetal anomalies, fetal death, spontaneous abortion, toxemia, or maternal or fetal disease were present. Thus data were included only if the fetus was considered normal. Fetuses with oligohydramnios or polyhydramnios were included if outcome was good.

The data were collected either from published tables or figures. For the latter, photographic enlargement combined with linear measurement of X-Y coordinates was used to convert points in the figures into numeric values.

Data analysis. Initially, the data are presented as the relationship between volume and age on a linear scale. For statistical analyses, amniotic fluid volumes were log transformed (base 10) because their variability was greater late in gestation than early in gestation and because the volumes at any given age were not normally distributed largely because of the presence of a few high volumes. The data were then analyzed with standard polynomial regression techniques. In addition, mean values were computed for each 2-week period (i.e., 8 to 9.99, 10 to 11.99, etc., weeks). In the figures containing 2-week averages, volumes were plotted at the computed mean age rather than at the midpoint; for example, 8.2 was the mean for the 8.0 to 9.99 week window and 38.6 was the mean for 38 to 39.99 weeks. Statistical differences in volumes among age groups were tested by means of a one-way analysis of variance with Fisher's least significant difference for multiple comparisons.[15] Variability was analyzed for the residuals calculated from the log of amniotic fluid volume minus the log as determined with the best-fit polynomial regression equation. To avoid biasing as a result of the different number of observations at each gestational age, the polynomial regression equation was determined from the means at 2-week intervals. Of 706 collected values, one volume of zero at 43 weeks was excluded from the analysis because it could not be log transformed and because there must have been at least a little fluid present.

It should be noted that it was possible to statistically define confidence intervals only for log-transformed data because volumes were not normally distributed about the mean before log transformation. To convert these into easily interpreted values, antilogs must be taken. When this is done, the confidence interval is no longer equal to the mean ± a constant but rather equals the mean multiplied and divided by a constant. For example, if the mean and 95% confidence interval were 2 ± 0.3 for the log data, the mean volume would be 100 ml (antilog of 2), with a confidence interval of

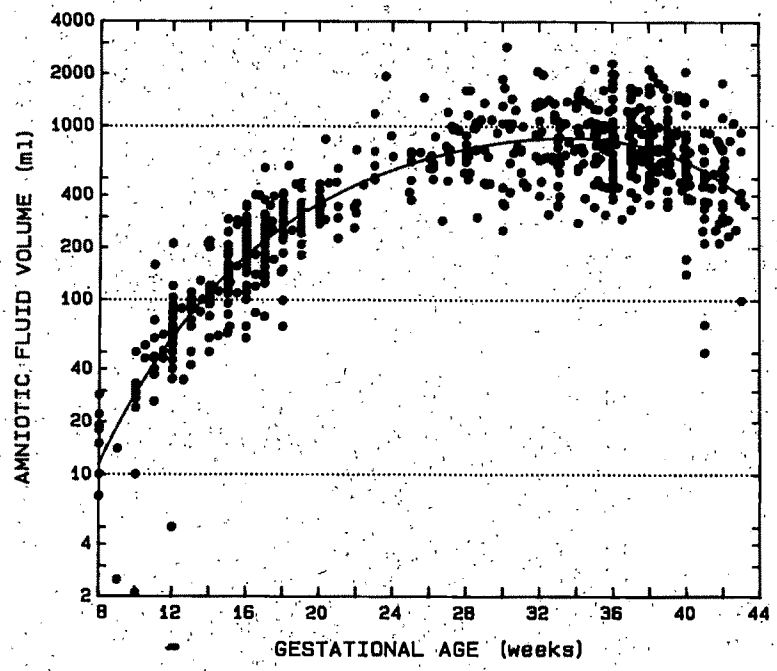


Fig. 2. Amniotic fluid volumes on a log scale in human pregnancies with normal fetuses (n = 705). *Solid line* is polynomial regression line (see text).

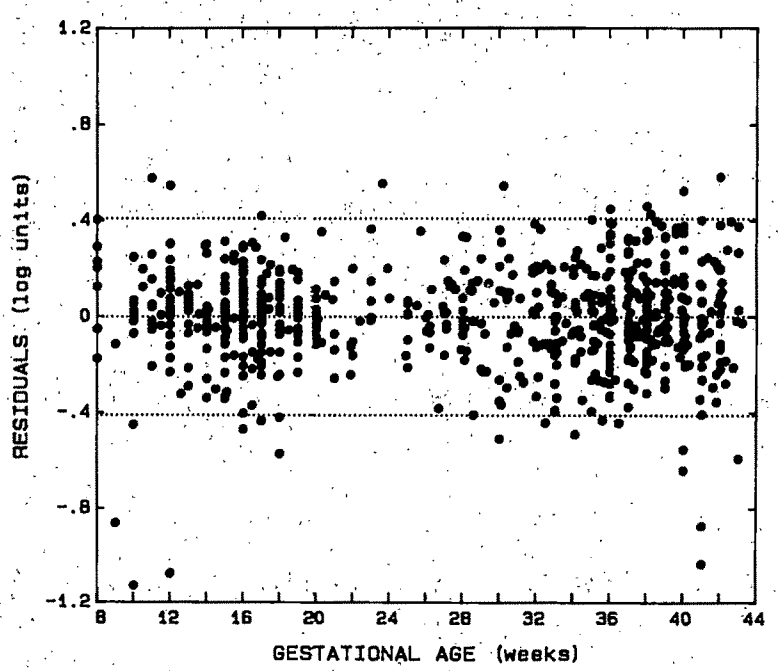


Fig. 3. Variability in amniotic fluid volumes as a function of gestational age. Data expressed as residuals calculated by subtracting value determined with polynomial regressional equation from individual points. Area between upper and lower dotted lines represents 95% confidence interval.

1/2 × 100 to 2 × 100, or 50 to 200 ml, since the antilog of 0.3 is 2.

Results

A total of 705 amniotic fluid volumes from normal fetuses were collected from 8 to 43.2 weeks' gestation. These are shown on a linear scale in Fig. 1 as a function of gestational age, along with the line of best fit as determined by polynomial regression. From the regression equation, the peak was 931 ml and occurred at 33.8 weeks.

Two major problems occur with the use of polynomial regression to describe the changes in amniotic fluid volume. First, as seen in Fig. 1, the scatter about the

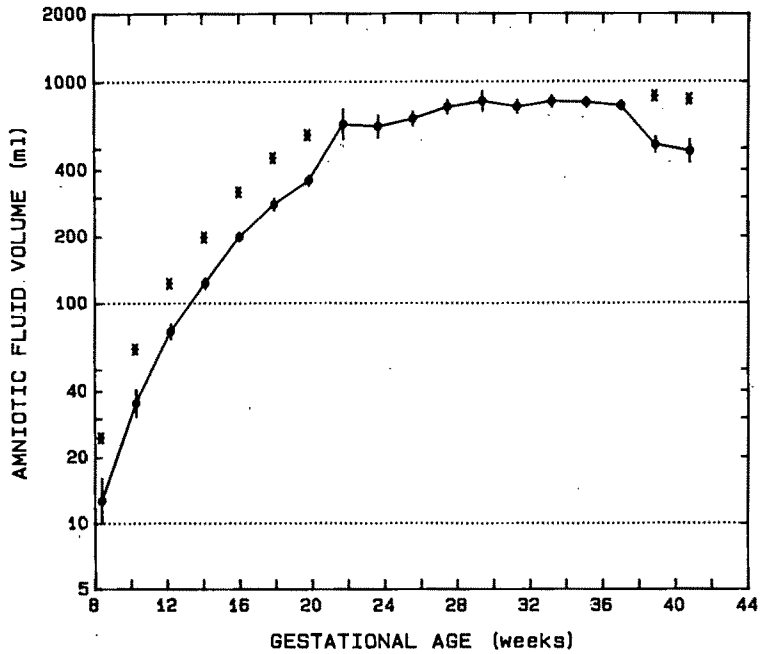


Fig. 4. Statistical analysis of gestational changes in amniotic fluid volume. Data expressed as mean ± SE for each 2-week interval. *$p < 0.0001$ compared with value at 39 weeks' gestation.

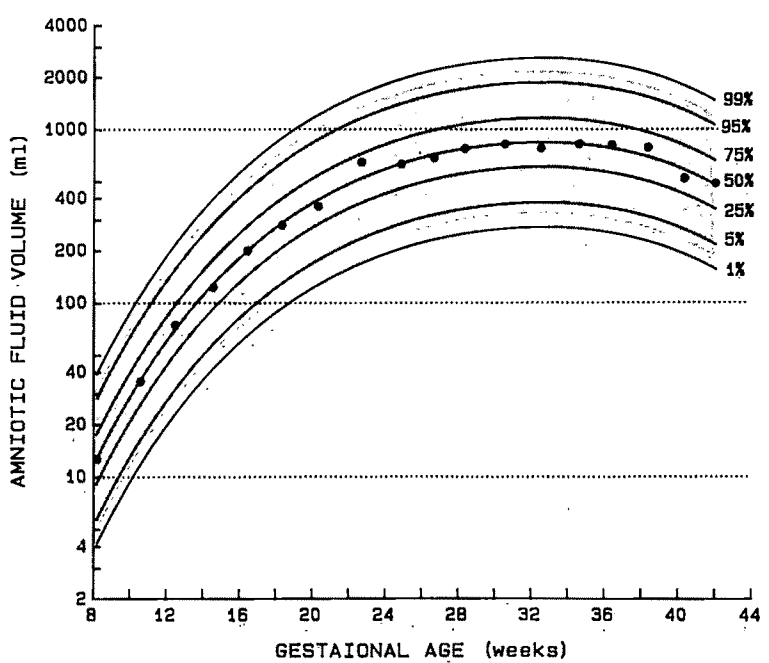


Fig. 5. Nomogram shows mean amniotic fluid volumes *(dots)* as a function of gestational age and percentiles. Volumes are plotted on a log scale. Fiftieth percentile line is polynomial regression calculated from biweekly means ($\log Y = -1.3916 + 0.4176X - 0.01622X^2 + 0.0003144X^3 - 0.0000026X^4$, $R = 0.998$, $F = 811$, $p < 10^{-6}$). *Shaded area* covers 95% confidence interval.

mean was much greater late in gestation compared with early in gestation. Thus the underlying assumption of uniform variability has been violated. Second, as readily seen in Fig. 1 at 30 to 40 weeks, the scatter above the mean (i.e., the regression line) was much greater than the scatter below the mean. Thus the assumption of a normal distribution was violated.

In an attempt to overcome these problems, amniotic fluid volumes were log transformed and are shown on a log scale in Fig. 2. Because it is difficult to visually perceive variability about a curved line, Fig. 3 replots the data of Fig. 2 after subtracting the regression line. An analysis of variance of the data in Fig. 3 found no statistically significant changes in the residuals (i.e.,

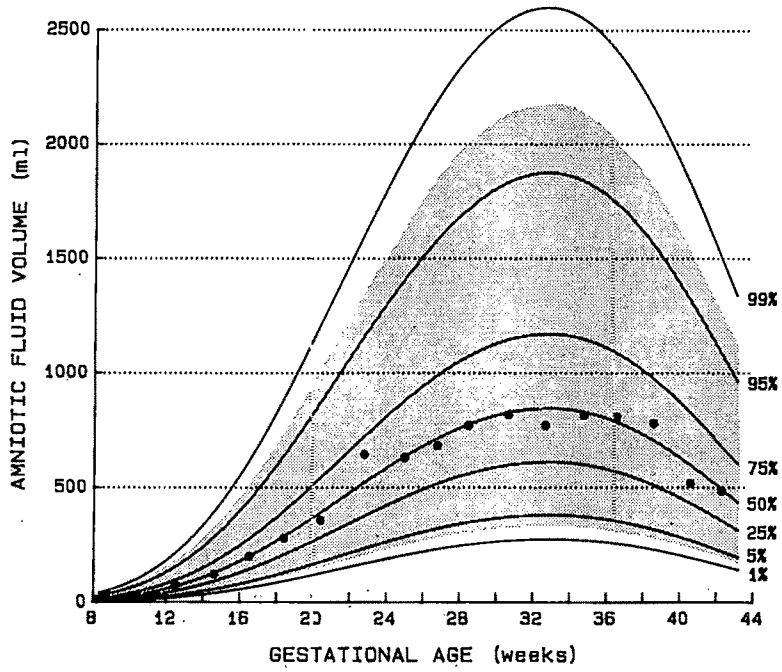


Fig. 6. Nomogram shows amniotic fluid volumes as a function of gestational age on a linear scale. *Dots* are means for each 2-week interval. Percentiles are calculated from polynomial regression equation and SD of residuals. *Shaded area* covers 95% confidence interval.

variability) with gestational age. In addition, after log transformation, amniotic fluid volumes were normally distributed about the mean for any given gestational age.

There are three major benefits of these results. First, polynomial regression now provides an adequate characterization of the changes in amniotic fluid volume with advancing gestation. When analyzed with this technique, each of the first four regression coefficients were statistically significant ($p < 0.002$). Thus the regression equation relating amniotic fluid volume (AFV) to gestational age (X) was log (AFV) = $-1.5595 + 0.4676\ X - 0.02014\ X^2 + 0.0004359\ X^3 - 0.0000039\ X^4$, with R = 0.896, F = 713, and $p < 10^{-6}$. This equation is represented by the solid line in Fig. 2.

The second benefit is that standard statistical methods can be used to compare the changes in mean volumes. As determined with an analysis of variance, the changes in amniotic fluid volume (AFV) with gestational age (Fig. 4) were highly significant (F = 160, $p < 10^{-6}$). However, there were no statistically significant changes in volume between 22 and 39 weeks' gestation; volume averaged 777 ml, and individual means ranged from 630 to 817 ml (Fig. 4).

The third benefit is that the variability of the data in Fig. 3 provides an overall estimate of the variability in amniotic fluid volume. Thus the standard deviation from the data in Fig. 3 can be used to characterize the normal range of amniotic fluid volumes throughout the range of gestational ages considered. From Fig. 3 the SD is 0.2094 so that the 95% confidence interval (1.96 X SD) is 0.4105 (log units). This means that 95% of all women with a normal fetus have amniotic fluid volumes within the range of 1/2.57 to 2.57 times, or within 39% to 257% of the mean volume for any gestational age over 8 to 43 weeks' gestation (2.57 is the antilog of 0.4105).

Nomograms showing the changes in amniotic fluid volume as a function of gestational age are shown in Fig. 5. As can be seen, these were calculated by adding or subtracting various multiples of the overall SD of the residuals to the mean volume calculated from the polynomial regression equation (i.e., the fiftieth percentile). The shaded area covers the 95% confidence interval (i.e., the 2.5 to 97.5 percentiles). These same data are replotted on a linear scale in Fig. 6 to facilitate comparisons.

The mean changes in amniotic fluid volume calculated on a weekly basis (from the polynomial regression equation) are shown in Fig. 7. At 8 weeks, volume is increasing at 10 ml/week. This increases to 25 ml/week at 13 weeks and reaches a maximum of 60 ml/week at 21 weeks' gestation. The weekly volume increment then decreases and reaches zero at 33 weeks' gestation, that is, volume is at maximum at this time. In contrast to this pattern, the weekly volume change when expressed relative to total volume decreases throughout weeks 8 to 43 of gestation (dashed line in Fig. 7). Thus mean amniotic fluid volume is increasing 45% per week at 8 weeks, 25% per week at 15 weeks, 10% per week at 24

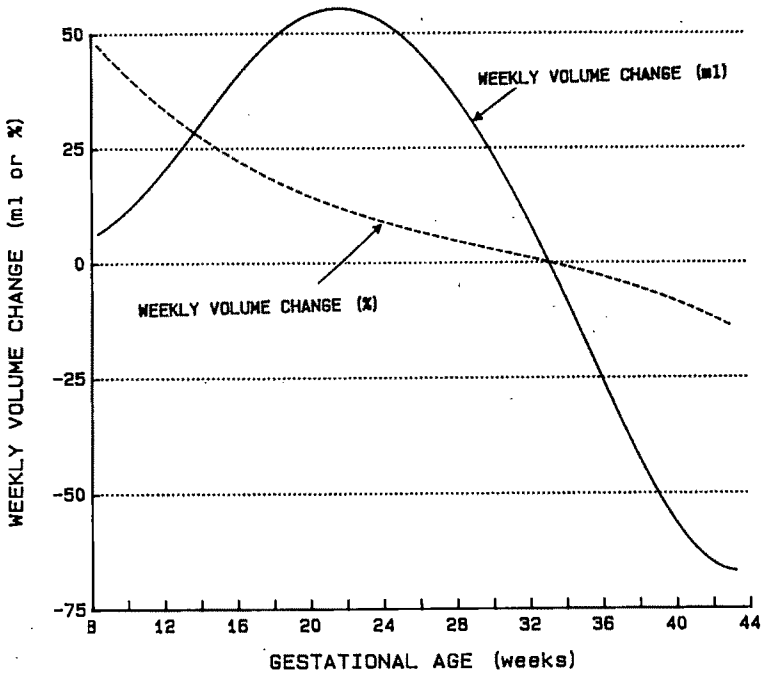


Fig. 7. Weekly changes in mean amniotic fluid volume as calculated from best-fit polynomial regression equation.

weeks, and 0% at 33 weeks. At 40 weeks, volume is decreasing an average of 8% per week.

Comment

The major significance of this study is that we were able to statistically characterize for the first time normal amniotic fluid volumes over the last 80% of gestation. This also provides a statistical definition of abnormal amniotic fluid volumes so that limits can be set for oligohydramnios and polyhydramnios. For example, as seen in Figs. 5 and 6, at 30 weeks' gestation, the 95% confidence interval about the mean (817 ml) covers the range of 318 to 2100 ml. Thus with <318 ml of fluid oligohydramnios would be present and with more than 2100 ml polyhydramnios would be present. Each of these occur 2.5% of the time.

Another observation is that after a progressive rise from 8 weeks' gestation, amniotic fluid volume reaches its statistical maximum at 22 weeks' gestation and remains at this level (630 to 817 ml) through 39 weeks' gestation, declining thereafter. Although Fuchs[16] had reported that mean amniotic fluid volume was relatively constant during the last trimester, it has been more widely accepted that amniotic fluid volume reached a peak at 34 weeks' gestation and declined thereafter.[10] This difference is largely attributable to the different methods of averaging (absolute values versus logs) in the studies; that is, a few cases of hydramnios had biased the mean value upward at 34 weeks' gestation. In addition, a statistical analysis of the volume changes with time was not performed in the previous studies so that these two statements are not necessarily inconsistent with each other. This same contradiction arises in the present study in that the analysis of variance revealed no significant changes in volume between 22 and 39 weeks (Fig. 4). On the other hand, the mean volume as determined from the regression equation reached its peak at 33 weeks and declined thereafter, which is in agreement with Queenan et al.[10] In this case, the polynomial regression equation most likely provides a better estimate of the trends in amniotic fluid volume. Thus with a much larger data set, a peak around 33 weeks' gestation would be more easily identified.

A final new observation is that from 8 to 43 weeks' gestation, the variability in amniotic fluid volume about the mean is constant when expressed as log-transformed data. Thus 95% of the time amniotic fluid volume will be within the range of 1/2.57 to 2.57 times the mean volume at any given gestational age. At present we do not know why the variability is constant, but it probably relates to the volume regulatory mechanisms. It also should be noted that this variability is quite large. Factors, such as maternal age, gravidity and parity, and ethnicity and race, may have contributed to the variability, but we were unable to explore these factors because they were absent from the original reports.

In summary, this study provides a statistical characterization of the changes in amniotic fluid volume that occur in normal human pregnancy. This also provides

a definition of oligohydramnios and polyhydramnios over 8 to 43 weeks' gestation.

REFERENCES

1. Chamberlain PF, Manning FA, Morrison I, Marman CR, Lange IR. Ultrasound evaluation of amniotic fluid volume. I. The relationship of marginal and decreased amniotic fluid volumes to perinatal outcome. AM J OBSTET GYNECOL 1984;150:245-9.
2. Chamberlain PF, Manning FA, Morrison I, Marman CR, Lange IR. Ultrasound evaluation of amniotic fluid volume. II. The relationship of increased amniotic fluid volumes to perinatal outcome. AM J OBSTET GYNECOL 1984;150:250-4.
3. Abramovich DR. The volume of amniotic fluid in early pregnancy. J Obstet Gynaecol Br Comm 1968;75:728-31.
4. Charles D, Jacoby HE, Burgess F. Amniotic fluid volumes in the second half of pregnancy. AM J OBSTET GYNECOL 1965;93:1042-7.
5. Gadd RL. The volume of the liquor amnii in normal and abnormal pregnancies. J Obstet Gynaecol Br Commonw 1966;73:11-22.
6. Gillibrand PN. Changes in amniotic fluid volume with advancing pregnancy. J Obstet Gynaecol Br Commonw 1969;76:527-9.
7. Haswell GL, Morris JA. Amniotic fluid volume studies. Obstet Gynecol 1973;42:725-32.
8. Marsden D, Huntingford PJ. An appraisal of the Coomassie blue dilution technique for measuring the volume of liquor amnii in late pregnancy. J Obstet Gynaecol Br Commonw 1965;72:65-8.
9. Nelson MM. Amniotic fluid volumes in early pregnancy. J Obstet Gynaecol Br Commonw 1972;79:50-3.
10. Queenan JT, Thomson W, Whitfield CR, Shah SI. Amniotic fluid volumes in normal pregnancies. AM J OBSTET GYNECOL 1972;114:34-8.
11. Rhodes P. The volume of liquor amnii in early pregnancy. J Obstet Gynaecol Br Commonw 1966;73:23-6.
12. Sinha R, Carlton M. The volume and composition of amniotic fluid in early pregnancy. J Obstet Gynaecol Br Commonw 1970;77:211-4.
13. Van Otterlo LC, Wladimiroff JW, Wallenburg HC. Relationship between fetal urine production and amniotic fluid volume in normal pregnancy and pregnancy complicated by diabetes. J Obstet Gynaecol Br Commonw 1977;84:205-9.
14. Wagner G, Fuchs F. The volume of amniotic fluid in the first half of human pregnancy. J Obstet Gynaecol Br Commonw 1962;69:131-6.
15. Dowdy S, Wearden S. Statistics for Research. New York: John Wiley & Sons, 1983.
16. Fuchs F. Volume of amniotic fluid at various stages of pregnancy. Clin Obstet Gynecol 1966;9:449-60.

Doppler velocimetry and placental disease

Luis A. Bracero, MD, Debra Beneck, MD, Nancy Kirshenbaum, MD, Marianne Peiffer, RDMS, Patricia Stalter, CDMS, and Harold Schulman, MD

Valhalla and Mineola, New York

Quantitative placental examinations were performed on 47 women who had Doppler flow velocity studies of the umbilical artery during their pregnancy. The systolic-diastolic ratio of the umbilical artery was used as the measurement parameter to divide the study population into two groups. Group 1 consisted of women with normal systolic-diastolic ratios (systolic-diastolic < 3), and group 2 consisted of women with an elevated systolic-diastolic ratio (systolic-diastolic ≥ 3). The group with an increase in systolic-diastolic ratio had more perinatal complications as demonstrated by two stillbirths, a higher incidence of cesarean deliveries for fetal distress, and more admissions to the neonatal intensive care unit. Significant differences were found when gestational age at delivery, placental weight, birth weight, and the number of small muscular arteries in the placenta were compared. Since gestational age may have accounted for the difference in placental findings, patients were matched for gestational age. The placental weights were comparable, but there were fewer small muscular arteries in those patients with an increase in systolic-diastolic ratio ($p < 0.001$). In addition, when these findings were examined to determine the influence of diminished uterine flow velocity, none was found. (AM J OBSTET GYNECOL 1989;161:388-93.)

Key words: Umbilical artery velocity waveforms, Doppler ultrasound, placenta

From the Department of Obstetrics and Gynecology and the Department of Pathology, New York Medical College, Westchester County Medical Center, and the Department of Obstetrics and Gynecology, Winthrop University Hospital.

Received for publication April 1, 1988; revised February 22, 1989; accepted March 3, 1989.

Reprint requests: Luis A. Bracero, MD, Department of Obstetrics and Gynecology, Westchester County Medical Center, Valhalla, NY 10595.

Umbilical artery velocity waveform analysis has been proposed as a new placental function test. It has been reported that an umbilical artery systolic-diastolic ratio of <3 during the third trimester implies normal placental function, whereas a systolic-diastolic ratio ≥ than 3 implies a strong probability of abnormal outcome.[1] These outcomes include small-for-gestational age fe-

tuses, poorly controlled diabetes, discordant twins, low Apgar scores, trisomy 13 and 18, cesarean section for fetal distress, and more admissions to the neonatal intensive care unit.[2-5] Previous studies have suggested a correlation between umbilical artery velocity waveforms and placental morphology.[6,7] The purpose of this study was to confirm and expand these observations to understand better the significance of an elevated systolic-diastolic ratio.

Material and methods

Forty-seven women who had Doppler flow velocity studies of the umbilical artery during pregnancy had quantitative placental examinations. There were various indications for performing umbilical artery Doppler flow velocity studies (Table I). A continuous-wave Doppler ultrasound scan with a 4 MHz transducer, a power density of 6.5 mW/cM^2, and a spectrum analyzer were used to identify and measure velocity waveforms. When the umbilical artery signal is obtained, it is freeze-framed and a systolic peak-to-diastolic trough ratio is calculated. Four separate fields of waveforms are measured, and an average systolic-diastolic ratio is calculated for each examination. In addition, we studied the velocity waveform of the uterine artery to determine if maternal circulation influences placental findings. This is done by placing the ultrasound transducer on the abdomen on the right and left paracervical areas. The uterine artery has a distinct contour that allows it to be easily differentiated from the other pelvic vessels. A systolic-to-diastolic ratio was obtained for each uterine artery, and these measurements were then averaged. One set of measurements was obtained at each examination. A uterine artery systolic-diastolic ratio >2.6 after 26 weeks is considered abnormal.[8] The systolic-diastolic ratio of the umbilical and uterine arteries obtained during the last examination before delivery was used for statistical analysis.

The study population was divided into two groups. Group 1 was made up of 29 women with normal umbilical artery systolic-diastolic ratios (systolic-diastolic < 3). Group 2 consisted of 18 women with an elevated umbilical systolic-diastolic ratio (systolic-diastolic ≥ 3). A two-tailed *t* test for independent samples or a two-tailed paired *t* test was used to test differences between the normal and abnormal systolic-diastolic ratio groups for all interval variables. A two-tailed χ^2 was used to test for differences in categoric variables, such as admissions to the neonatal intensive care unit and cesarean section deliveries for fetal distress. In addition, a multivariate analysis of variance was performed on the data, holding the arterial count as the dependent variable. The statistical package SPSS/PC+ was used for all statistical analysis.

Table I. Indication for Doppler velocimetry testing

Condition	*No.*
Preeclampsia	10
Chronic hypertension	7
Chronic hypertension and preeclampsia	5
Diabetes	9
Preterm labor	8
Suspected growth retardation	2
Lupus erythematosus	2
Other	4

The methodology for the study of the placenta is similar to that described by Giles et al.[6] and McCowan et al.[7] The placentas were weighed fresh, with cord and membranes attached, and then immediately fixed in 10% formalin for at least 1 day. After adequate fixation, longitudinal cuts through the maternal surface were made at approximately 2 cm intervals. At least two blocks were prepared from each placenta, one from an area 2 cm from the insertion of the cord and another from an area 2 cm from the disk margin. Areas of obvious infarction were avoided, and additional sections were submitted in cases in which there was extensive infarction. The paraffin-embedded tissue was cut and then stained with hematoxylin and eosin for preliminary evaluation, followed by Masson trichrome, for better demonstration of the vascular musculature and surrounding collagen.

Five fields at × 100 magnification were scanned from each block by one pathologist, who was unaware of the results of the umbilical artery velocity waveform. Subchorionic and basal areas and areas of infarction were avoided, as were artifactually distorted areas. Tertiary stem villi and small muscular arteries were counted in each field. Small muscular arteries were defined as vessels with a diameter between 20 and 90 μm and with a muscular coat greater than a single cell in thickness.[6] Vessel diameter was measured as the greatest external transverse diameter in a section at right angles to the line of the vessel. A micrometer disk (Bausch & Lomb, Rochester, N.Y.), calibrated at × 100 was used to obtain this measurement. The results are reported as the total number of tertiary stem villi and small muscular arteries counted in the 10 fields studied in each placenta.

Results

The mean systolic-diastolic ratio for group 1 was 2.5 ± 0.4 and 4.0 ± 0.9 for group 2. Five patients in group 2 had absent end-diastolic velocity. When gestational age at delivery, placental weight, birth weight, and number of small muscular arteries were compared, statistically significant differences were found between

Table II. Comparison of placental and perinatal data between normal and abnormal umbilical artery systolic-diastolic ratios

	Group 1 (mean ± SD)	*Group 2 (mean ± SD)*	*p value*
n	29	18	
Systolic-diastolic (mean)	2.5 ± 0.4	4.0 ± 0.9	0.0005
Gestational age (wk)	36 ± 5	32 ± 4	0.007
Birth weight (gm)	2869 ± 1066	1623 ± 859	0.0005
Placental weight (gm)	511 ± 173	379 ± 148	0.01
Tertiary stem villi count (mean)	88 ± 17	79 ± 22	0.095, NS
Small muscular arterial count (mean)	81 ± 25	45 ± 21	0.0005

Table III. Comparison of placental and perinatal data between normal and abnormal umbilical artery systolic-diastolic ratios matched for gestational age

	Group 1 (mean ± SD)	*Group 2 (mean ± SD)*	*p value*
n	16	16	
Systolic-diastolic ratio (mean)	2.6 ± 0.2	4.0 ± 0.9	0.0005
Gestational age (wk)	33.5 ± 4	33.3 ± 4	0.897, NS
Birth weight (gm)	2201 ± 947	1740 ± 841	0.155, NS
Placental weight (gm)	411 ± 148	399 ± 144	0.824, NS
Tertiary stem villi count (mean)	87 ± 18	79 ± 23	0.272, NS
Small muscular arterial count (mean)	79 ± 24	46 ± 22	0.0005

Table IV. Effect of uterine flow velocity on perinatal and placental parameters

	Normal uterine S/D ratio (mean ± SD)	*Abnormal uterine S/D ratio (mean ± SD)*	*p value*
n	35	12	
Uterine S/D ratio	1.9 ± 0.3	3.7 ± 0.9	0.0005
Umbilical S/D ratio	2.8 ± 0.7	3.5 ± 1.5	0.03
Gestational age (wk)	36 ± 5	32 ± 4	0.01
Birth weight (gm)	2671 ± 1089	1579 ± 983	0.004
Placental weight (gm)	500 ± 158	344 ± 174	0.006
Tertiary stem villi count	84 ± 15	86 ± 21	0.764, NS
Small muscular arterial count	70 ± 29	60 ± 31	0.304, NS

S/D, Systolic-diastolic ratio.

these two groups (Table II). Newborn infants from patients in group 2 had lower 1-minute Apgar scores (group 1 = 7 ± 2.6; group 2 = 5 ± 3.2; two-tailed *t* test, $p = 0.017$). This group had two antepartum fetal deaths because of abruptio placentae. The incidence of cesarean deliveries performed for fetal distress was 55% in group 2 and 10% in group 1 ($p < 0.001$). Seventy-eight percent of the newborns delivered to patients in group 2 required admission to the neonatal intensive care unit versus 38% of the newborns delivered to patients in group 1 ($p < 0.01$).

The data were corrected for gestational age (Table III) because this variable may have accounted for the significant differences seen between groups 1 and 2. The only difference that remained significant was in the number of small muscular arteries. This difference can be seen in a sample of a placenta from a patient with a normal ratio (Fig. 1) and that of a patient with an abnormal ratio (Fig. 2).

The most abnormal umbilical artery velocity waveform is that in which there is no end-diastolic velocity. We compared those patients with elevated umbilical systolic-diastolic ratios to those with absent end-diastolic velocity and found no significant differences in the number of small muscular arteries. The group with absent end-diastolic velocity tended to deliver earlier (29 ± 2.7 versus 34 ± 3.4 weeks; two-tailed *t* test, $p = 0.006$), have lower birth weight infants (776 ± 242

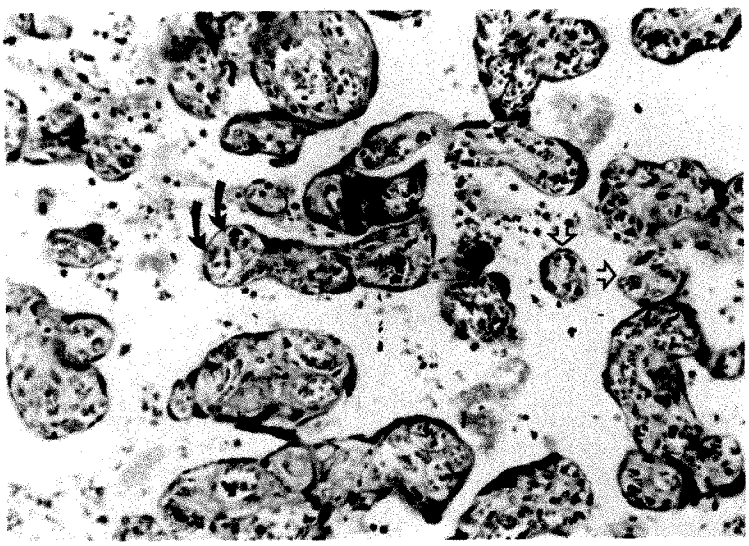


Fig. 1. Placental photomicrograph of patient with systolic-diastolic ratio of 2.4. Note abundance of well-vascularized terminal villi *(open arrows)*. Tertiary stem villi contain abundant small muscular arteries *(curved arrows)*. (Original magnification ×200; Masson trichrome.)

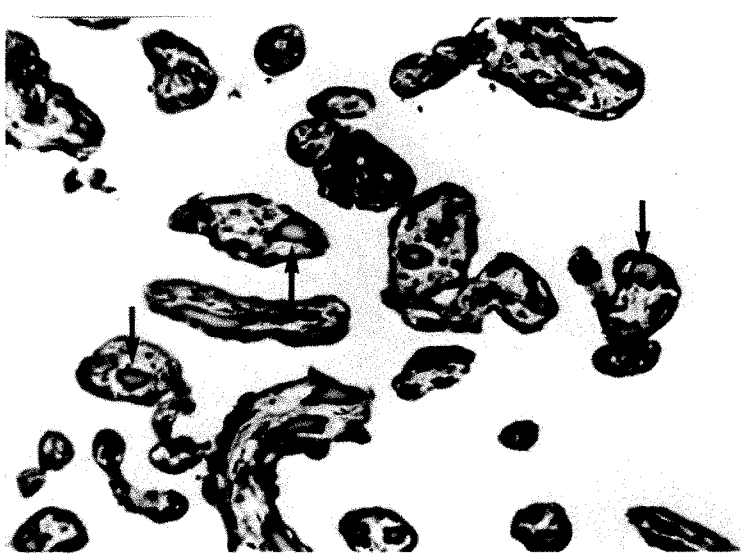


Fig. 2. Placental photomicrograph of patient with absent end-diastolic velocity. Poorly vascularized tertiary stem villi with few capillaries *(straight arrows)*. There is a lack of small muscular ateries in this field, which is representative of the vascular condition of this placenta. (Original magnification, ×200; Masson trichrome.)

versus 1950 ± 783 gm; two-tailed *t* test, $p = 0.0005$), and smaller placentas (244 ± 90 versus 431 ± 133 gm; two-tailed *t* test, $p = 0.01$).

To determine if the difference seen in the arterial count could be accounted for by one disease process or could only be identified by the systolic-diastolic ratio of the umbilical artery, we independently compared those patients with diabetes, preeclampsia, chronic hypertension, and preterm labor with the members of our study population without these conditions. No significant differences were found in the number of tertiary stem villi or the number of small muscular arteries in any of these groups.

The patient population was also divided on the basis of the systolic-diastolic ratio value of the uterine arteries (Table IV) to see if uterine flow affected placental histologic findings. Women who had an elevated systolic-diastolic ratio of the uterine artery had higher umbilical artery velocity waveform systolic-diastolic ratios than those with normal uterine artery systolic-diastolic ratios. There was no significant difference in the placental weight, number of tertiary stem villi, or number of

Table V. Arterial count (mean ± SD) in the four vascular patterns obtained with Doppler ultrasound studies

	Normal umbilical	*Abnormal umbilical*	*p value*
Normal uterine	82 ± 26 (n = 24)	44 ± 13 (n = 11)	$p < 0.001$*
Abnormal uterine	77 ± 21 (n = 5)	48 ± 31 (n = 7)	$p = 0.06$

*The p value denotes a two-tailed t test for independent samples.

small muscular arteries. Next, women with abnormal velocity waveform systolic-diastolic ratios of the umbilical artery were subdivided according to the uterine systolic-diastolic ratio. Again, there were no significant differences in the number of tertiary stem villi or small muscular arteries. The four possible combinations of vascular patterns obtained with Doppler ultrasound examination are analyzed with regard to the number of small muscular arteries seen in the placenta (Table V). The data suggest that uterine flow was not a critical factor in the development of the small muscular arteries.

Comment

Giles et al.[6] performed placental examinations in patients who underwent Doppler flow velocity studies before delivery. They found that there was a decrease in the number of small muscular arteries in those patients with elevated systolic-diastolic ratios of the umbilical artery. In the group with abnormal systolic-diastolic ratios, placental weights were significantly lower than those of patients with normal systolic-diastolic ratios. There also was a disproportionately large number of intrauterine growth-retarded newborn infants in the group with abnormal systolic-diastolic ratios. The fact that they studied smaller placentas and smaller infants in the abnormal systolic-diastolic ratio group may account for the decrease seen in the number of small muscular arteries.

McGowan et al.[7] have performed a similar study with a smaller patient population and used the pulsatility index as the measure of umbilical artery velocimetry. They found a decrease in the number of small muscular arteries in those patients with a high pulsatility index. In their study corrections were not made for gestational age (31 weeks in the high pulsatility index group versus 37 weeks in the low pulsatility index group) or for birth weight (1053 gm in the high pulsatility index group versus 3054 gm in the low pulsatility index group). Again, these significant differences between the two groups could account for the differences seen in the number of small muscular arteries.

The purpose of our study was to try and correct for all variables that could theoretically influence the small muscular arterial count, since clinically we find that the group with elevated systolic-diastolic ratios has preterm deliveries, smaller placentas, and smaller newborn infants. After correcting for the encountered variables, we could then compare the two groups whose only difference was in the systolic-diastolic ratio value (Table III). Our findings suggest that in this group the increase in the systolic-diastolic ratio is associated with a decrease in the number of small muscular arteries within the placental vascular tree. This decrease in the number of vessels appears to be independent of gestational age, birth weight, placental weight, disease process complicating pregnancy, and uterine artery flow velocity (multivariate analysis of variance, $p > 0.05$).

Giles et al. hypothesized that the decrease in the number of placental vessels is caused by an obliterative process. They based this opinion on two observations. First, they saw that as pregnancy progressed, the systolic-diastolic ratio became higher in pregnancies, resulting in growth-retarded newborns. Second, they saw collections of nuclear material in some abnormal placentas, which they believed represented obliteration of a small artery. However, we observed systolic-diastolic ratios that decreased as pregnancy progressed, although they still remained in the abnormal range. We also saw obliteration of vessels in placentas with both normal and abnormal systolic-diastolic ratios. This finding was seen in a small number of placentas and cannot be used to make a general statement. It is possible that this vessel obliteration may represent a normal aging process in some placentas, but our data on vessel number between 33 and 36 weeks show no differences. Furthermore, Giles et al. demonstrated that the average small muscular arterial count increases slightly with gestational age in those patients with normal and abnormal systolic-diastolic ratios of the umbilical artery. Thus the decrease in the number of vessels in the abnormal umbilical artery group must occur at a much earlier period of gestation. Vascular resistance occurs primarily across the arterioles. If there are diminished arterioles with no change in the radii of the remaining vessels, it would still not be clear whether velocity would be affected because of the net diminution in area (velocity = flow/area). The associated elevated systolic-diastolic ratio indicates that velocity is diminished. Since most ancillary studies on umbilical vein flow in intrauterine growth-retarded newborns indicate a reduced volume of blood flow,[9] it can be hypothesized that in most of these cases there is a developmental arrest of placental angiogenesis. The stimuli for angiogenesis are un-

known, but these data suggest that successful therapy would have to be carried out early in the second trimester of pregnancy because an unchanging pattern emerges by 25 weeks.

This is the third study demonstrating an inverse relationship between an elevated umbilical artery systolic-diastolic ratio and the small muscular arterial count of the stem villi. Since these findings occurred in varying clinical situations (hypertension, diabetes, lupus erythematosus, etc.), it appears that the decrease in arterial counts is a response that could be initiated by varying stimuli.

REFERENCES

1. Schulman H, Fleischer A, Stern W, et al. Umbilical velocity wave ratios in human pregnancy. AM J OBSTET GYNECOL 1984;148:985.
2. Fleischer A, Schulman H, Farmakides G, et al. Umbilical artery velocity waveforms and intrauterine growth retardation. AM J OBSTET GYNECOL 1985;151:502.
3. Farmakides G, Schulman H, Saldana LR, et al. Surveillance of twin pregnancy with umbilical arterial velocimetry. AM J OBSTET GYNECOL 1985;153:789.
4. Bracero L, Schulman H, Fleischer A, et al. Umbilical artery velocimetry in diabetes and pregnancy. Obstet Gynecol 1986;68:654.
5. Rochelson B, Schulman H, Farmakides G, et al. The significance of absent end-diastolic velocity waveforms. AM J OBSTET GYNECOL 1987;156:1213.
6. Giles WB, Trudinger BJ, Baird PJ. Fetal umbilical artery flow velocity waveforms and placental resistance: pathological correlation. Br J Obstet Gynecol 1985;92:31.
7. McCowan LM, Mullen BM, Ritchie K. Umbilical artery flow velocity waveforms and the placental vascular bed. AM J OBSTET GYNECOL 1987;157:900.
8. Fleischer A, Schulman H, Farmakides G, et al. Uterine artery Doppler velocimetry in pregnant women with hypertension. AM J OBSTET GYNECOL 1986;154:806.
9. Gill RW, Kosoff G, Warren PS, et al. Umbilical venous flow in normal and complicated pregnancy. Ultrasound Med Biol 1984;10:349.

Local methotrexate injection: A nonsurgical treatment of ectopic pregnancy

Mordechai Pansky, MD, Ian Bukovsky, MD, Abraham Golan, MD, Rami Langer, MD, David Schneider, MD, Shlomo Arieli, MD, and Eliahu Caspi, MD

Zerifin, Israel

Twenty seven patients with unruptured tubal pregnancy were selected for nonsurgical treatment with the use of one injection of 12.5 mg of methotrexate into the ectopic site at laparoscopy. No adverse reactions were observed. In three patients (11%), a laparotomy was performed because of rising β–human chorionic gonadotropin titers. In the other patients, serum β–human chorionic gonadotropin levels decreased to the nonpregnant range with no further intervention, and the patients recovered uneventfully. This method is suggested as an alternative to surgery in selected cases of early unruptured tubal pregnancy. (AM J OBSTET GYNECOL 1989;161:393-6.)

Key words: Local methotrexate, tubal pregnancy

Surgical removal of the tube or products of conception has been the treatment of choice for tubal pregnancy. However, postoperative morbidity exists, and the procedure requires as long as 6 days of hospitalization and 4 to 6 weeks of recovery. It has recently been shown that surgery can be avoided in carefully selected cases.[1, 2] Future fertility after nonoperative management seems to be better than after conventional surgery.[3]

Methotrexate, a cytotoxic drug that is largely used in cases of gestational trophablastic disease,[4] had previously shown promise in resolving unruptured ectopic pregnancies when given systemically, thereby eliminating the need for surgery.[2, 5, 6] However, its systemic use exposes the patients to serious side effects and toxicity. To minimize the risk of these side effects and maximize benefit from high tissue concentrations of methotrexate in the fallopian tube, the drug was locally applied by one injection to the pregnancy site at laparoscopy.

We present our experience with treating unruptured tubal pregnancy by a local methotrexate injection at laparoscopy.

From the Department of Obstetrics and Gynecology, Assaf Harofe Medical Centre, affiliated with the Sackler School of Medicine, University of Tel-Aviv.
Received for publication December 21, 1988; revised January 1, 1989; accepted February 24, 1989.
Reprint requests: Abraham Golan, MD, Department of Obstetrics and Gynecology, Assaf Harofe Medical Centre, Zerifin, 70300, Israel.

Table I. Clinical patient data

Case no.	*Age (yr)*	*Gestational age (days)*	*hCG level on admission (IU/L)*	*Size of pregnancy (cm)*	*Days of hospitalization*	*Day of resolution of hCG*
1	30	46	55	1 by 2	3	4
2	33	42	460	1 by 1	8	—
3	33	45	56	1 by 1	3	11
4	30	39	240	1 by 1.5	4	8
5	24	35	800	1 by 2	5	14
6	37	47	415	1 by 2	5	35
7	34	56	100	1 by 2	3	5
8	31	52	1320	1 by 1	10	—
9	3	42	680	1 by 1.5	5	10
10	32	50	375	2 by 2	4	12
11	30	56	408	1 by 1	3	6
12	31	46	560	3 by 3	3	12
13	32	39	220	1 by 3	4	5
14	22	40	567	1 by 1	4	34
15	26	44	207	1 by 2	3	12
16	28	42	248	1 by 1	3	13
17	27	56	354	1 by 2	3	18
18	30	44	300	2 by 2	3	10
19	42	37	1860	2 by 2	11	—
20	28	60	131	3 by 2	3	7
21	27	56	540	1 by 1	2	10
22	36	49	580	2 by 2	4	20
23	24	42	260	3 by 3	3	9
24	35	49	105	2 by 2	3	5
25	27	42	50	2 by 1	2	4
26	25	52	200	2 by 1	2	15
27	26	53	129	2 by 1	4	8

Material and methods

Of the 73 ectopic pregnancies diagnosed at the Assaf Harofe Medical Centre during 1988, 70 were tubal. A diagnosis of ectopic pregnancy was made clinically and supported by (β–human chorionic gonadotropin) levels and ultrasonography. Final confirmation of the diagnosis and selection of treatment were made at laparoscopy. The following selection criteria were used: (1) The largest diameter of the tubal pregnancy did not exceed 3 cm; (2) the tubal serosa was intact and no active bleeding could be observed; and (3) the pelvis could be fully visualized.

Informed consent was obtained from each patient before laparoscopy after detailed explanation. Twenty-seven (38%) of the 70 cases of tubal pregnancy, which were diagnosed from January to December 1988, fulfilled the selection criteria.

After meticulous screening of the whole pelvis, the involved tube was grasped by an atraumatic forceps through a second puncture. Methotrexate, 12.5 mg, diluted in 2 ml of physiologic solution, was injected into the pregnancy site through the tubal wall by means of a 15 cm long spinal needle (18 gauge) introduced under laparoscopic guidance. Each patient was carefully observed for changes in pulse, blood pressure, and other vital signs for the first 48 hours. Daily serum β–human chorionic gonadotropin levels were determined after surgery. Patients remained in the hospital until β–human chorionic gonadotropin levels fell on 2 consecutive days or reached the level of 10 mIU/ml. Thereafter each patient was seen every 24 to 48 hours on an ambulatory basis, clinically examined, and serum β–human chorionic gonadotropin levels determined. Follow-up was terminated when β–human chorionic gonadotropin level dropped below 10 mIU/ml.

Results

Patient characteristics and clinical data are summarized in Table I. Ages ranged from 22 to 42 years (mean, 30 years). Mean gestational age of the group was 46 days with a range of 35 to 60 days. Range of serum β–human chorionic gonadotropin on admission was 55 to 1860 mIU/ml. The location of tubal pregnancy was ampullary in all patients: 20 on the right and seven on the left. All 27 patients underwent the procedure without any adverse reaction. Twenty-four patients were discharged 2 to 5 days later because of decreasing serum β–human chorionic gonadotropin levels.

Three patients (cases 2, 8, and 19) required a laparotomy because of a further rise in β–human chorionic gonadotropin levels. In the first two cases serum β–human chorionic gonadotropin levels dropped during the first 2 to 3 days after the injection, indicating an initially promising response. Although both women were asymptomatic and clinically stable, laparotomy was performed on days 8 and 5, respectively. The third patient (case 19) had a continuous rising serum β–

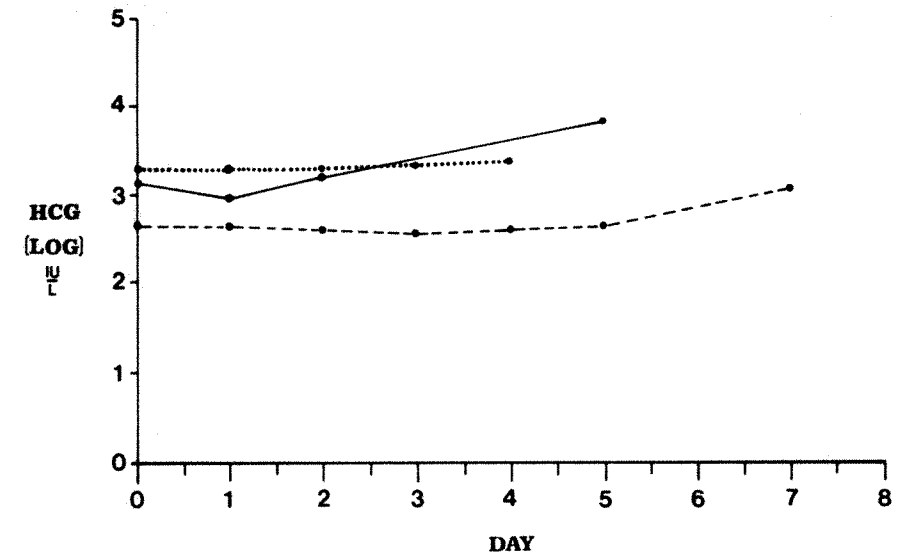


Fig. 1. β–Human chorionic gonadotropin levels of three patients requiring salpingectomy.

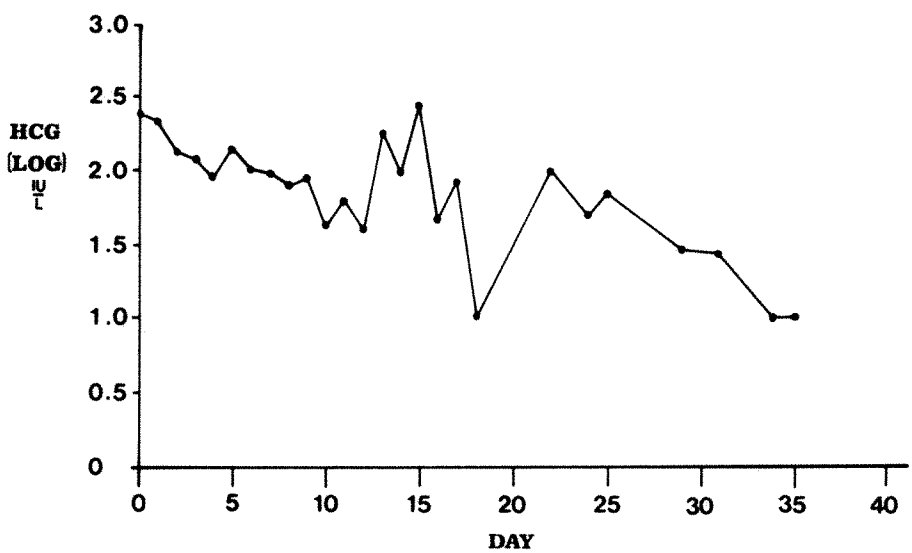


Fig. 2. β–Human chorionic gonadotropin levels (average) of 24 patients not having surgery.

human chorionic gonadotropin pattern and an acute onset of severe abdominal pain on the fourth day after the injection and immediately had surgery.

In all three failures, an unruptured tubal pregnancy was found, with no blood in the peritoneal cavity (not even in the latter case) and salpingectomy was performed. The β–human chorionic gonadotropin patterns for the three failures are presented in Fig. 1. Three patients requested tubal sterilization, which was performed by electrocautery after injection of methotrexate.

The pattern and average levels of serum β–human chorionic gonadotropin for the successful cases are presented in Fig. 2. In two patients it took as long as 34 and 35 days for hCG levels to drop to 10 mIU/ml. The average for all patients was 12 days. None of the treated patients had any adverse reactions related to methotrexate.

Hospitalization averaged 4 days (range, 2 to 11 days). So far seven patients have had postoperative hysterosalpingography, which showed normal patency of the affected tube in six and distal occlusion in the seventh patient.

Three patients conceived again; one is an ongoing normal intrauterine pregnancy. One had an early induced abortion and one an early spontaneous abortion.

Comment

The introduction of various new diagnostic measures, such as rapid β–human chorionic gonadotropin assays and vaginal ultrasonography, has significantly expedited the diagnosis of ectopic pregnancy. More

and more tubal pregnancies are diagnosed early in an unruptured state.[7]

Methotrexate had proved to be well suited for the eradication of trophoblastic tissue in ectopic locations.[5, 6, 8] Ory et al.[8] reports on six patients with ampullary ectopic pregnancies treated with intravenous methotrexate followed by leucoverin. Only one patient required laparotomy, and two received blood transfusions. Ichinoe et al.[9] report a 95.7% success rate with systemic methotrexate alone, showing high rates of patency of the affected tube.

Sauer et al.[2] expanded the series of Ory et al.[8] and report high success rate by treating tubal pregnancies with systemic methotrexate in combination with citrovorum factor.

However, with systemic administration of methotrexate toxicity is expected as many as 20% to 30% of patients.[2, 8, 9] An increased incidence of congenital anomalies in subsequent pregnancies was also reported.[10]

Recently nine cases of unruptured tubal pregnancy were successfully treated with laparoscopy by local injection of prostaglandin $F_{2\alpha}$.[11]

Use of local injection of methotrexate under laparoscopic control enabled us to avoid abdominal surgery in 24 of 27 (89%) patients and thus reduce postoperative recovery time. Applied in this manner methotrexate probably achieves higher tissue concentration in the affected tube, thereby avoiding the systemic route and thus reducing the risk of adverse reactions and side effects.

Our three failures may be related to technical problems, such as leakage of the injected methotrexate through the injection site or an additional puncture in the posterior wall of the tube, which could be responsible for the failures. In such a case, an additional dose of methotrexate can be given. We used a fixed dose of methotrexate for all patients. Since two of these three patients had the highest preoperative β–human chorionic gonadotropin levels, perhaps a higher dose is necessary in such cases.

We believe and suggest that this mode of treatment provides a safe and relatively effective alternative to surgery in patients with early unruptured tubal pregnancy. It is very tempting to be able to establish an alternative to surgery in ectopic pregnancies. Hospital stay is shortened and surgical risks and complications are avoided. Such an alternative should be acceptable if the incidence of side effects and complications remains low. It seems that the suggested protocol conforms with such demands.

REFERENCES

1. Mashiach S, Carp HJA, Serr DM. Nonoperative management of ectopic pregnancy. J Reprod Med 1982;27:127.
2. Sauer MV, Gorill MJ, Rodi IA, et al. Nonsurgical management of unruptured ectopic pregnancy. Fertil Steril 1987;46:752.
3. Carp HJA, Oelsmer G, Serr DM, Mashiach S. Fertility after nonsurgical treatment of ectopic pregnancy. J Reprod Med 1985;31:119.
4. Berkowitz RS, Goldstein DP. Methotrexate with citrovorum factor rescue for non metastatic gestational trophoblastic neoplasia. Obstet Gynecol 1979;54:725.
5. Tanaka T, Hayashi H, Kutzuzawa T, Ichinoe K. Treatment of interstitial ectopic pregnancy with methotrexate. Fertil Steril 1982;37:851.
6. Lathrop JC, Bowles GE. Methotrexate in abdominal pregnancy. Report of a case. Obstet Gynecol 1968;37:81.
7. Sherman D, Langer R, Sadovsky G, Bukovsky I, Caspi E. Improved fertility following ectopic pregnancy. Fertil Steril 1982;37:497.
8. Ory SJ, Villaneuva AL, Sand PK, Tamura RK. Conservative treatment of ectopic pregnancy with methotrexate. Am J Obstet Gynecol 1986;154:1299.
9. Ichinoe K, Wake N, Shinkai N, Shiina Y, Miatzaki Y, Tanaka T. Nonsurgical therapy to preserve oviduct function in patient with tubal pregnancies. Am J Obstet Gynecol 1987;156:484.
10. Ross GT. Congenital anomalies among children born to mothers receiving chemotherapy for gestational trophoblastic neoplasia. Cancer 1976;37:1043.
11. Linblom B, Kallfelt B, Hahlin M, Hamberger L. Local prostaglandins $F_{2\alpha}$ injection for termination of ectopic pregnancy. Lancet 1987;4:776.

Make researching easier with the Ten-Year Cumulative Index (1978-1987) to the American Journal of Obstetrics and Gynecology

The Ten-Year Cumulative Index is a complete guide to more than 30,000 pages of original articles, case reports, letters, and editorials published in the American Journal of Obstetrics and Gynecology from 1978 through 1987 (volumes 130 to 157). Numbering more than 800 pages, the Cumulative Index includes both a subject and an author index. This hard-cover volume is $64.50 ($69.00 international). Prices include shipping. *Payment must accompany all orders and must be in U.S. funds, drawn on a U.S. bank.*

Contact The C.V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146 USA; phone (314) 872-8370, ext. 394. In the U.S., call toll-free: 1-800-325-4177, ext. 394. Please allow six weeks for delivery.

Serial human chorionic gonadotropin determinations by fluoroimmunoassay for differentiation between intrauterine and ectopic gestation

Bo Lindblom, MD, PhD, Mats Hahlin, MD, and Peter Sjöblom, PhD

Göteborg, Sweden

A new means for differentiation between ectopic and early intrauterine pregnancy—the human chorionic gonadotropin score—is described. The score relates the rate of serum hCG rise per day to the initial human chorionic gonadotropin level. The positive predictive value for ectopic pregnancy was 94.7%, based on human chorionic gonadotropin scores from 41 women with increasing human chorionic gonadotropin levels in the range of 10 to 4000 IU/L. The method may be useful for identification of ectopic pregnancy in a category of women in whom ultrasonography is of limited diagnostic value. (Am J Obstet Gynecol 1989;161:397-400.)

Key words: Human chorionic gonadotropin, ectopic pregnancy, fluoroimmunoassay, diagnosis

Ectopic pregnancy can lead to massive intraabdominal bleeding; the danger increases with the gestational length.[1] Early recognition of ectopic pregnancy reduces the risk of medical emergency and makes it possible to use conservative therapy (e.g., laparoscopic surgery)[2] and medical treatment.[3, 4]

The proportion of cases that are seen early in the course of ectopic pregnancy is increasing in our population, probably as a result of a better knowledge among clinicians with regard to risk determinants[5, 6] and possibly also an increasing awareness among patients about ectopic pregnancy. Since ultrasonography is of limited diagnostic value in very early pregnancy,[7, 8] we have considered the use of serial human chorionic gonadotropin (hCG) assays as a single diagnostic tool in patients with "low" hCG levels (i.e., <4000 IU/L).

As in intrauterine pregnancy, the serum levels of hCG in ectopic pregnancy are related to the gestational length but the levels of hCG are usually lower.[9] Consequently, the mean rate of hCG increase in serum, the hCG slope, is lower in ectopic pregnancy.[7, 10]

Thus serial determination of hCG in early pregnancy can be used as a single tool to distinguish pathologic gestation from viable intrauterine pregnancies.[7, 10] However, these methods did not account for the fact that the hCG increase is not linear.[11, 12] The purpose of our study was to develop an improved method for early recognition of ectopic pregnancies that takes into account the nonlinear hCG increase.

From the Department of Obstetrics and Gynecology, University of Göteborg.

Supported by the Swedish Medical Research Council (grant 2019 and 8683) and the Göteborg Medical Society.

Received for publication April 14, 1988; revised February 15, 1989; accepted February 23, 1989.

Reprint requests: Bo Lindblom, MD, PhD, Department of Obstetrics and Gynecology, University of Göteborg, S-413 45 Göteborg, Sweden.

Material and methods

Serum hCG levels were determined in 181 women admitted to the hospital for suspicion of ectopic pregnancy. Many of these patients were asymptomatic, but because of anamnestic factors such as previous ectopic pregnancy or a history of infertility were considered at high risk for developing ectopic pregnancy.[6] The initial hCG value was <10 IU/L in 51 of these 181 women; these subjects were considered nonpregnant. Twenty-eight cases had initial hCG values >4000 IU/L and were excluded from further hormonal analyses and alternatively had ultrasonographic examination. Sixty of the remaining 102 women with initial hCG values of between 10 and 4000 IU/L had decreasing levels, excluding a viable pregnancy. The majority of the latter cases had curettage and, in cases of suspected ectopic pregnancy, diagnostic laparoscopy. The remaining 42 women had an initial hCG value of 10 to 4000 IU/L with rising levels and thus constituted a diagnostic problem. These women were carefully monitored clinically to establish the final diagnosis. One single case was excluded from further analysis because the diagnosis could not be settled.

The criteria for the diagnosis of normal intrauterine pregnancy (n = 22) were normal fetal development, including heart activity in the eighth gestational week, studied by endovaginal ultrasonography (Brüel and Kjaer, Naerum, Denmark). The diagnosis of ectopic

Table I. Serum hCG readings and slopes of rise for ectopic and intrauterine pregnancies

Patient	*Sampling interval (days)*							*Relative slope*	*Absolute slope*
	0	*+1*	*+2*	*+3*	*+4*	*+5*	*+6*		
Ectopic pregnancies									
1	103			109				0.02	2
2	877			940				0.02	21
3	3541	3677						0.04	136
4	186		203					0.05	8
5	849			1056				0.08	69
6	1034		1203					0.08	84
7	2265			2878				0.09	204
8	473				670			0.10	49
9	90	100						0.11	10
10	43	48						0.12	5
11	89			124				0.13	12
12	670		848					0.13	89
13	3098			4502				0.15	468
14	2193		2923					0.17	365
15	79	93						0.18	14
16	1511	1791						0.18	280
17	1157					3843		0.46	537
18	251			656				0.54	135
19	258		560					0.58	151
Mean	988							0.17	139
Intrauterine pregnancies									
20	2223						4330	0.16	351
21	2815		3965					0.20	575
22	3575			6273				0.25	899
23	849			1788				0.37	313
24	1075				2754			0.39	419
25	3688		7037					0.45	1674
26	1919				5621			0.48	926
27	3097		6130					0.50	1533
28	2232			5878				0.54	1215
29	1357		3173					0.67	908
30	1556			5010				0.74	1151
31	856					2942		0.80	695
32	1172			4003				0.80	943
33	586					3172		0.88	517
34	309			1175				0.93	289
35	620		1826					0.97	603
36	506					3172		1.05	533
37	758						6489	1.26	955
38	328					2702		1.45	475
39	332				2377			1.54	511
40	468						6850	2.27	1064
41	94						1698	2.84	267
Mean	1382							0.89	764

hCG values in international units per liter. Day 0 is initial value.

pregnancy (n = 19) was based on laparoscopy, in the majority of cases followed by laparotomy. Regardless of the operative method (laparoscopy or laparotomy), the presence of extrauterine trophoblast was confirmed by histopathologic examination. Information about the last menstrual bleeding is often misleading in patients with pathologic pregnancies because of spotting and for this reason we made no evaluation of gestational length.

hCG determinations. Blood sera, stored at −20° C for up to 7 days, were analyzed for hCG by the time-resolved fluoroimmunoassay described by Pettersson et al.[18] This nonisotopic immunoassay method involves a double antibody reaction with the second antibody labeled with europium. The sensitivity of the assay is approximately 0.5 IU/L; the upper limit for quantitation without dilution is approximately 8000 IU/L. Assay kits were purchased from Pharmacia Diagnostics, Sweden. The intraassay coefficient of variation was 5%. The interassay coefficient of variation was 8% (at 10 to 10,000 IU/L). The crossreactivity against human luteinizing hormone was less than 0.5%.

Computations and statistics. For each patient, the two hCG values were used in calculation of the slope of hCG rise.

The absolute slope was defined as the difference be-

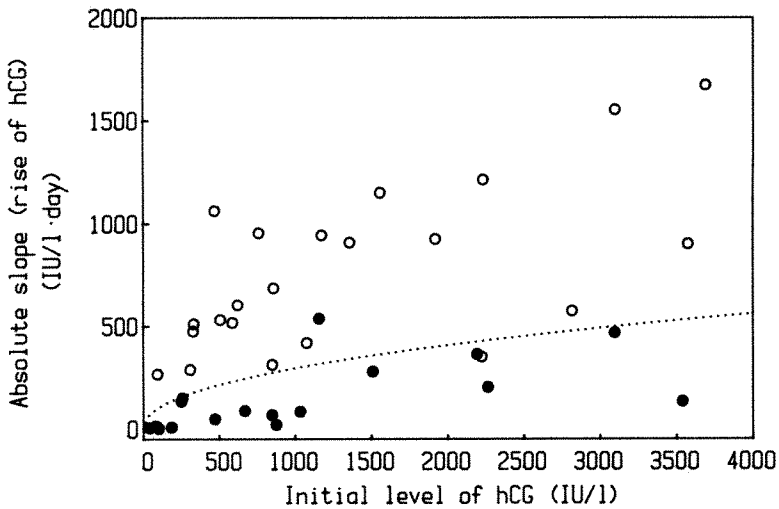


Fig. 1. Slope of serum hCG rise as function of initial hCG level. *Open circles,* Intrauterine pregnancies. *Filled circles,* Ectopic pregnancies. *Dotted line,* Discriminatory function (see text).

Table II. Positive predictive values for different methods of analyzing hCG data in early pregnancy

	Intrauterine pregnancy (n = 22)		*Ectopic pregnancy (n = 19)*	
Method	*%*	*No.*	*%*	*No.*
Absolute slope	95.4	21	84.2	16
Relative slope	95.4	21	84.2	16
hCG score	95.4	21	94.7	18

Table III. Cases incorrectly classified by either of the three methods for identification of ectopic pregnancy and intrauterine pregnancy

		Classification by		
Patient No.	*True diagnosis*	*Relative slope*	*Absolute slope*	*hCG score*
13	EP	EP	IUP	EP
14	EP	EP	IUP	EP
17	EP	IUP	IUP	IUP
18	EP	IUP	EP	EP
19	EP	IUP	EP	EP
20	IUP	EP	EP	EP

EP, Ectopic pregnancy; *IUP,* intrauterine pregnancy.

tween the final and the preceding ("initial") readings, divided by the time interval (days) between the sample collection: Absolute slope = final hCG − initial hCG/sample interval.

The relative slope of hCG rise was defined as the difference between the final and the initial hCG values divided by the initial value and the time interval (days) between the sample collection: Relative slope = final hCG − initial hCG/initial hCG × sample interval.

The hCG score is the data point obtained by plotting the initial hCG value against its corresponding absolute slope. To separate the two categories of patients (intrauterine or ectopic pregnancy), a discriminatory curve was drawn betwen the two clusters of data points.

The Mann-Whitney U test was used for statistical comparisons. A p value <0.05 was considered significant.

Results

The hCG readings for each of the patients are shown in Table I, which also includes the absolute and relative slopes for each patient. The 41 patients were ranked according to the value of the relative slope. The medians of the initial values for the two groups of patients were not significantly different. Conversely, the medians of both the absolute and relative slope differed significantly (Table I).

By use of the absolute slope to differentiate between normal intrauterine pregnancy and ectopic pregnancy, an hCG rise of 285 IU/L/day was found to give the best discrimination. Thus 95.4% (21 of 22) of normal intrauterine pregnancies had slopes above this limit, whereas 84.2% (16 of 19) of ectopic pregnancies showed an hCG rise below this value (Table I).

Similarly, at a discriminatory level of 0.19, the relative slope had a positive predictive value for normal intrauterine pregnancies and ectopic pregnancies of 95.4% (21 to 22) and 84.2% (16 to 19), respectively.

Fig. 1 shows both the hCG score and the discriminatory function for each patient. The equation of the discriminatory function was: $y = 12.31 \cdot x^{0.46}$, where y is the absolute slope and x is the initial hCG value. Twenty-one of the 22 intrauterine pregnancies had val-

ues above the discriminatory curve, whereas one was below. Of the ectopic pregnancies, 18 of 19 had values below the line and one above. Thus the positive predictive value for intrauterine pregnancy in the case of an hCG score above the discriminatory curve was 95.4%. Values below the discriminatory curve had a positive predictive value for ectopic pregnancy of 94.7%.

The positive predictive values of the three methods of analyzing hCG data are summarized in Table II. The two cases incorrectly classified by the hCG score were also incorrectly classified by the other two methods (Table III).

Comment

Compared with the absolute and relative slopes of hCG increase, the hCG score appears to give better discrimination between early viable intrauterine pregnancies and ectopic pregnancies. The hCG score seems to be of specific value for identification of ectopic pregnancies with relatively rapid rise in hCG values (case 13, 14, 18, 19). This is of particular interest because such ectopic pregnancies generally are associated with a dramatic clinical presentation, including a high risk of tubal rupture and massive intraabdominal bleeding.

Abdominal ultraasonographic examination has a great documented value for differentiation of pregnancies when hCG values are >6500 IU/L.[7] With an endovaginal ultrasonographic transducer, good accuracy is achieved at still lower hCG levels[8, 14]; we arbitrarily chose an upper limit of 4000 IU/L for the initial hCG value.

No spontaneous abortions were encountered in this group of early pregnancies. This is explained by the exclusion of all cases with falling hCG titers, although it is realized that a small proportion of miscarriages may occur with rising hCG levels for limited periods of time.[12]

When the sample interval is extended, an increasing number of ectopic pregnancies will exhibit a subnormal hCG rise and, hence, an hCG score below the discriminatory curve. Although extension of the interval provides better differentiation, this delays the recognition of ectopic pregnancies with the associated risks for acute hemorrhage. In our opinion, the sampling interval should be in the range of 1 to 2 days. Shorter sample intervals may be less reliable because of the normal fluctuation of the serum hCG concentration and the assay variability.

One of the 19 ectopic pregnancy cases could not be correctly classified as such with the discriminatory curve chosen. If the discriminatory curve was moved upward to include this woman, four instead of one woman with normal intrauterine pregnancies would have been incorrectly classified. Not surprisingly, an increased sensitivity thus implies a reduced specificity. This phenomenon is even more pronounced when the absolute and relative slopes are used.

We conclude that the hCG score appears to give clinically useful information to diagnose ectopic pregnancy in a group of women in whom ultrasonography is of limited diagnostic value.

REFERENCES

1. Lundström V, Bremme K, Eneroth P, Nygård I, Sundvall M. Serum beta-human chorionic gonadotrophin levels in the early diagnosis of ectopic pregnancy. Acta Obstet Gynecol Scand 1979;58:2.
2. Pouly JL, Mahnes H, Mage G, Canis M, Bruhat MA. Conservative laparoscopic treatment of 321 ectopic pregnancies. Fertil Steril 1986;46:1093.
3. Tanaka T, Hayashi H, Kutsuzawa T, Fujimoto S, Ichinoe K. Treatment of interstitial ectopic pregnancy with methotrexate: report of a successful case. Fertil Steril 1982;37:851.
4. Lindblom B, Källfelt B, Hahlin M, Hamberger L. Local prostaglandin $F_{2\alpha}$ injection for termination of ectopic pregnancy. Lancet 1987;1:776.
5. Thorburn J, Berntsson C, Philipson M, Lindblom B. Background factors of ectopic pregnancy. I. Frequency distribution in a case-control study. Eur J Obstet Gynecol Reprod Biol 1986;23:321.
6. Thorburn J, Philipson M, Lindblom B. Background factors of ectopic pregnancy. II. Risk estimation by means of a logistic model. Eur J Obstet Gynecol Reprod Biol 1986;23:333.
7. Romero R, Kadar N, Copel JA, Jeanty P, DeCherney AH, Hobbins JC. The value of serial human chorionic gonadotropin testing as a diagnostic tool in ectopic pregnancy. Am J Obstet Gynecol 1986;155:392.
8. Enk L, Wikland M, Hammarberg K, Lindblom B. Early recognition of ectopic pregnancy by means of vaginal ultrasound and semiquantitative HCG assays. In: Proceedings of the third meeting of the European Society of Human Reproduction and Embryology. Cambridge: European Society of Human Reproduction and Embryology, 1987:72.
9. Barnea ER, Oelsner G, Benveniste R, Romero R, DeCherney AH. Progesterone, estradiol and α-human chorionic gonadotropin secretion in patients with ectopic pregnancy. J Clin Endocrinol Metab 1986;62:529.
10. Cartwright PS, DiPietro D. Ectopic pregnancy: changes in serum human chorionic gonadotropin concentration. Obstet Gynecol 1984;63:76.
11. Pittaway DE, Reish RL, Wentz AC. Doubling times of human chorionic gonadotropin increase in early viable intrauterine pregnancies. Am J Obstet Gynecol 1985; 152:199.
12. Lenton EA, Neal LM, Sulaiman R. Plasma concentrations of human chorionic gonadotropin from the time of implantation until the second week of pregnancy. Fertil Steril 1982;37:773.
13. Pettersson K, Siitari H, Hemmilä I, et al. Time-resolved fluoroimmunoassay of human choriogonadotropin. Clin Chem 1983;29:60.
14. Fossum GT, Davajan V, Kletzky OA. Early detection of pregnancy with transvaginal ultrasound. Fertil Steril 1988;49:788.

Dandy-Walker syndrome: A review of fifteen cases evaluated by prenatal sonography

Paul D. Russ, MD,[a] Dolores H. Pretorius, MD,[b] and Mark J. Johnson, MD[c]

Denver, Colorado, San Diego, California, and Columbus, Ohio

Fifteen cases of the Dandy-Walker syndrome evaluated by prenatal sonography were reviewed retrospectively. A posterior fossa cyst communicating with the fourth ventricle was a feature in each case. Hydrocephalus was present in 53% of fetuses. Extracranial congenital malformations were documented in 60% of cases. Cardiac, genitourinary, gastrointestinal, and skeletal anomalies were noted. Of 12 available karyotypes, 4 (33%) were abnormal, including two cases of trisomy 18. Excluding terminated pregnancies, there was an overall mortality of 55%. Associated congenital defects contributed to 83% of the postnatal deaths. The Dandy-Walker syndrome can be accurately diagnosed in utero by sonographic demonstration of characteristic morphologic changes in the fetal posterior fossa. The prenatal examination should include an evaluation of associated supratentorial and extracranial defects. Coexisting structural and chromosomal anomalies occur frequently and adversely affect survival. (Am J Obstet Gynecol 1989;161:401-6.)

Key words: Fetus, ultrasonographic studies, central nervous system, Dandy-Walker syndrome

The Dandy-Walker syndrome is a congenital brain malformation involving the fourth ventricle and cerebellum.[1, 2] Cystic dilatation of the fourth ventricle is associated with aplasia or hypoplasia of the cerebellar vermis.[1, 3-5] Obstructive hydrocephalus is a common feature.[3, 4, 6]

The Dandy-Walker syndrome is estimated to complicate approximately one in 25,000 to 35,000 pregnancies.[6] The usefulness of prenatal sonography in the detection and diagnosis of the Dandy-Walker syndrome has been demonstrated.[1, 2, 5, 7, 8-10] We present 15 cases of the Dandy-Walker syndrome evaluated by sonography in utero. The purposes of this study were to further examine prenatal sonographic findings, to describe related intracranial and extracranial anomalies, and to review obstetric and neonatal outcome.

Material and methods

Fifteen fetuses with the Dandy-Walker syndrome were evaluated by prenatal sonography in our departments from 1980 through 1987. Sonograms and medical records were reviewed retrospectively. If several sonographic examinations had been performed during the pregnancy, data were compiled from the first available study. Subsequent scans were evaluated for significant longitudinal changes.

Sonograms were reviewed for fetal gestational age in menstrual weeks as determined by measurements of femur length and abdominal circumference. Biparietal diameter and head circumference measurements were noted. Head circumference measurements were compared with normal values compiled by Jeanty and Romero,[11] and macrocephaly was diagnosed if the head circumference exceeded the 95th percentile. The fetal posterior fossa was analyzed for features of the Dandy-Walker malformation, including: (1) a central cyst communicating with the fourth ventricle, (2) agenesis or dysgenesis of the vermis, and (3) splaying of the cerebellar hemispheres with anterolateral displacement against the tentorium (Fig. 1, *A* and *B*). The midline anteroposterior dimension of the posterior fossa cyst was measured from axial images (Fig. 1, *A*). Hydrocephalus and other intracranial structural abnormalities were noted (Fig. 1, *C*). Ventriculomegaly was graded subjectively as normal, mild, moderate, or severe. Extracranial congenital anomalies were recorded. Amniotic fluid volume was assessed subjectively as decreased (oligohydramnios), normal, or increased (polyhydramnios).

Maternal records were evaluated to establish the following: (1) the reason for sonography, (2) gestational age at delivery, and (3) mode of delivery. The clinical outcome was determined in each case. Autopsy reports and neonatal medical records were reviewed for confirmation of the Dandy-Walker syndrome. Associated structural and chromosomal anomalies were noted.

From the Department of Radiology, University of Colorado Health Sciences Center,[a] the Department of Radiology, University of California San Diego Medical Center,[b] and the Ohio State University College of Medicine.[c]

Received for publication September 7, 1988; revised January 17, 1989; accepted February 8, 1989.

Reprint requests: Paul D. Russ, MD, c/o Mary Montague, Department of Radiology A-030, University of Colorado Health Sciences Center, 4200 East Ninth Ave., Denver, CO 80262.

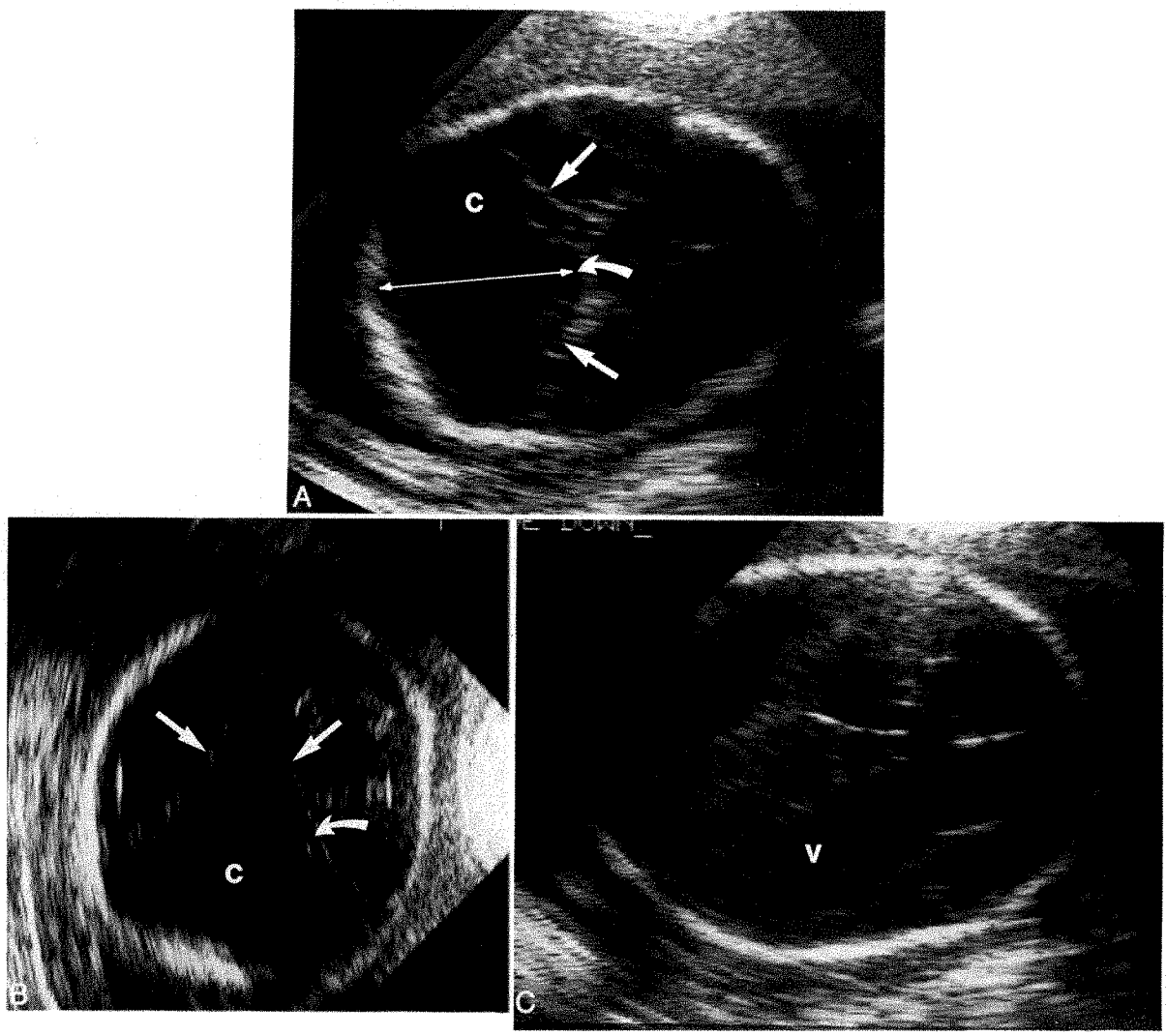


Fig. 1. Prenatal sonogram demonstrates intracranial features of Dandy-Walker syndrome in 28-week fetus. **A,** Axial scan through posterior fossa shows large cyst *(C)* communicating with fourth ventricle through defect of vermis *(curved arrow)*. Hypoplastic cerebellar hemispheres are anterolaterally displaced against tentorium *(straight arrows)*. Sagittal anteroposterior dimension of cyst *(double arrow)* measures 44 mm. **B,** Coronal scan through posterior fossa shows symmetric triangular Dandy-Walker cyst *(C)* and elevation of tentorium *(straight arrows)*. Note one of the rudimentary cerebellar hemispheres adjacent to tentorium *(curved arrow)*. **C,** Axial scan through cerebral hemispheres demonstrates moderate hydrocephalus with dilatation of the lateral ventricles *(V)*. Upside ventricle is obscured by artifact.

Results

Initial sonograms had been obtained for the following indications: size-dates discrepancy ($n = 6$), previous child with the Dandy-Walker syndrome ($n = 3$), premature labor ($n = 1$), premature rupture of membranes ($n = 1$), and routine gestational dating ($n = 1$). The reason for the initial scan was unknown in three cases. At the time of referral, the gestational age determined by sonography ranged from 22 to 36 weeks.

Posterior fossa morphologic features consistent with the Dandy-Walker syndrome were identified at the time of referral in 14 of 15 cases (93%). In one fetus with mild hydrocephalus at 24 weeks' gestation, the cerebellum and posterior fossa appeared normal, but a follow-up sonogram obtained 10 weeks later demonstrated features of the Dandy-Walker malformation (Fig. 2).

The anteroposterior dimension of the posterior fossa cyst ranged from 7 to 45 mm. This measurement was >10 mm in 14 of 15 cases (93%) at the time of referral. The single exception occurred in the fetus with a posterior fossa that appeared normal at initial examination. In this case the corresponding measurement was 7 mm at presentation, but as gestation pro-

gressed, it exceeded 10 mm before the vermian defect could be detected.

Ventriculomegaly was present in 8 of 15 fetuses (53%) and was graded as mild (n = 4), moderate (n = 2), or severe (n = 2). Macrocephaly was present in 3 of 15 fetuses (20%). In 4 fetuses, enlargement of the Dandy-Walker cyst and worsening hydrocephalus was documented by serial sonograms.

Oligohydramnios complicated 4 of 15 pregnancies (27%), including one case of premature rupture of the membranes. Polyhydramnios was present in 4 of 15 pregnancies (27%). Amniotic fluid volume was normal in 7 of 15 cases (46%).

Elective abortion was done in 3 pregnancies. An induced vaginal delivery with cephalocentesis performed in 1 case resulted in a stillbirth. The gestational age at delivery of nonterminated pregnancies ranged from 27 to 41 weeks. Eleven live births resulted from 3 vaginal deliveries and 8 cesarean sections.

There were 6 subsequent deaths among the 11 live births corresponding to a mortality rate of 55%. Five of the six deaths (83%) were secondary to coexisting extracranial congenital anomalies, whereas one postnatal death (17%) resulted from neurologic sequelae of the Dandy-Walker syndrome. Three neonatal deaths occurred within 5 days of delivery, whereas three infants died between 5 and 24 months of age.

Five survivors have been followed up from 2 to 60 months. Treatment has included neurosurgical shunting in 4 of these cases. Neurologic development has ranged from normal to severely delayed among the survivors.

Intracranial abnormalities occurring in conjunction with the Dandy-Walker syndrome noted at prenatal sonography, autopsy, or neonatal evaluation are listed in Table I. Extracranial malformations were documented in 9 of 15 cases (60%) and are summarized in Table II. Four of twelve available karyotypes (33%) are abnormal. Chromosomal anomalies included 2 cases of trisomy 18, a partial 11q trisomy, and an interstitial deletion of chromosome 2.

Comment

The pathogenesis of the Dandy-Walker syndrome was originally postulated to be congenital atresia of the foramina of Magendie and Luschka with obstructive hydrocephalus predominantly affecting the fourth ventricle.[12] Subsequent experimental and clinicopathologic studies suggested that the Dandy-Walker syndrome represents a complex developmental anomaly of the rhombencephalon rather than the sequelae of foraminal obstruction.[3, 4, 9, 13] Because of associated facial, cardiac, and limb anomalies, it has been suggested that the Dandy-Walker malformation originates before the sixth or seventh week of embryonic development.[4, 6, 14]

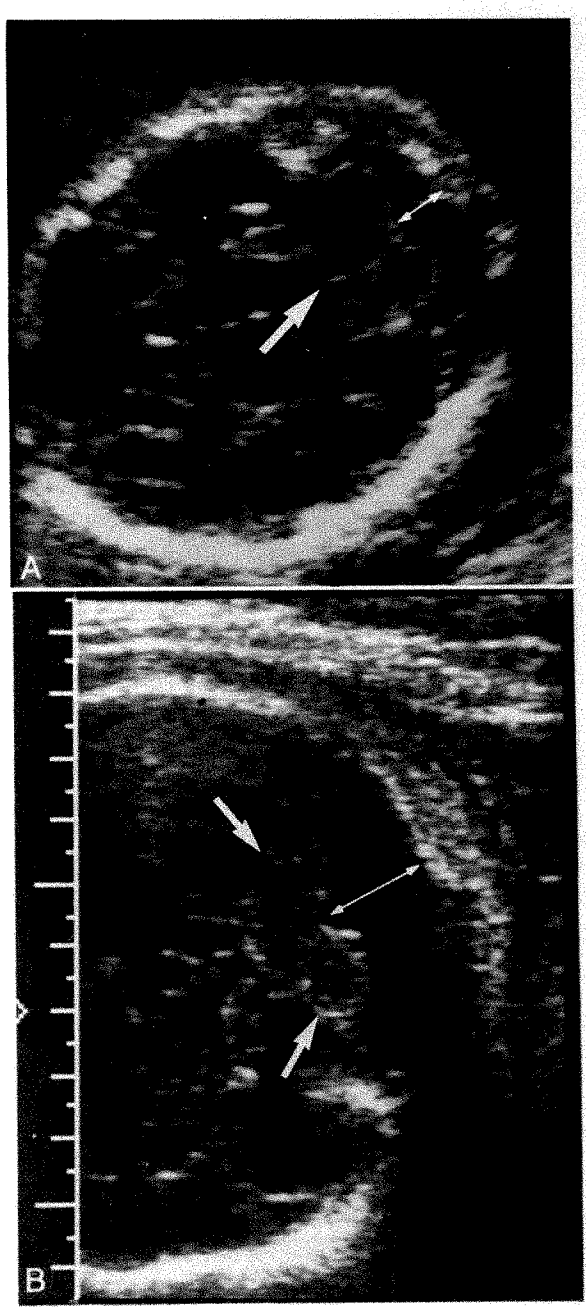


Fig. 2. Progressive morphologic changes in fetus with Dandy-Walker syndrome. **A,** Axial scan at level of cerebellum *(straight arrow)* demonstrates normal-appearing posterior fossa at 24 weeks' gestation. Sagittal depth of retrocerebellar fluid *(double arrow)* measures 7 mm and does not indicate presence of a cyst. **B,** Axial scan at 34 weeks demonstrates mild splaying of hypoplastic cerebellar hemispheres *(straight arrows)*. Abnormal midline measurement *(double arrow)* of 19 mm helps to confirm presence of posterior fossa cyst.

The prenatal diagnosis of the Dandy-Walker syndrome depends on the demonstration of a posterior fossa cyst communicating with the fourth ventricle through a defect of the cerebellar vermis (Fig. 1, *A*).[9] Although present from very early in gestation,[4, 6, 14]

Table I. Associated intracranial abnormalities

Malformation	*No. of cases*
Ventriculomegaly	8
Agenesis of corpus callosum	4
Aqueductal stenosis	1
Malformed inferior olivary nuclei	2
Malformed dentate nuclei	1
Abnormal gyral pattern	1
Heterotopic gray matter	1

Table II. Extracranial congenital abnormalities

Malformation	*No. of cases*
Cardiac	
Double-outlet right ventricle	1
Atrioventricular canal	1
Ventricular septal defect	4
Patent ductus arteriosus	2
Patent foramen ovale	2
Atrial septal defect	1
Genitourinary	
Obstructive uropathy	3
Urethral stenosis	1
Microphallus	2
Hypospadias	2
Cryptorchidism	2
Gastrointestinal	
Malrotation	1
Meckel diverticulum	1
Umbilical hernia	1
Miscellaneous	
Dysmorphic facies	5
Syndactyly	1
Clinodactyly	1
Cleft lip	1

small vermian defects can be difficult to detect in utero.[9] Additional findings variably manifested in the posterior fossa include separation, anterolateral displacement, and hypoplasia of the cerebellar hemispheres (Fig. 1, *A*), elevation of the torcula and tentorium (Fig. 1, *B*), and enlargement of the occiput.[1, 2, 7, 9, 12]

In our study posterior fossa abnormalities characteristic of the Dandy-Walker syndrome were noted in all cases. The spectrum of morphologic changes ranged from a subtle vermian defect to massive cystic dilatation of the fourth ventricle with minimal remnants of cerebellar tissue. In one fetus with hydrocephalus, the posterior fossa anatomy appeared normal at 24 weeks' gestation, but typical findings of the Dandy-Walker syndrome became apparent over the next 10 weeks of follow-up (Fig. 2).

Although hydrocephalus is usually described as a feature of the Dandy-Walker syndrome, it is not invariably present.[2, 4, 6] Hydrocephalus was demonstrated in only 53% of our cases. Comparable observations have been made by others. Pilu et al.[9] observed enlarged ventricles in 3 of 5 fetuses (60%) with the Dandy-Walker syndrome examined in utero, whereas Nyberg et al.[10] noted ventriculomegaly in 71% of 7 fetuses evaluated by prenatal sonography.

As noted by others [2, 4, 7, 15], the degree of ventricular dilatation can be variable among cases of the Dandy-Walker syndrome. In our study ventriculomegaly ranged from mild enlargement of only the third ventricle to severe dilatation of the lateral ventricles with pronounced thinning of the cerebral mantle. In one fetus with gross hydrocephalus, associated aqueductal stenosis was confirmed at autopsy. Macrocephaly was noted to correlate with moderate to severe ventriculomegaly.

The differential diagnosis of the Dandy-Walker syndrome includes retrocerebellar arachnoid cyst and prominent fetal cisterna magna.[7, 9, 13, 16] Because the normal depth of the fetal cisterna magna does not exceed 10 mm, measuring the sagittal dimension of a suspected posterior fossa cyst helps to establish the presence of an abnormality.[16] Unlike the Dandy-Walker malformation, arachnoid cyst and large cisterna magna are not associated with a vermian defect or other intrinsic cerebellar or cerebral abnormality.[1, 13, 16] Retrocerebellar arachnoid cysts can compress but do not communicate with the fourth ventricle.[7, 13, 15] Whereas a Dandy-Walker cyst is seen as a triangular midline fluid collection with symmetric splaying of the cerebellar hemispheres (Fig. 1, *B*), an arachnoid cyst can be asymmetrically positioned in the posterior fossa and tends to be rounded.[7, 15] Although these features can aid in diagnosis, the sonographic differentiation between the Dandy-Walker syndrome and a retrocerebellar arachnoid cyst can be difficult.[5, 7]

The Dandy-Walker syndrome has been associated with additional brain abnormalities in approximately 68% of cases.[4] These include aqueductal stenosis, gyral anomalies, heterotopias, and malformations of inferior olivary and dentate nuclei.[4, 12] Agenesis of the corpus callosum has been reported in 7% to 17% of cases.[4, 6, 15] Instances of the Dandy-Walker syndrome occurring with holoprosencephaly, occipital meningocele, and encephalocele have been described.[6, 9, 13, 15] In our study a variety of intracranial anomalies were documented (Table I), including agenesis of the corpus callosum in 4 cases.

Extracranial congenital anomalies have been associated with Dandy-Walker syndrome in approximately 25% of cases.[4] Facial abnormalities that have been reported include dysmorphism, cleft palate, and hemangioma.[4, 6, 9, 10] Congenital heart lesions that have been described include ventricular septal defect, patent foramen ovale, patent ductus arteriosus, pulmonary stenosis, pulmonary atresia, and coarctation.[6, 9, 14, 15, 17] More complex cardiac malformations have occurred, such as tetralogy of Fallot, Ebstein anomaly, hypoplastic right ventricle, transposition with single ventricle, and cor biloculare.[10, 14, 17] Associated skeletal abnormalities

have included polydactyly, syndactyly, vertebral segmentation anomalies, and lethal short-limbed dwarfism.[4, 6, 14, 17] Bowel malrotation, Meckel diverticulum, and urogenital malformations have occurred sporadically.[4, 12, 15, 17]

Extracranial structural anomalies were present in 60% of the fetuses that we studied. Cardiac, genitourinary, gastrointestinal, facial, and skeletal malformations were noted (Table II). Fetal echocardiography detected the double-outlet right ventricle and the atrioventricular canal. Obstructive uropathy secondary to urethral stenosis or abnormal external genitalia occurred in 3 fetuses. Oligohydramnios and pulmonary hypoplasia contributed to neonatal death in two of these cases.

The frequency of chromosomal defects in the Dandy-Walker syndrome is uncertain.[10] Hirsch et al.[6] noted two cases of trisomy 21 and one abnormality of chromosome 5, in their review of 40 pediatric patients. Nyberg et al.[10] reported one trisomy 18 and one mosaic trisomy 13 among 4 cases with chromosomal analysis. In our series 33% of 12 available karyotypes were abnormal, including 2 cases of trisomy 18, a partial 11q trisomy, and an interstitial deletion of chromosome 2. Although central nervous system malformations are uncommon in trisomy 18,[18] the Dandy-Walker syndrome has been associated with this chromosomal duplication syndrome.[10, 19]

Familial recurrences of the Dandy-Walker syndrome are infrequent. Although 3 of our cases had previously affected siblings, Murray et al.[19] have estimated the prevalence of the Dandy-Walker syndrome among sibs to be 2%. Hirsch et al.[6] reported only six instances of familial clustering among 300 cases compiled from the literature. Although not considered a hereditary disorder,[6] the Dandy-Walker syndrome can be a manifestation of certain autosomal recessive and X-linked dominant syndromes.[19] Excluding these disorders, the recurrence risk for the Dandy-Walker syndrome in subsequent pregnancies is approximately 1% to 5%.[19]

The natural history of the Dandy-Walker syndrome is not well known.[9, 10] As noted in this report and by others,[5, 7, 9] enlargement of the posterior fossa cyst and worsening hydrocephalus can be observed in utero. Since progressive changes are often slow, conservative management is usually possible.[8] However, severe or rapidly increasing ventriculomegaly can necessitate aggressive obstetric intervention.[9, 20] Of the cases not detected prenatally, 80% to 85% will present before 1 year of age with symptoms of hydrocephalus.[6, 15]

Overall mortality rates of 12% to 50% have been reported for the Dandy-Walker syndrome in the pediatric neurosurgical literature.[6] A mortality rate of 55% was noted for nonterminated pregnancies in our study. Excluding elective abortion, Nyberg et al.[10] documented a mortality of 67%. Fetal and neonatal deaths are often attributable to extracranial malformations.[10, 15] Associated congenital anomalies contributed to 83% of postnatal deaths among our cases.

The functional outcome for survivors is variable. Subnormal intelligence (IQ < 83) is manifested in 41% to 71%.[6, 15] More severe intellectual impairment has been observed in cases of agenesis of the corpus callosum.[15]

In conclusion, the Dandy-Walker syndrome can be diagnosed accurately with prenatal sonography by demonstrating a posterior fossa cyst communicating with the fourth ventricle through a defect of the cerebellar vermis.[9, 10] There is great variability of intracranial findings in fetuses with the Dandy-Walker syndrome. A sagittal measurement exceeding 10 mm helps to confirm the presence of a Dandy-Walker cyst. The sonographic examination, including fetal echocardiography, should be directed toward identifying additional supratentorial and extracranial anomalies. Follow-up sonograms are useful for monitoring cyst size and ventriculomegaly. Fetal karyotyping is recommended for chromosomal analysis.[9, 10, 19] The presence of multiple congenital defects adversely affects survival.

REFERENCES

1. Dempsey PJ, Koch HJ. In utero diagnosis of the Dandy-Walker syndrome: differentiation from extra-axial posterior fossa cyst. JCU 1981;9:403-5.
2. Newman GC, Buschi AI, Sugg NK, Kelly TE, Miller JQ. Dandy-Walker syndrome diagnosed in utero by ultrasonography. Neurology 1982;32:180-4.
3. Benda CE. The Dandy-Walker syndrome or the so-called atresia of the foramen Magendie. J Neuropathol Exp Neurol 1954;13:14-29.
4. Hart MN, Malamud N, Ellis WG. The Dandy-Walker syndrome: a clinico-pathological study based on 28 cases. Neurology 1972;22:771-80.
5. Hatjis CG, Horbar JD, Anderson GG. The in utero diagnosis of a posterior fossa intracranial cyst (Dandy-Walker cyst). AM J OBSTET GYNECOL 1981;140:473-5.
6. Hirsch JF, Pierre-Kahn A, Renier D, Sainte-Rose C, Hoppe-Hirsch E. The Dandy-Walker malformation: a review of 40 cases. J Neurosurg 1984;61:515-22.
7. Taylor GA, Sanders RC. Dandy-Walker syndrome: recognition by sonography. AJNR 1983;4:1203-6.
8. Serlo W, Kirkinen P, Heikkinen E, Jouppila P. Ante- and postnatal evaluation of the Dandy-Walker syndrome. Childs Nerv Syst 1985;1:148-51.
9. Pilu G, Romero R, De Palma L, et al. Antenatal diagnosis and obstetric management of Dandy-Walker syndrome. J Reprod Med 1986;31:1017-22.
10. Nyberg DA, Cyr DR, Mack LA, Fitzsimmons J, Hickok D, Mahony BS. The Dandy-Walker malformation: prenatal sonographic diagnosis and its clinical significance. J Ultrasound Med 1988;7:65-71.
11. Jeanty P, Romero R. Obstetrical ultrasound. New York: McGraw-Hill, 1984:88.
12. Taggart JK, Walker AE. Congenital atresia of the foramens of Luschka and Magendie. Arch Neurol Psychiatry 1942;48:583-612.
13. Raybaud C. Cystic malformations of the posterior fossa: abnormalities associated with the development of the roof of the fourth ventricle and adjacent meningeal structures. J Neuroradiol 1982;9:103-33.

14. Olson GS, Halpe DCE, Kaplan AM, Spataro J. Dandy-Walker malformation and associated cardiac anomalies. Childs Brain 1981;8:173-80.
15. Sawaya R, McLaurin RL. Dandy-Walker syndrome: clinical analysis of 23 cases. J Neurosurg 1981;55:89-98.
16. Mahony BS, Callen PW, Filly RA, Hoddick WK. The fetal cisterna magna. Radiology 1984;153:773-6.
17. Moerman Ph, Vandenberghe K, Fryns JP, Haspeslagh M, Lauweryns JM. A new lethal chondrodysplasia with spondylocostal dysostosis, multiple internal anomalies and Dandy-Walker cyst. Clin Genet 1985;27:160-4.
18. Passarge E, True CW, Sueoka WT, Baumgartner NR, Keer KR. Malformations of the central nervous system in trisomy 18 syndrome. J Pediatr 1966;69:771-8.
19. Murray JC, Johnson JA, Bird TD. Dandy-Walker malformation: etiologic heterogeneity and empiric recurrence risks. Clin Genet 1985;28:272-83.
20. Depp R, Sabbagha RE, Brown JT, Tamura RK, Reedy NJ. Fetal surgery for hydrocephalus: successful in utero ventriculoamniotic shunt for Dandy-Walker syndrome. Obstet Gynecol 1983;61:710-4.

The frequency and management of uterine perforations during first-trimester abortions

Steven G. Kaali, MD, Ivan A. Szigetvari, MD, PhD, and George S. Bartfai, MD, PhD
Dobbs Ferry, New York

The frequency and management of uterine perforation during first-trimester abortions remain a matter of continuing debate among gynecologists. The rate of uterine perforations was 1.3/1000 procedures (eight cases) in 6408 women undergoing first-trimester abortions at our clinic. We also performed 706 first-trimester abortions at the time of laparoscopic sterilization. Two perforations (2.8/1000 procedures) were reported before laparoscopy. Twelve (15.6/1000 procedures) unsuspected perforations were discovered during direct laparoscopic visualization. This represents a 19.8/1000 procedure rate of perforation (14 cases). All 22 patients with perforations were managed conservatively, and no immediate or late complications were noted. Our data suggest that the true incidence of uterine perforations is significantly underestimated and serious complications caused by perforations are rare. Conservative therapy is recommended rather than early surgical intervention. (Am J Obstet Gynecol 1989;161:406-8.)

Key words: Abortion, uterine perforation, management

First-trimester abortion is a simple and commonly performed procedure. However, complications, including uterine perforations, occur. The incidence of uterine perforation varies between 0.8 and 6.4/1000 procedures.[1, 2]

Uterine perforations are usually diagnosed as a result of physician suspicion. Definite diagnosis can be made on rare occasions when abdominal contents are brought out through the cervix or invading instruments reach beyond the uterine cavity. According to Mittal and Misra,[3] unrecognized perforations are rather common, and the true incidence of uterine perforations is underestimated without direct pelvic visualization. White et al.[4] described 19 unsuspected uterine perforations during 624 laparoscopic sterilizations, making the frequency 30/1000 procedures. Similar conclusions were reached by Chi and Feldblum[5] during 20,000 laparoscopic and minilaparotomy sterilizations.

The frequency of and therapy for uterine perforations remain a matter of continuing debate among gynecologists. This propective study describes our experiences with the rate of recognized and unsuspected uterine perforations and their management.

Material and methods

Between Jan. 1, 1986, and Dec. 31, 1987, 6408 first-trimester abortions, including termination of three molar gestations, were performed by vacuum aspiration at our free-standing surgical facility. The average age of the patients was 21 years, and parity was 0.7 in this study group. All procedures were performed on an outpatient basis either under general anesthesia (89%) or paracervical block (11%). The cervix was gradually dilated with Pratt dilators without uterine sounding, and the products of conception were evacuated by plastic suction cannula. This was followed by sharp curettage to ascertain that the uterine cavity was empty. In

From the Women's Medical Pavilion.
Received for publication November 6, 1988, revised February 23, 1989, accepted March 3, 1989.
Reprint requests: Steven G. Kaali, MD, Women's Medical Pavilion, 88 Ashford Ave., Dobbs Ferry, NY 10522.

cases of continuous bleeding, oxytocin was used in lieu of repeat curettage.

An additional 706 first-trimester abortions were performed with the same method at the time of laparoscopic sterilization. The average age of the patients in this group was 35 years and parity was 2.3. General anesthesia was used in 687 (97.3%) cases and local anesthesia was used in 19 (2.7%) cases. The largest Pratt dilator used for dilatation was reinserted just beyond the internal os of the cervix. It was securely attached to the tenaculum with adhesive tape to mobilize the uterus. Tubal sterilizations were carried out through a single incision by means of bipolar cautery or Falope rings with the aid of video laparoscopy. The procedures were performed by four experienced surgeons.

In the abortion group, uterine perforations were diagnosed by physician suspicion after an instrument was passed beyond the expected distance. This perforation rate was compared with those described in the literature.

In the abortion-sterilization group, the surgeons were asked to report perforations before laparoscopy. The reported rate of uterine perforations could then be compared with those recognized only during laparoscopy.

The p values < 0.05 were considered significant. Calculation of statistical significance between the abortion and laparoscopy-abortion groups was made by the χ^2 test.

Results

Eight uterine perforations were reported during 6408 first-trimester abortions, resulting in a perforation rate of 1.3/1000 procedures. The average age of these patients was 22 years and parity was 0.8. Six of the eight perforations were sustained under general anesthesia.

When the abortions were performed at the time of laparoscopic sterilizations, two perforations were reported before direct visualization (2.8/1000 procedures). Twelve additional perforations were recognized only through the laparoscope. Thus the true incidence of uterine perforations in the laparoscopic group was 14 (two + 12) per 706 cases, or 19.8/1000 procedures. This is sevenfold higher ($p < 0.001$) than the perforation rate recognized with traditional methods. The average age of these 14 patients was 36 years and parity was 2.2. All received general anesthesia. The location of perforations and uterine positions are shown in Table I. Most perforations occurred in the fundus.

Of 22 uterine perforations, three were recognized before and two were discovered during the suction procedure. In five patients the perforation became evident at the time of curettage. Twelve perforations went undetected until laparoscopic sterilization, and the perforating instrument could not be identified.

Table I. Uterine position and location of perforations in the abortion-laparoscopy group

	Uterine position	
Site of perforations	*Anteverted, No.*	*Retroverted, No.*
Fundus or cornua	5	4
Anterior wall	1	3
Posterior wall	1	—
Cervix	—	—

In the 10 patients in whom perforations were recognized without laparoscopy, abortions were completed under sonographic control. In the 12 patients in whom unsuspected uterine perforations were diagnosed at the time of laparoscopic sterilization, no further treatment was required. All 22 patients were discharged on antibiotics an average of 4 hours after the procedure and returned to their normal activities in 48 hours. With the exception of one woman, all returned for postoperative checkup within 2 weeks, which revealed a benign postoperative course and normal pelvic findings.

Comment

The rates of recognized perforations in our abortion group and abortion-sterilization group were 1.3 and 2.8/1000 procedures, respectively, which are similar to those reported in the literature. Twelve additional perforations (15.6/1000 cases) were discovered only during laparoscopic sterilizations, resulting in a true uterine perforation rate of 19.8/1000 procedures in this study group. The secure placement of the Pratt dilator during uterine manipulation prevented accidental perforations. Therefore most traumatic uterine perforations during first-trimester abortions are unreported or even unsuspected. Similar conclusions were reached by White et al.[3] during diagnostic curettage in patients who had subsequent laparoscopy.

On the basis of these data, it is clear that most uterine perforations inadvertently escape medical attention and seldom result in serious complications. We would not deny that serious injuries can be sustained at a time of uterine perforations, but we disagree with Lauersen and Birnbaum[6] and Mittal and Misra's[3] suggestion that all patients with uterine perforations should immediately have laparoscopy or even laparotomy.

If the perforation occurs while the gestational tissue is still present in the uterine cavity, the abortion should be completed under sonographic guidance, avoiding more invasive surgical procedures. If intraoperative ultrasound studies are unavailable or the surgeon is in-

experienced with suction curettage, laparoscopic control is indicated to complete the abortion. Of course, these patients need careful surveillance after surgery.

Our data also confirmed the previously known fact that general anesthesia, advanced age, and parity have been associated with increased incidence of uterine perforations during abortion. However, surgical experience and proper technique can reduce the risk of the perforation, even in these high-risk cases.[7] We recommend a preoperative linear or vaginal ultrasound scanning and careful bimanual pelvic examination under suitable anesthesia. We suggest the use of Pratt dilators without uterine sounding. The suction cannula should only be passed through the internal os of the cervix, and the conceptus should be sucked from this position. The curettage should always be gentle and only to confirm the intended result. An optimally evacuated uterus after abortion seldom bleeds, and the use of uterotonics is recommended rather than repeat over-zealous curettage. Reinsertion of the Pratt dilator for uterine mobilization before laparoscopy should not increase the risk of perforation if it is securely attached to the tenaculum and not passed significantly beyond the internal os of the cervix.

In conclusion, we feel that most uterine perforations during first-trimester abortions go unrecognized and untreated. Therefore we suggest restraint and observation in managing recognized perforations, unless intra-abdominal contents have been brought through the cervix. Careful postoperative monitoring in the recovery room and instructions after discharge will help identify patients requiring more invasive treatment. Our experiences suggest that premature exploratory laparotomies after uterine perforations are unnecessary. We recognize that in the current medicolegal climate, it is a hard decision to make, but a well-balanced judgment in this critical time period on the part of the physician will benefit the overwhelming majority of young women seeking first-trimester abortions.

REFERENCES

1. Nathanson BN. Management of uterine perforations suffered at elective abortion. Am J Obstet Gynecol 1972; 114:1054.
2. Moberg PJ. Uterine perforation in connection with vacuum aspiration in legal abortion. Int J Gynecol Obstet 1976; 14:77.
3. Mittal S, Misra SL. Uterine perforation following medical termination of pregnancy by vacuum aspiration. Int J Gynecol Obstet 1985;23:45.
4. White MK, Ory HW, Goldenberg LA. A case-control study of uterine perforations documented at laparoscopy. Am J Obstet Gynecol 1977;129:623.
5. Chi IC, Feldblum P. Uterine perforation during sterilization by laparoscopy and minilaparotomy. Am J Obstet Gynecol 1981;139:735.
6. Lauersen NH, Birnbaum S. Laparoscopy as a diagnostic and therapeutic technique in uterine perforations during first-trimester abortions. Am J Obstet Gynecol 1977; 117:522.
7. Grimes DA, Shulz KF, Cates W. Prevention of uterine perforation during curettage abortion. JAMA 1984;251:2108.

DEPARTMENT OF OBSTETRICS AND GYNECOLOGY
UNIVERSITY OF WISCONSIN MEDICAL SCHOOL, MILWAUKEE CLINICAL CAMPUS AT SINAI SAMARITAN MEDICAL CENTER

Excellent opportunities for **MATERNAL FETAL MEDICINE** and **GENERAL OBSTETRICS AND GYNECOLOGY** with an interest in **AMBULATORY CARE, UROGYNECOLOGY,** or **STATEWIDE FAMILY PLANNING.** Sinai Samaritan Medical Center is the largest obstetrical unit (4,800 deliveries) in Wisconsin and is a tertiary care referral center in downtown Milwaukee. The University of Wisconsin Medical School, Milwaukee Clinical Campus, supports an active ob/gyn department with consultative ultrasound, genetic, infectious disease and reproductive/endocrine services.

Qualifications include board eligibility or certification in Obstetrics and Gynecology and/or subspecialty. Commitment to teaching residents, medical students, consultative services and clinical research.

Salary open and dependent on qualifications and experience. For more information, interested candidates should contact:

Fredrik F. Broekhuizen, M.D.
Chairman and Chief
Department of Obstetrics and Gynecology
University of Wisconsin Medical School
Milwaukee Clinical Campus
Sinai Samaritan Medical Center
Mount Sinai Campus
950 North 12th Street, G405
Milwaukee, WI 53233
(414) 283-7931

2V MEDTECH INTERNATIONAL CORP.

DIAGNOSIS TREATMENT

2V STAT™

PRESENTS

2V STAT Medical Decision Support Software Covering 69 Specialities – Algorithms and Text.

PLUS

COMPUTER 80286/10 MHz Turbo CPU, 80 MB HD, EGA Color Monitor, Color Graphic Card, 40 MB Back-up Drive, and Printer.

$5962
(for complete system)

ORDER NOW!
1•800•228•STAT
Technical Support Call 1•404•956•1855

Add volume to your practice and make an extra $500K with only a $5,962 one-time investment with 2V STAT including managerial support, brochures, +/- one-day seminar.

2480 Windy Hill Road, Suite 201 • Marietta, Georgia 30067
Fax 404•956•7068 Telex 551341 MedVideo Easy Link 622779660

Obstetrics and Gynecology
Maternal/Fetal Medicine Specialist

The Western Pennsylvania Hospital is seeking a second BC/BE specialist in Maternal/Fetal Medicine to join a rapidly growing division of Maternal/Fetal Medicine in a 550 bed tertiary care center. This is a unique opportunity in that major teaching, clinical and research opportunities are available in this university affiliated center. A fully accredited independent Ob/Gyn Residency training program is in place. This is a level III tertiary care center with a 30 bed neonatal intensive care unit. The Maternal/Fetal Medicine service accepts high risk maternal transports from throughout the tri-state area. A full range of genetic services are also performed at the institution, including chorionic villi sampling, amniocentesis, and genetic counseling. A research building is currently being built, and opportunities are available for both basic and clinical research. This position offers a state of the art practice opportunity, an attractive compensation package, and the chance for one to be creative. Send your CV to:

Mark Caine, M.D.
Director, Maternal/Fetal Medicine
Department of Obstetrics and Gynecology
West Penn Hospital
4800 Friendship Avenue
Pittsburgh, PA 15224

Maternal-Fetal Medicine

The Department of Obstetrics and Gynecology at the University of Tennessee Medical Center at Knoxville, Tennessee is currently accepting applications for an additional Maternal-Fetal Medicine Specialist.

This position requires Board certification or eligibility in Maternal-Fetal Medicine. Responsibilities include resident and medical student education, participation in Regionalization Program and clinical or basic research. Specialized areas of interest such as critical care, ultrasonography and Doppler studies, and prenatal diagnosis are encouraged. A private practice opportunity is available.

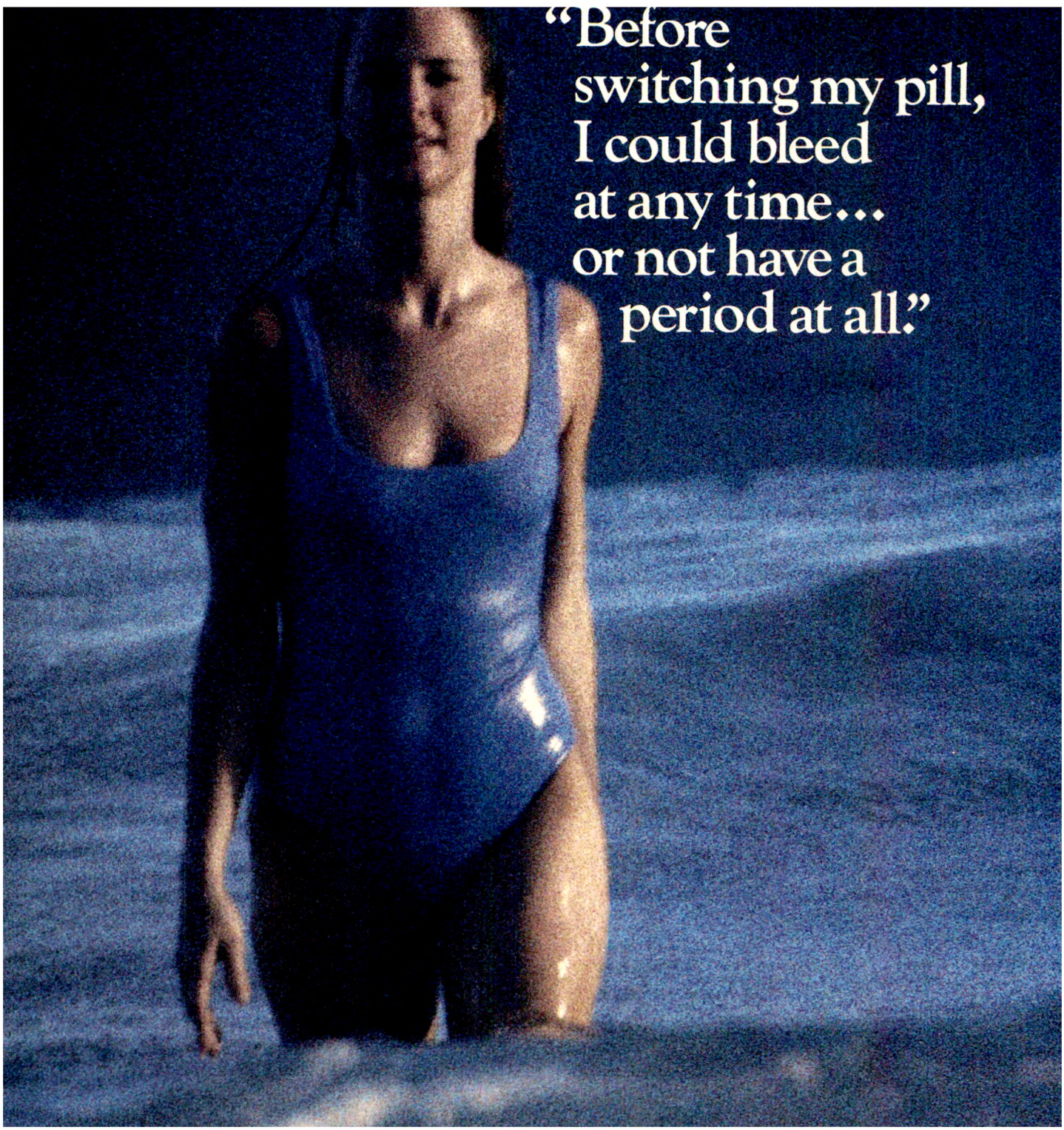
Academic rank is commensurate with qualifications and experience.

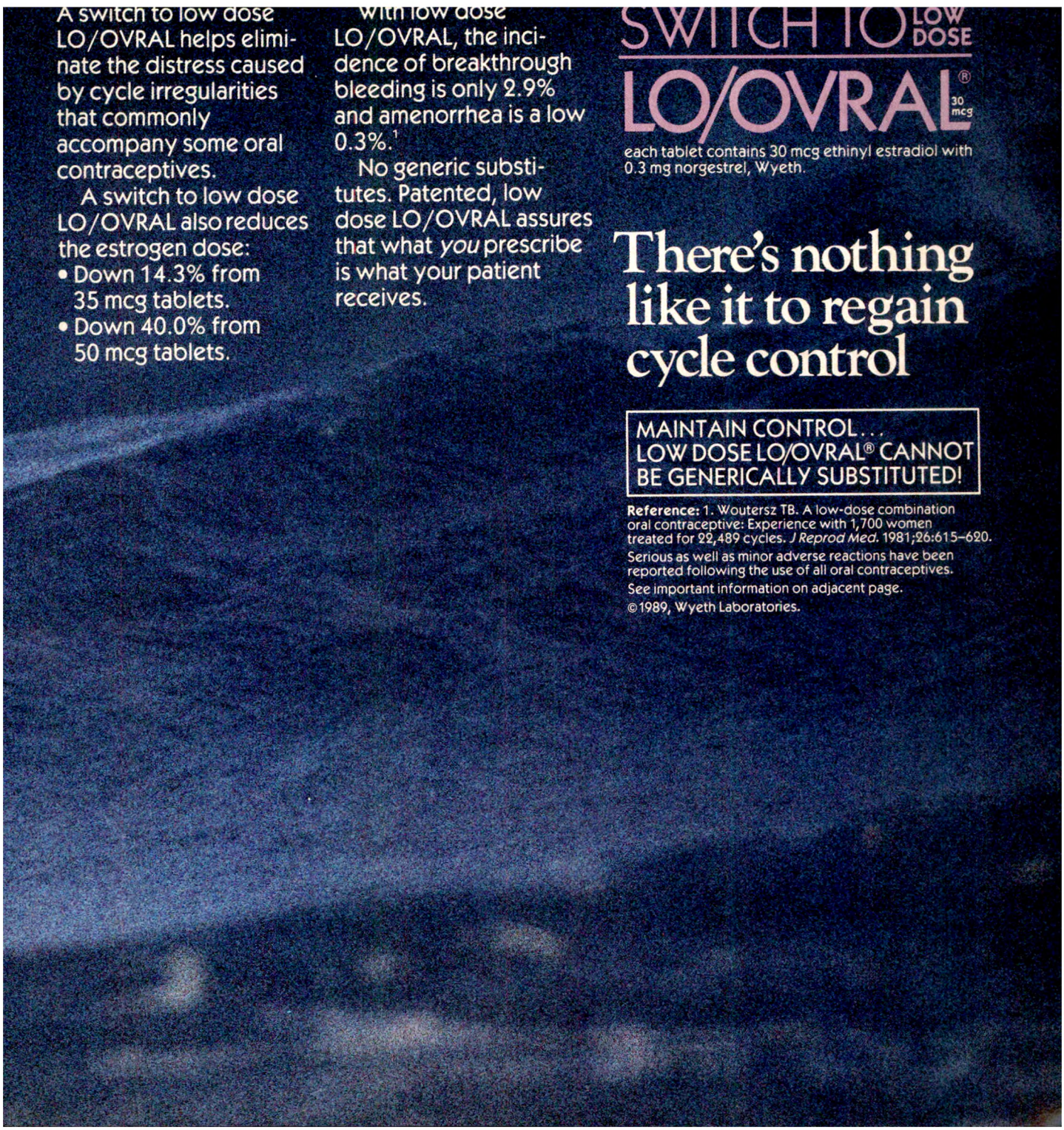
Interested persons should send curriculum vitae to:

Michael R. Caudle, M.D.
Associate Professor and Gynecology
Department of Obstetrics and Gynecology
For more information call (615) 544-9306

THE UNIVERSITY OF TENNESSEE
Medical Center at Knoxville

1924 ALCOA HIGHWAY
KNOXVILLE, TENNESSEE 37920
EEO/An Affirmative Action/Title IX/Section 504 Employer


"Before
switching my pill,
I could bleed
at any time...
or not have a
period at all."


A switch to low dose LO/OVRAL helps eliminate the distress caused by cycle irregularities that commonly accompany some oral contraceptives.
A switch to low dose LO/OVRAL also reduces the estrogen dose:
• Down 14.3% from 35 mcg tablets.
• Down 40.0% from 50 mcg tablets.
With low dose LO/OVRAL, the incidence of breakthrough bleeding is only 2.9% and amenorrhea is a low 0.3%.[1]
No generic substitutes. Patented, low dose LO/OVRAL assures that what *you* prescribe is what your patient receives.
SWITCH TO LOW DOSE
LO/OVRAL® 30 mcg
each tablet contains 30 mcg ethinyl estradiol with 0.3 mg norgestrel, Wyeth.
There's nothing like it to regain cycle control
MAINTAIN CONTROL... LOW DOSE LO/OVRAL® CANNOT BE GENERICALLY SUBSTITUTED!
Reference: 1. Woutersz TB. A low-dose combination oral contraceptive: Experience with 1,700 women treated for 22,489 cycles. *J Reprod Med.* 1981;26:615–620.
Serious as well as minor adverse reactions have been reported following the use of all oral contraceptives.
See important information on adjacent page.
©1989, Wyeth Laboratories.

IN BRIEF:

Indications and Usage—LO/OVRAL® is indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OC's) as a method of contraception.

Contraindications—OC's should not be used in women with any of the following conditions:

1. Thrombophlebitis or thromboembolic disorders.
2. A past history of deep-vein thrombophlebitis or thromboembolic disorders.
3. Cerebral-vascular or coronary-artery disease.
4. Known or suspected carcinoma of the breast.
5. Known or suspected estrogen-dependent neoplasia.
6. Undiagnosed abnormal genital bleeding.
7. Known or suspected pregnancy (see Warning No. 5).
8. Benign or malignant liver tumor which developed during use of OC's or other estrogen-containing products.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems*—An increased risk of thromboembolic and thrombotic disease associated with use of OC's is well established. Three principal studies in Great Britain and 3 in the U.S. have demonstrated increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OC's are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION (MI)—An increased risk of MI associated with use of OC's has been reported, confirming a previously suspected association. These studies, conducted in the UK, found, as expected, that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclamptic toxemia) the higher the risk of developing MI, regardless of whether the patient was an OC user or not. OC's, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to MI) are about twice as likely to have a fatal MI as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, amount of smoking is also an important factor. In determining importance of these relative risks, however, baseline rates for various age groups must be given serious consideration. Importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; quite likely the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE—In an analysis of data derived from several national adverse-reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to dose of estrogen in OC's. Preparations containing 100 mcg or more of estrogen were associated with higher risk of thromboembolism than those containing 50-80 mcg. Their analysis did suggest, however, that quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the U.S.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the UK estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OC's according to age, smoking habits, and duration of use. Overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), risk being concentrated in older women, in those with long duration of use, and in cigarette smokers. It was not possible, however, to examine interrelationships of age, smoking, and duration of use, nor to compare effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess magnitude of relative risk for this younger group. Available data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates of risk of death for each method include combined risk of contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OC's) plus risk attributable to pregnancy or abortion in event of method failure. This latter risk varies with effectiveness of method. The study concluded that mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OC's in women over 40 who smoke. Lowest mortality is associated with condom or diaphragm backed up by early abortion. Risk of thromboembolic and thrombotic disease associated with OC's increases with age after about 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of pre-eclamptic toxemia, and especially cigarette smoking. Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A 4- to 6-fold increased risk of postsurgery thromboembolic complications has been reported in OC users.

If feasible, OC's should be discontinued at least 4 weeks before surgery of a type associated with increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Britain involving cerebrovascular disease and another in the U.S. concerning MI suggest an increased risk of these conditions in users of OC's persists after discontinuation of the OC's. In the British study, risk of cerebrovascular disease remained elevated in former OC users for at least 6 years after discontinuation. In the U.S. study, increased risk of MI persisted for at least 9 years in women 40- to 49-years-old who had used OC's for 5 or more years. Findings in both studies require confirmation since they are inconsistent with other published information.

2. *Ocular Lesions*—There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with use of OC's. Discontinue OC's if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. *Carcinoma*—Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in OC's, have been noted to increase incidence of mammary nodules, benign and malignant, in dogs. In humans, 3 case-control studies have reported an increased risk of endometrial carcinoma associated with prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OC's. Of cases found in women without predisposing risk factors (e.g., irregular bleeding at the time OC's were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These are no longer marketed. No evidence has been reported suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only OC's. Several studies have found no increase in breast cancer in women taking OC's or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women on OC's, found an excess risk in subgroups of OC users with documented benign breast disease. Reduced occurrence of benign breast tumors in users of OC's has been well documented. In summary, there is at present no confirmed evidence from human studies of increased risk of cancer associated with OC's. Close clinical surveillance of all women on OC's is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or abnormal mammograms should be monitored with particular care if they elect to use OC's.

4. *Hepatic Tumors*—Benign hepatic adenomas have been found to be associated with use of OC's. One study showed that OC's with high hormonal potency were associated with higher risk than lower potency OC's. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users. Two studies relate risk with duration of use of OC's, the risk being much greater after 4 or more years' use. While hepatic adenoma is rare, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women on OC's. Relationship of these drugs to this type of malignancy is not known.

5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring*—Use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have increased risk of developing in later life a form of vaginal or cervical cancer ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence now that OC's further enhance risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OC's. Furthermore, 30 to 90% of such exposed women have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with use of other estrogens, it cannot be presumed they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with use of sex hormones, including OC's, in pregnancy. One case control study estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OC's, hormonal withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some exposures involved only a few days. Data suggest that risk of limb-reduction defects in exposed fetuses is somewhat less than 1 in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OC's. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OC's is unknown. It is recommended that, for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing OC's. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at time of first missed period, and further use of OC's should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and advisability of continuation of the pregnancy should be discussed. It is also recommended that women who discontinue OC's with intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this. The administration of progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease*—Studies report increased risk of surgically confirmed gallbladder disease in users of OC's and estrogens. In one study, increased risk appeared after 2 years' use and doubled after 4 or 5 years' use. In one of the other studies, increased risk was apparent between 6 and 12 months' use.

7. *Carbohydrate and Lipid Metabolic Effects*—Decrease in glucose tolerance has been observed in a significant percentage of patients on OC's. For this reason, prediabetic and diabetic patients should be carefully observed while on OC's. Increase in triglycerides and total phospholipids has been observed in patients on OC's; clinical significance of this finding remains to be defined.

8. *Elevated Blood Pressure*—Increase in blood pressure has been reported in patients on OC's. In some women, hypertension may occur within a few months of beginning OC's. In the 1st year of use, prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. Prevalence in users increases, however, with longer exposure, and in the 5th year of use is 2½ to 3 times the reported prevalence in the 1st year. Age is also strongly correlated with development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure on OC's. Hypertension that develops as a result of taking OC's usually returns to normal after discontinuing the drug.

9. *Headache*—Onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of OC's and evaluation of the cause.

10. *Bleeding Irregularities*—Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing OC's. In breakthrough bleeding, as in all cases of irregular vaginal bleeding, nonfunctional causes should be borne in mind.

In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another OC may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase risk of thromboembolic disease. Women with past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuing OC's. Women with these pre-existing problems should be advised of this possibility and encouraged to use other methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. *Ectopic Pregnancy*—Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. *Breast-feeding*—OC's given in the postpartum period may interfere with lactation and decrease quantity and quality of breast milk. Furthermore, a small fraction of the hormones in OC's has been identified in the milk of mothers on OC's; effects, if any, on the breast-fed child have not been determined. If feasible, defer OC's until infant has been weaned.

Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OC's. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Pap smear and relevant laboratory tests.

As a general rule OC's should not be prescribed for longer than 1 year without another physical examination.

2. Under influence of estrogen-progestogen preparations, pre-existing uterine leiomyomata may increase in size.

3. Patients with history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while on OC's should stop OC's and use an alternate method to try to determine whether the symptom is drug-related.

4. OC's may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency.

5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while on OC's. If jaundice develops, OC's should be discontinued.

6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution.

7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. Clinical significance is undetermined.

8. Serum folate levels may be depressed by OC's. Since the pregnant woman is predisposed to development of folate deficiency and incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OC's, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency.

9. The pathologist should be advised of OC therapy when revelant specimens are submitted.

10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OC's: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test.

Information for the Patient—See Patient Package Labeling.

Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Carcinogenesis—See Warnings on carcinogenic potential of OC's.

Pregnancy—Category X. See Contraindications, Warnings.

Nursing Mothers—See Warnings.

Adverse Reactions—An increased risk of these serious adverse reactions has been associated with use of OC's (see Warnings): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies.

There is evidence of an association between the following conditions and use of OC's although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients on OC's and are believed to be drug-related: nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea, amenorrhea during and after treatment, temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement, and secretion; change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening), intolerance to contact lenses.

The following adverse reactions have been reported in users of OC's, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, hemolytic uremic syndrome.

Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OC's by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

2/27/85 3209-3 **54541**

LO/OVRAL® each tablet contains 0.3 mg norgestrel with 0.03 mg ethinyl estradiol.

WYETH AYERST™

Histologic and immunologic study of uterine biopsy tissue of women with incipient abortion

Magdy Michel, MB, MRCOG,[a] Jenny Underwood, BSc, MA,[b] David A. Clark, MD, PhD, FRCPC,[c] James F. Mowbray, MD, FRCP,[b] and Richard W. Beard, MD, FRCOG[a]

London, England, and Hamilton, Ontario, Canada

Biopsy specimens taken from the region of the placental bed were examined for the presence of phloxinophilic granulated mononuclear cells in women with a history of recurrent miscarriage and who would eventually miscarry a current pregnancy. They were compared with biopsy specimens from women with intact pregnancies presenting for elective termination of pregnancy and those with "missed abortion." Cells with large cytoplasmic granules (≥1 μm) were abundant in the group of ongoing pregnancies whereas cells with smaller granules (<1 μm) that were similar to large granular lymphocytes were more abundant relative to cells with large granules in the biopsy specimens from failing pregnancies. Immunosuppressive activity was tested in the supernatants of cultured biopsy samples of each group and found to be significantly lower in the incipient miscarriage group. These findings could represent alterations associated with the process of miscarriage, such as inflammation, or there may be deficient suppressor cell activity at the fetomaternal interface as the reason for "rejection" of the early embryo. (Am J Obstet Gynecol 1989;161:409-14.)

Key words: Aborting women, uterine biopsies in pregnancy, immunologic suppression, fetomaternal relations

The success or failure of the mammalian fetus in an immunologically hostile host has been shown to be correlated to the properties of the tissue at the maternal-fetal interface. Certain types of pregnancies in animals tend to have a high failure rate.[1] Intrauterine destruction of the embryo may be associated with an infiltration by cytotoxic maternal mononuclear cells at the deciduatrophoblastic interface reminiscent of the histologic picture of graft rejection. Both specific cytotoxic T cells and nonspecific natural killer–type cells may be involved. It is proposed that normal human pregnancy involves recognition of the foreign antigen on the conceptus and the development of a subsequent immunologic response. This response is protective to the growing fetus and inhibits maternal cytotoxic cell infiltration by boosting local active suppressor cell activity.[1] Disturbance of this sequence of protective events may lead to an initial miscarriage and recurrence in subsequent pregnancies.

Experimental studies of pregnancy in mice suggest the presence of nonspecific suppressor cells in mechanically disaggregated decidua. These are predominantly small granulated lymphocytic cells that lack T-cell surface markers and elaborate soluble factor(s) that inhibit the response to T-cell growth factor (interleukin-2).[1] Similar interleukin-2 blocking factor(s) has been obtained from human decidua.[2, 3]

In pregnant women distinct differences in the cellular composition of the decidua were noted when healthy and failing pregnancies were compared in 10 samples of placental bed biopsies of four normal women and six women who had begun to miscarry. A paucity of small lymphocytic cells with large granules (stained by phloxine-tartrazine) was evident in the decidua of a failing pregnancy, whereas cells with large granular lymphocytes were prominent in the decidua of miscarrying women.[4] However, this study provided only qualitative rather than quantitative data, and no information was obtained concerning altered immunosuppressor activity in these specimens.

In the study reported here, we quantitatively measured changes in granulated maternal mononuclear cell populations in uterine samples from the area of the placental bed in women who would eventually miscarry and measured the level of suppressor activity in biopsy culture supernatants from this group of patients.

Patients and methods

The study included 59 pregnant women. Group A was a control group consisting of 27 women who un-

From the Department of Obstetrics and Gynaecology[a] and the Department of Experimental Pathology,[b] St. Mary's Hospital Medical School, London, and the Molecular Virology and Immunology Programme, Department of Medicine, McMaster University, Hamilton.[c]

Supported by grants from Birthright, United Kingdom, and Physicians' Services Incorporated Foundation, Ontario, Canada.

Received for publication June 27, 1988; revised January 28, 1989; accepted March 10, 1989.

Reprint requests: Dr. David A. Clark, McMaster University, 1200 Main St. West, Hamilton, Ontario, Canada L8N 3Z5.

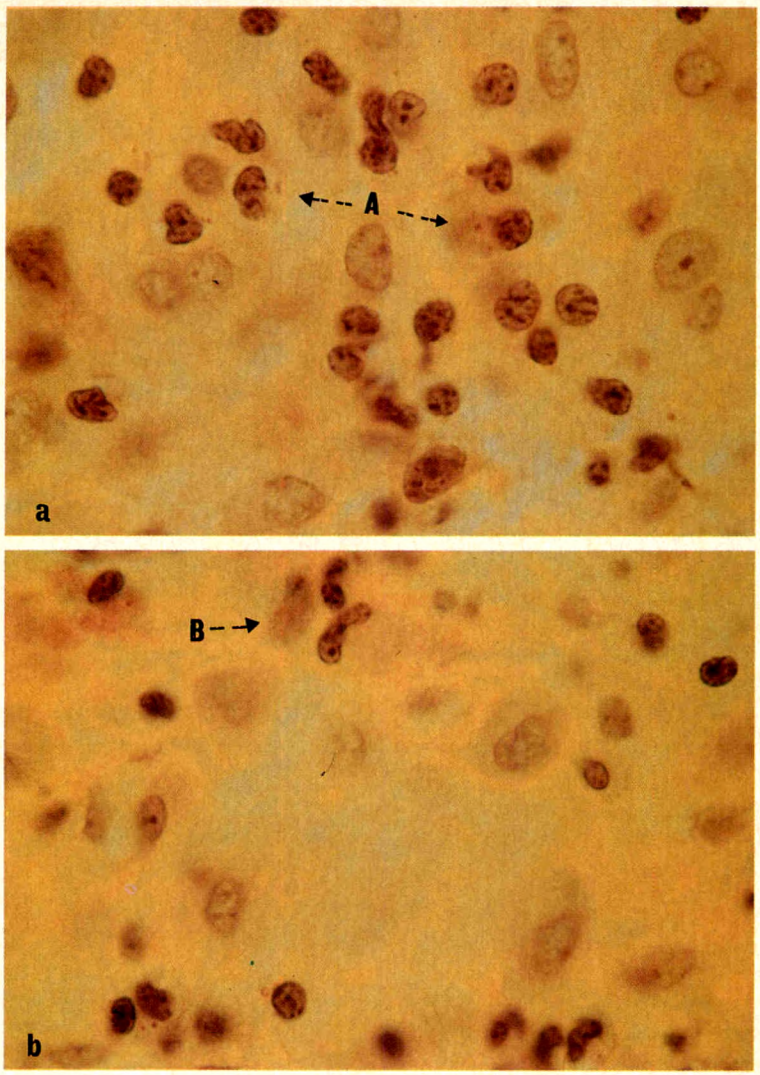


Fig. 1. Photomicrographs of phloxine-tartrazine–stained placental bed biopsy specimens. **a,** Sample from elective termination of pregnancy. **b,** Sample from patient with "anembryonic" pregnancy. *Arrows* point to granulated cells. *A,* Cells with large granules. *B,* Large granular lymphocytes. (Oil immersion; original magnification ×1000.)

derwent legal abortion. Group B consisted of 26 women who had a history of recurrent miscarriage (i.e., three or more unexplained miscarriages with the same partner and not more than one live birth). Group C consisted of six women with an ultrasonographically diagnosed "missed abortion." The average maternal age was 24.9, 33.4, and 29.7 years, respectively, in the three groups.

Group A patients were scanned in the operating room before legal abortion and the gestational age was determined (crown-rump length or biparietal diameter). The presence of fetal heart activity was confirmed and localization of the placenta established.

Group B patients were all investigated at the Recurrent Miscarriage Clinic at the Samaritan Hospital for Women, London, and those found to be lacking antipaternal cytotoxic antibodies were given paternal lymphocyte immunization either before or during pregnancy.[5] Four patients were not immunized in this group, two because antipaternal cytotoxic antibodies were detectable in the sera and the other two because of "anembryonic" pregnancies at the time they were seen for immunization. All patients were followed up by weekly ultrasonographic examination as soon as the test result for the β-subunit of human chorionic gonadotropin was positive. "Anembryonic" pregnancy was diagnosed in all patients in this group and the placental site was determined by ultrasonography. Group C patients had threatened miscarriage (i.e., vaginal bleeding) and were assessed by ultrasonography. Absence of a fetal pole and fetal heart on two separate occasions was considered diagnostic of "anembryonic pregnancy."

Consent was sought from all patients for evacuation

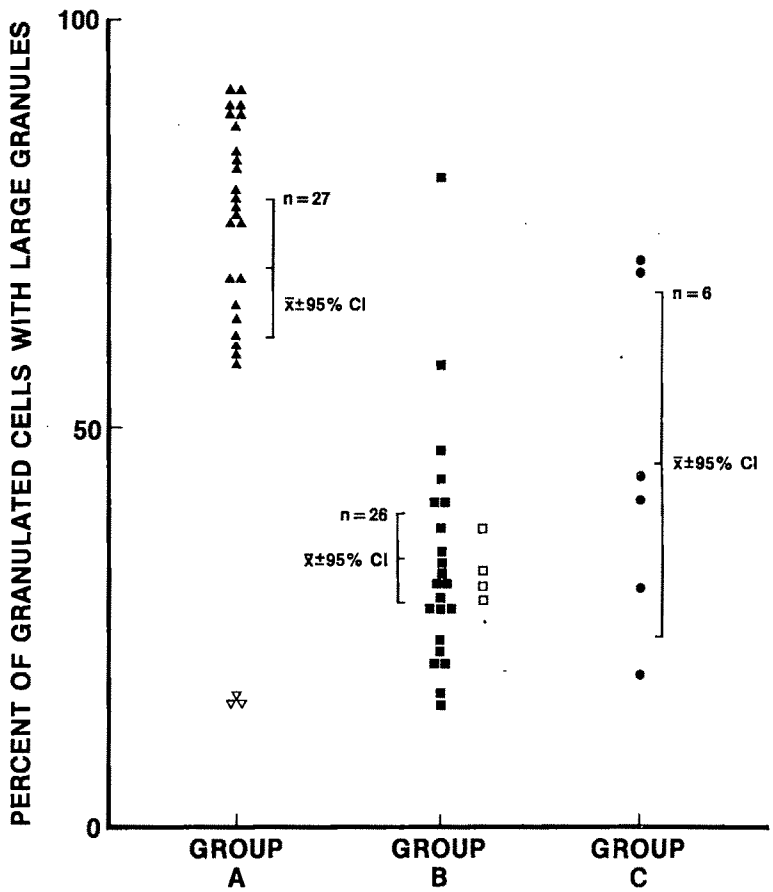


Fig. 2. Percent of granulated mononuclear cells with at least one granule >1 μm. *Group A*, Controls (▲,▽). Geometric mean value ± 1 SEM = 69.4% ± 4.19% (n = 27). Three low values (▽) were from areas with significant increase in numbers of polymorphonuclear leukocytes suggestive of early spontaneous abortion. Excluding these three gives a geometric mean ± 1 SEM = 76.2% ± 2.23%. *Group B*, Women with recurrent spontaneous miscarriage who had not received immunization (□) or had received paternal lymphocyte immunization (■). Mean ± 1 SEM = 33.8% ± 2.65% (n = 26; $p < 0.05$ compared with group A). The value of 81% represented a patient with a trisomy 22. Excluding this value gave a mean ± 1 SEM = 31.9% ± 1.94%. *Group C*, Sporadic abortions (●). Mean ± 1 SEM = 46.5% ± 8.4% (n = 6; $p < 0.05$ compared with control group A).

of the uterus and uterine biopsy under ultrasonographic guidance from the region of the placental bed. With the patient under general anesthesia, the placental site was determined by ultrasonography, the cervix was dilated with Hegar dilators, and cervical biopsy forceps was introduced through the cervical canal to the placental site under direct ultrasonographic guidance. Biopsy specimens including decidual tissue (decidual basalis and superficial myometrium) were taken from the thickest part of the placenta. There were no reported complications from the procedure.

Material obtained was either fixed in Bouin's solution for histologic study or placed in sterile normal saline solution for preparation of supernatant. Fetoplacental tissue was sent for chromosomal analysis. The fixed samples were paraffin embedded, sectioned at 4 μm and stained with phloxine-tartrazine for demonstration of intracellular granules.[4, 6, 7] The slides were scored by two different observers (M. M. and D. A. C.) without knowledge of the clinical details. Large granulated lymphocytes (all granules <1 μm) and cells with large granules (at least one granule ≥1 μm), as described elsewhere,[6] were counted on each slide. Since the biopsy sample was too small to permit disaggregation of the cells for immunologic studies, each sample was weighed and then incubated at 37° C for 48 hours in 1 ml RPMI 1640 medium with antibiotics (100 μg/ml streptomycin, 100 IU/ml penicillin) and 10% fetal bovine serum (Gibco).[4] The cell-free supernatant was then removed and stored at 4° C. The suppressor activity of the supernatant was tested at a 1:4 dilution for suppression of human lymphocyte proliferation. Briefly, sterile heparinized peripheral blood from one of the authors was centrifuged on Isopaque-ficoll (lymphocyte separation medium, density of 1.077) to isolate the mononuclear cells. Viable cells (2×10^5) were incubated with or without test supernatant in replicate round-bottom culture wells (96-well plates, Linbro) in a total volume of 100

Table I. Changes in cell count per slide in biopsy specimens of study population

Patient group	*Cells with large granules*	*Large granular lymphocytes*
Group A		
Log mean ± 1 SEM*	3.72328 ± 0.18744	2.49321 ± 0.17735
Geometric mean†	41.4	12.1
Group B		
Log mean ± 1 SEM*	2.96527 ± 0.21747‡	3.66099 ± 0.16758‡
Geometric mean†	19.4	38.9
Group C		
Log mean ± 1 SEM*	2.19722 ± 0.57479‡	2.91777 ± 0.20384‡
Geometric mean†	9.0	18.5

group A, Termination of pregnancy; *group B*, recurrent spontaneous miscarriage (excluding patient with trisomy 22 in Fig. 1); *group C*, missed abortion.

*Data were converted to natural logarithms for calculation of mean and SEM.

†Mean reexpresed arithmetically as the geometric mean (median).

‡$p < 0.05$ by Student's t test, compared with control group A.

μl (minimum essential medium Eagle with alpha modification, 10% fetal bovine serum [Gibco], and antibiotics) for 48 hours with 10 μg/ml concanavalin A. tritiated thymidine was then added (25 μl, final concentration 1 μCi/ml), and the following morning the labeled cells were harvested on glass fiber filters (Skatron cell harvester). The filters were dried and processed for liquid scintillation counting in a β-counter.

The incorporation in counts per minute (average ± SEM) of four replicate wells was calculated, and percentage suppression determined by the formula:

$$\% \text{ Suppression} = 100\left(1 - \frac{\text{counts/min with test supernatant}}{\text{counts/min with medium}}\right)$$

No correction was made for background proliferation occurring in the absence of concanavalin A. Chromosome analysis was not performed on fetoplacental tissue obtained from group A, but 32 samples were cultured from the other two groups. Thirteen samples failed to grow, and two (10.5%) were abnormal (trisomy 47,XX + 22 and 47,XY + 20), both from group B. Chromosome analysis of both parents was normal.

The significance of differences was determined by Student's t test. Log transformation was used where the data followed a log normal distribution.

Results

Cells with large granules were abundant in the decidua of successful pregnancy (Fig. 1, *a*). In contrast, large granulated lymphocytes were frequently seen infiltrating the decidua of women in groups B and C (Fig. 1, *b*). The percentage of granulated cells with large granules for each patient is shown in Fig. 2. It can be seen that the percentage of cells that had large granules was significantly lower in group B than in control group A. The cell population of one of the trisomies, trisomy 22, was clearly in the normal range in contrast to those of the other patients. Group C also showed a reduction in cells with large granules but the number of patients was small.

To determine whether the percentage of cells with large granules had changed in aborting women as the result of a decrease in the number of cells with large granules or rather because of dilution from an increase in the number of large granular lymphocytes, we counted the number of each cell type per slide. There was considerable difference between patients in the total number of granulated cells per slide. In part, this was due to variation in size of the biopsy specimen, but an even greater variation was noted among patients in frequency of granulated cells per microscopic field, most likely caused by sampling differences. In spite of this variability, when the geometric mean of number of cells with large granules per slide and the number of large granular lymphocytes (paired with cells with large granules) per slide were compared for each group of patients, some interesting information was obtained relevant to explaining the decreased percentage of cells with large granules. Where the relative frequency of cells with large granules dropped (in group B), it can be seen (Table I) that the number of cells with large granules decreased and number of large gramular lymphocytes increased compared with group A (i.e., the control group). Group C also showed a decrease in cells with large granules with a smaller increase in large granular lymphocytes. The latter was due in part to somewhat smaller biopsy specimens taken from these six patients.

Suppressive activity in supernatants from the biopsy specimens is shown in Table II. The geometric mean weight was equivalent in groups A and B. Group C samples, as already described, were lighter. Supernatants from group B (excluding one case of trisomy 22 with the percentage of cells with large granules in the normal range and 100% suppression) were significantly

Table II. Suppressor activity in supernatants of placental bed biopsy tissue (donors 7 to 14 weeks' gestation)

Patient group	*Specimen weight (mg)*	*No. of supernatants*	*Suppression of placental bed biopsy tissue (%)*
Group A		17	
Mean ± SEM*	4.85203 ± 0.10545		4.27667 ± 0.06176
Geometric mean†	128		72.0
Group B		13	
Mean ± SEM*	4.84419 ± 0.14954		3.88362 ± 0.19308‡
Geometric mean†	127		48.6
Group C		5	
Mean ± SEM*	4.41884 ± 0.26950		4.08598 ± 0.17974
Geometric mean†	83		59.5
All with abortion (groups B and C)		18	
Mean ± SEM*	4.71850 ± 0.14255		3.93964 ± 0.14834‡
Geometric mean†	112		51.4

The groups are the same as shown in Table I; the patient with trisomy 22 was omitted from group B.
*Data were converted to natural logarithms for calculation of mean and SEM.
†Mean reexpressed arithmetically as the geometric mean (median).
‡Significantly less than the value in control group A. ($p < 0.05$ by Student's t test).

less suppressive than control (group A) specimens. Group C was less suppressive than control group A, but this difference did not reach statistical significance because of small numbers and lighter samples.

Comment

The data in this paper show a decreased number of cells with large granules and an increase in large granulated lymphocytes in placental bed biopsy specimens from women with incipient miscarriage. There was also a decrease in the suppressor activity in these women in comparison with the control group. Women with sporadic miscarriage showed a similar pattern, but the numbers were small. These data confirm earlier reports that a decrease in cells with large granules occurs in association with miscarriages.[4] Although we confined this study to miscarriages <14 weeks' gestation, we have seen similar changes in granulated cell populations in women with gestational ages up to 20 weeks.

There was a low percentage of cells with large granules in three women presenting for termination of pregnancy. These changes were restricted to areas of the biopsy specimen showing focal necrosis, edema, and polymorphonuclear cell infiltration typical of biopsy specimens from women with incipient miscarriage. Low percentages of cells with large granules in biopsy specimens from women who are experiencing a miscarriage could be due to inflammation, which might cause either a dilution by an increase in the number of large granular lymphocytes or a decrease in the granule size related to partial degranulation of the mononuclear cells.

In the mouse, local suppressor cell activity, granulated small suppressor cells, and natural killer cell activity in decidua have been shown to require an implanted embryo for induction.[1, 8] Natural killer cell activity and immunosuppression also have been shown to be due to different cell populations on the basis of size and presence or absence of the asialoganglioside-G_{m1} activation marker.[1] In human decidua, granulated mononuclear cells found near sites of cytotrophoblastic invasion as described by Bulmer and Johnson[9] are phenotypically similar to T-lineage lymphocytes but lack the classic surface markers of mature T cells. Trophoblast cell products appear to activate suppressor function of mononuclear cells in human endometrial-decidual tissue.[10]

Both suppressor cell activity and natural killer cell activity have been partially associated with granulated cells.[11, 12] It has been known for some time that HNK-1^+ (Leu-7^+) human cells (including classic natural killer cells) may be suppressive in vitro.[12] However, cells bearing the HNK-1 (Leu-7) antigen are infrequent in first-trimester human decidua; the Leu-19^+ (NKH-1^+) cell is present and may have both natural killer cell and suppressive activity.[11] However, Leu-19^+ cells isolated by fluorescence-activated cell sorting do not seem to account for all of the suppressor cell activity.[11] This is not entirely surprising because both cells and functional activity may be lost to varying degrees with fluorescence-activated cell sorting. Further, it has been shown in the murine system that there may be a large-sized suppressor cell population in addition to the granulated small lymphocytic suppressor cells.[1] This may also be true in humans.[10] Indeed it is not yet clear if cells with large granules are actually suppressor cells rather than a correlate of a decidua rich in suppressor factors. On the basis of murine data, we would not have expected classic natural killer cells (large granular lymphocytes) (bearing Leu-7 and OKM1) to correlate with suppressor activity.[2] Classic natural killer cells might infiltrate at the time of abortion; alternatively the properties of the Leu-19^+ Leu-7^- cells might change and

granule size decrease. Recently, Starkey et al.[13] described three populations of granulated lymphocytes in human decidua: (1) Leu-19$^+$ CD16$^-$ (Fc receptor), CD2$^-$ (E-rosette receptor); (2) Leu-19$^+$ CD16$^-$, CD2$^+$; and (3) a population of CD16$^+$ Leu-19$^\pm$ cells similar to natural killer cells in peripheral blood (and biopsy specimens from our aborting patients, Fig. 1, *b*). The CD16$^+$ subset was larger and more granular, which is typical of large granular lymphocytes in peripheral blood. All of these cells lacked the CD5 marker of mature T cells and CD3 T-cell receptor. Whether cells with large granules and large granular lymphocytes, as we have defined them, belong to the same lineage or different lineages, as defined by the above phenotypic markers, will ultimately be answered by isolating these cells and correlating their morphologic features with surface markers and function.

It was interesting to find that one of the two trisomies in the group studied had a percentage of cells with large granules in the normal range and high suppressive activity. A similar phenomenon has been noted in mice with genetic abortion where the fetus died as the result of lethal effects of T/t locus genes.[6] It is tempting to speculate from our data that a decrease in total number of decidual suppressor cells and cells with large granules is associated with miscarriage caused by "rejection." Recent studies in aborting mice suggest that natural killer–like nonspecific effector cells may be involved in rejection and that the suppressive factor in decidual supernatants is closely related to transforming growth factor-β that blocks interleukin-2, interleukin-3, and interferon-γ activation of nonspecific effector cells including lymphokine activated killer cells, natural killer cells, natural cytotoxic cells, and macrophages.[14] Natural killer cells, natural cytotoxic cells, and macrophages produce tumor necrosis factor-α that can cause cytotoxic effects on tumor cells and the local vascular supply.[15] Immunization with paternal cells of women with a history of recurrent miscarriage may prevent miscarriage and boost local suppression. The women we studied, however, were those who had failed treatment. Immunization apparently had failed to increase the local suppressive activity in this subset of women with recurrent miscarriage.

Defective hemochorial placentation as a result of inadequate trophoblast invasion of decidua also has been associated with miscarriages and is not necessarily linked to fetal chromosomal abnormality.[16] It will be important to determine if a decrease in the number of cells with large granules and suppressive activity also correlates with inhibition of trophoblastic invasion by tumor necrosis factor-α or related toxins in aborting women.

Biopsies of uterine decidua from the placental bed could give useful information regarding whether pregnancy failure is caused by failure of the trophoblast or rather is a result of genetic fetal death. The cellular and molecular basis for protection by local suppressor cells and factor is now under further investigation and should lead to further understanding of the mechanism of pregnancy failure and how immunotherapy may rescue a potentially "doomed" embryo.

REFERENCES

1. Clark DA, Damji N, Chaput A, Daya S, Rosenthal KL, Brierley J. Decidua-associated suppressor cells and suppressor factors regulating interleukin 2: their role in the survival of the "fetal allograft." In: Cinader B, Miller RG, eds. Progress in immunology. New York: Academic Press, 1986, vol 6:1089-99.
2. Golander A, Zakuth V, Shechter Y, Spirer Z. Suppression of lymphocytes reactivity in vitro by a soluble factor secreted by explants of human decidua. Eur J Immunol 1981;1:849-51.
3. Daya S, Rosenthal KL, Clark DA. Immunosuppressor factor(s) produced by decidua-associated suppressor cells: a proposed mechanism for fetal allograft survival. Am J Obstet Gynecol 1987;156:344-50.
4. Clark DA, Mowbray J, Underwood J, Lidell H. Histopathologic alterations in the decidua in human spontaneous abortion: fetal allograft survival. Am J Reprod Immunol Microbiol 1987;13:19-22.
5. Mowbray JF, Underwood JL, Michel M, Forbes PB, Beard RW. Immunisation with paternal lymphocytes in women with recurrent miscarriage. Lancet 1987;2:679-80.
6. Clark DA. Host immunoregulatory mechanisms. The success of the conceptus fertilized in vivo and in vitro. In: Beard RW, Sharp F, eds. Proceedings 18th RCOG study group. Early pregnancy—physiology, diagnosis and treatment. Ashton-under-Lyne, England: Peacock Press, 1988: 215-27.
7. Luna LG, ed. Manual of histologic staining methods of the Armed Forces Institute of Pathology. 3rd ed. New York: McGraw-Hill, 1968:234.
8. Croy BA, Gambel P, Rossant J, Wegmann TG. Characterization of murine decidual natural killer (NK) cells and their relevance to the success of pregnancy. Cell Immunol 1985;93:315-26.
9. Bulmer JN, Johnson PM. Immunohistological characterization of the decidual leukocyte infiltrate related to endometrial gland epithelium in early pregnancy. Immunology 1985;55:3-44.
10. Daya S, Johnson PM, Clark DA. Trophoblast induction of suppressor type cell activity in human endometrial tissue. Am J Reprod Immunol Microbiol 1989 (In press).
11. Bulmer JN, Ritson A. The decidua in early pregnancy. In: Beard RW, Sharp F, eds. Proceedings 18th RCOG study group. Early pregnancy—physiology, diagnosis and treatment. Ashton-under-Lyne, England: Peacock Press, 1988:171-80.
12. Tilden AB, Abo T, Balch CM. Suppressor cell function of human granular lymphocytes identified by the HNK-1 (Leu 7) monoclonal antibody. J Immunol 1983;130: 1171-5.
13. Starkey PM, Sargent I, Redman CWG. Cell populations in human early pregnancy decidua; characterization and isolation of large granular lymphocytes by flow cytometry. Immunology 1988;65:129-34.
14. Clark DA, Falbo M, Rowley RB, Banwatt D, Stedronska-Clark J. Active suppression of host-versus-graft reaction in pregnant mice. IX. Soluble suppressor activity obtained from allopregnant mouse decidua that blocks the response to interleukin 2 is related to TGF-β. J Immunol 1988;141:3833-40.
15. Shimomura K, Manda T, Mukumoto S, Kobayashi K, Nakamo K, Mori J. Recombinant human tumour necrosis factor-alpha: thrombus formation is a cause of antitumour activity. Int J Cancer 1988;41:243-7.
16. Khong TY, Liddell HS, Robertson WB. Defective haemochorial placentation as a cause of miscarriage: a preliminary study. Br J Obstet Gynaecol 1987;94:649-55.

Randomized trial of diet versus diet plus cardiovascular conditioning on glucose levels in gestational diabetes

Lois Jovanovic-Peterson, MD,[a] Eric P. Durak, MSc,[a,b] and Charles M. Peterson, MD[a]
Santa Barbara, California, and New York, New York

We studied the impact of a training program on glucose tolerance in gestational diabetes mellitus. Women with gestational diabetes mellitus ($N = 19$) were randomized into either group I, a 6-week diet alone group (24 to 30 kcal/kg/24 hours; 20% protein, 40% carbohydrate, 40% fat), or group II, which followed the same diet plus exercise (20 minutes three times a week for 6 weeks). An arm ergometer was used to maintain heart rate in the training range. Glycemic response was monitored by glycosylated hemoglobin, a 50 gm oral glucose challenge with a fasting and 1-hour plasma glucose, and blood glucose self-monitoring, fasting and 1 hour after meals. Week 1 glycemic parameters were the same for both groups. Week 6 data (mean ± SD) were as follows: group I glycosylated hemoglobin, 4.7% + 0.2% versus group II, 4.2% ± 0.2%; $p < 0.001$. The group I glucose challenge fasting value was 87.6 ± 6.2 versus 70.1 ± 6.6 mg/dl, $p < 0.001$ for group II. The group I 1-hour plasma glucose challenge result was 187.5 ± 12.9 mg/dl versus 105.9 ± 18.9 mg/dl for group II, $p < 0.001$. The glycemic levels diverged between the groups at week 4. We conclude that arm ergometer training is feasible in women with gestational diabetes mellitus and results in lower glycosylated hemoglobin, fasting, and 1-hour plasma glucose concentrations than diet alone. Arm ergometer training may provide a useful treatment option for women with gestational diabetes mellitus and may obviate insulin treatment. (Am J Obstet Gynecol 1989;161:415-9.)

Key words: Gestational diabetes, exercise, glucose tolerance, pregnancy, arm ergometry

Gestational diabetes mellitus is the most common medical complication of pregnancy. Current management consists of diet and careful monitoring of fasting and postprandial glucose levels. The goal of therapy is maintenance of euglycemia. When euglycemia is not achieved by diet alone, insulin therapy is recommended.[1] Gestational diabetes mellitus is considered in part to be a disease of glucose clearance, although it has been shown that this disorder is a heterogeneous entity.[2] Initiation of insulin therapy per se is palliative, but does not address the primary defect(s) that may include hyperinsulinemia. Rather, treatment modalities that overcome a peripheral resistance to insulin such as sulfonylurea treatment or exercise might be preferable. Although the sulfonylurea agents are reported to increase insulin sensitivity,[3] these agents are contraindicated for use in gestational diabetes in the United States.[4]

Cardiovascular conditioning exercise facilitates glucose utilization inter alia by increasing insulin binding to and affinity for its receptor.[5] Although exercise during pregnancy has been gaining acceptance,[6] it remains controversial.[7-15] Specifically, maternal exercise on a bicycle ergometer has been associated with fetal bradycardia.[7,11] Therefore we designed a study that used arm ergometry, which has not been associated with either uterine activity or fetal bradycardia,[16] to evaluate the impact of a training program on glucose tolerance in gestational diabetes mellitus.

From the Sansum Medical Research Foundation, Santa Barbara[a], and the Sports Training Institute, New York.[b]
Received for publication October 26, 1988; revised March 1, 1989; accepted March 8, 1989.
Reprint requests: Lois Jovanovic-Peterson, MD, Sansum Medical Research Foundation, 2219 Bath St., Santa Barbara, CA 93105.

Material and methods

Subjects and study design. All women gave informed witnessed consent for the studies that were approved by appropriate institutional review. Table I characterizes the study population and infant outcome. Twenty untrained women with gestational diabetes diagnosed according to standard protocol[1] (Table I) were recruited. One woman was dropped from the study because she was found to have placenta previa and therefore could not be entered. Thus 19 women were randomized into two groups by drawing a number (group I or group II). The randomization process revealed that the peak plasma glucose response at 1 hour after a 50 gm glucose load was not significantly different in the two groups at the start of the program, whereas the peak 1-hour plasma glucose in group I on the 100 gm glucose tolerance test was significantly lower than in group II (Table I). The 50 gm glucose test with a fasting and 1-hour plasma glucose level will be referred to in this paper as the glucose challenge test.

Each woman was instructed to perform blood glucose

Table I. Clinical characteristics of nineteen study women and infant outcome

Patient No.	Glucose tolerance test at 28 wk (mg/dl)				Infant		Maternal age (yr)	C-peptide (pmol/ml)	Maternal weight at time of GTT (kg)
	Fasting	1 hr	2 hr	3 hr	Birth weight (gm)	Sex			
Group I									
1	90	199	168	115	3440	F	27	>3.00	67
2	85	207	169	133	3000	M	34	1.95	68
3	81	290	198	160	3100	F	32	2.75	93
4	90	191	168	152	4000	M	36	>3.00	82
5	107	223	185	162	3760	M	28	>3.00	70
6	98	217	168	148	3520	F	29	1.87	73
7	86	192	184	168	3370	M	31	2.43	79
8	85	201	190	145	3820	F	31	2.75	74
9	94	272	201	162	3170	F	32	>3.00	72
		Mean 221.3			Mean 3465		Mean 31.1		Mean 75.3
		SD 35.7			SD 343		SD 2.8		SD 8.2
Group II									
1	93	275	201	182	3560	F	27	>3.00	64
2	95	293	240	128	3212	M	26	>3.00	98
3	87	299	270	165	3473	F	29	1.95	82
4	106	282	180	120	3870	F	29	2.71	84
5	102	215	169	148	4000	M	31	2.53	87
6	87	275	170	122	3980	F	30	>3.00	72
7	84	263	172	117	3900	M	27	2.10	71
8	97	259	164	148	3127	M	30	1.98	73
8	98	245	187	136	3771	F	34	>3.00	79
10	102	229	192	108	3443	M	32	>3.00	74
		Mean 263.5			Mean 3634		Mean 29.5		Mean 78.4
		SD 27.2			SD 317		SD 2.5		SD 9.7
		$p = 0.01$*			$p = 0.282$*		$p = 0.203$*		$p = 0.471$*

GTT, glucose tolerance test.
*Group I versus group II.

self-monitoring four times daily (before breakfast and 1 hour after each meal). Each week all subjects were seen by a physician. Insulin was to be initiated if the fasting plasma glucose level rose higher than 105 mg/dl[1] and/or the 1-hour postprandial plasma glucose level was >140 mg/dl.[17] Serial evaluation of the glucose challenge test included a fasting plasma glucose, a 50 gm oral glucose load, and a 1-hour plasma glucose study. Plasma glucose was obtained by venipuncture and analyzed by the glucose oxidase method. The glucose challenge test was performed before and after the 6-week protocol. A fasting C-peptide specimen was drawn with the fasting plasma glucose determinations.

Group I was managed for 6 weeks with a standard diet that consisted of 40% carbohydrate, 20% protein, and 40% fat calculated at 24 to 30 kcal/kg/24 hours present pregnant weight divided into three meals and three snacks.[18] These women did not participate in any structured exercise program. Weekly questionnaires revealed that their only form of exercise was cleaning, shopping, and "running after toddlers." Group II received the same diet and participated in an exercise program of 6 weeks' duration that consisted of 20 minutes of supervised aerobic training on an arm ergometer (UBE-Cybex Fitness, Ronkonkoma, N.Y.). Six women exercised in the afternoon, between 4 PM and 5 PM (3 hours after the last meal) and four of the women exercised in the morning between 10 and 11 AM (3 hours after breakfast). These times were chosen because the blood glucose levels in the diet alone group were stable and comparable at these time points.

The patients were monitored for 20 minutes of cardiovascular work during which they maintained their target heart rate,[11] which was calculated to be equal to (220 − age in years) × 70% unless this heart rate exceeded 140 beats/min; then 140 beats/min was the target heart rate.[6] As each patient adapted to her workload in terms of perceived exertion and heart rate, the workload (or resistance) (kg × meters × min^{-1}) was increased or decreased by 10% to 20%; thus maternal heart rate was maintained in the training range. The exercise session never exceeded 50% maximal oxygen consumption ($\dot{V}O_{2\,max}$) in any case. Fifty percent oxygen consumption was estimated based on our previous work that related maternal heart rate in beats/min to the $\dot{V}O_2$ at steady state utilizing a metabolic cart (Beckman model S/N 614, D/N 11265, Fullerton, Calif.).[11] Blood glucose levels were measured before and after each exercise session. In over 150 sessions of exercise with the arm ergometer with pregnant women, no fetal

Table II. Group I data before and after 6-week dietary program

Patient No.	*Plasma glucose before diet*		*Plasma glucose 6 wk after diet*	
	Fasting	*1 hr after 50 gm oral glucose (mg/dl)*	*Fasting*	*1 hr after 50 gm oral glucose (mg/dl)*
1	92	194	90	174
2	105	195	94	179
3	110	215	98	168
4	117	257	82	184
5	105	285	80	190
6	98	247	92	210
7	82	205	82	192
8	79	240	87	192
9	92	195	84	199
Mean	97.8	225.8	87.6	187.5
SD	12.7	32.8	6.2	12.9
		$p = 0.048$		$p = 0.005$

Table III. Group II data before and after 6-week dietary program

Patient No.	*Plasma glucose before diet*		*Plasma glucose after 6 wk of training*	
	Fasting	*1 hr after 50 gm oral glucose (mg/dl)*	*Fasting*	*1 hr after 50 gm oral glucose (mg/dl)*
1	90	240	60	88
2	95	270	70	77
3	110	195	64	105
4	120	210	77	103
5	120	220	82	142
6	95	240	74	118
7	102	197	69	88
8	98	276	73	110
9	90	247	65	123
10	104	213	67	105
Mean	100.4	230.8	70.1	105.9
SD	9.3	28.5	6.6	18.9
		$p = 0.000$		$p = 0.000$

bradycardia or uterine activity has been observed. The caloric expenditure associated with this form of exercise is equal to the expenditure on a bicycle ergometer at the same heart rate for the same duration, provided that the test method utilizes the same muscle groups trained by the regimen.[19]

Laboratory evaluation. The plasma glucose level was measured by glucose oxidase (Yellow Springs Instruments, Yellow Springs, Ohio). Glycosylated hemoglobin was analyzed by high-pressure liquid chromatography.[20] The normal range for glycosylated hemoglobin in pregnancy is 3.5% to 4.5%, and the mean glycosylated hemoglobin is 4.0 ± 0.250, based on 100 pregnant women who had normal glucose tolerance tests. C-peptide was analyzed by radioimmunoassay according to the manufacturer's instructions (Novo Biolabs Antiserum M1221, Bagsverd, Denmark).[21] The normal range in pregnancy is 0.5 to 2.0 pmol/ml.

Fetal heart rate and uterine activity were measured continuously for 10 minutes before, during, and after exercise with a fetal monitor (Corometrics model 111 fetal monitor, Wallingford, Conn.). Uterine activity was expressed as a percent of total exercise time (minutes of contraction/total minutes of exercise × 100). A contraction was defined as a tocometer deflection >15 mm Hg above baseline.

Statistical analysis. Statistical analysis used the STATWORKS program on the Macintosh PC. Paired or unpaired *t* tests were used to analyze the data as appropriate. All mean values are expressed as ± 1 SD.

Results

Groups I and II did not differ (Table I) in maternal age (31.1 ± 2.8 vs. 29.5 ± 2.5 years, $p = 0.203$), maternal weight at the start of the program (75.3 ± 8.2 vs. 78.4 ± 9.7 kg, $p = 0.471$), maternal C-peptide level (range 1.87 to 3.0 vs. 1.95 to 3.0 pmol/ml), gestational age at delivery (range 39.4 to 40.0 vs. 39.5 to 40.5 weeks), neonatal birth weight (3465 ± 343 vs. 3634 ± 317 gm, $p = 0.282$), or gender of the infants (5 females

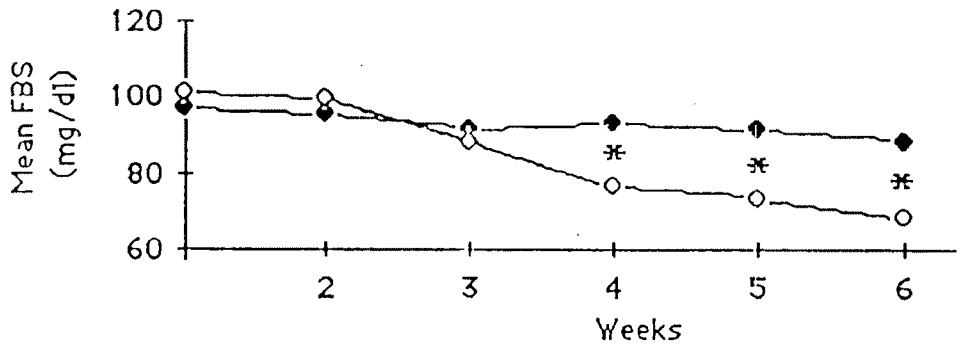


Fig. 1. Weekly mean fasting blood glucose (milligrams per deciliter) of blood glucose self-monitored results from week 1 to week 6 of protocol. *Closed circles* (●) represent mean values for group I (diet alone) and *open circles* (○) represent group II (diet and exercise). *Asterisk* signifies significance of $p < 0.001$.

vs. 5 females). The 1-hour plasma glucose value on the diagnostic glucose tolerance test did differ between the groups: group I, 221.3 ± 35.7 vs. group II, 263.5 ± 27.2 mg/dl ($p = 0.01$).

Despite the difference in the diagnostic glucose tolerance test results, at the beginning of the protocol groups I and II had equivalent 1-hour blood glucose responses (Tables II and III) to a glucose challenge test (group I = 225.8 ± 32.8 mg/dl vs. group II = 230.8 ± 28.5 mg/dl, p = NS). After 6 weeks, the mean 1-hour glucose response in group I on the glucose challenge test was 187.5 ± 12.9 mg/dl compared with the women in group II, who had a mean 1-hour response of 105.9 ± 18.9 mg/dl ($p < 0.001$, group I vs. group II). Group I did improve their 1-hour glucose response to a challenge with the program of diet alone (initial vs. final $p = 0.005$) but the mean 1-hour glucose response on the glucose challenge test was still greater than the current cutoff for the initial screening value for the diagnosis of gestational diabetes of 140 mg/dl[1] (Table II).

The chronic effect of exercise on hepatic glucose output was reflected by the fasting plasma glucose levels on the glucose challenge tests before and after the 6-week protocol. The mean fasting plasma glucose value in group I was 97.8 ± 12.7 mg/dl during the first glucose challenge test and 87.6 ± 6.2 mg/dl on the glucose challenge test after 6 weeks of diet alone ($p = 0.043$). The mean initial fasting plasma glucose value on the glucose challenge test in group II was 100.4 ± 9.3 mg/dl ($p = 0.478$ group I vs. group II). After 18 training sessions, the mean final fasting plasma glucose on the glucose challenge test performed between 15 to 45 hours after the eighteenth session for group II was 70.1 ± 6.6 mg/dl ($p < 0.001$ initial fasting plasma glucose in group II vs. the final). The mean fasting plasma glucose on the final glucose challenge test in group II was also significantly lower than the final value for group I ($p < 0.001$).

The glycosylated hemoglobin level improved in both groups. In group I the mean initial glycosylated hemoglobin value was 4.86% ± 0.39%; after 6 weeks this level fell to 4.67% ± 0.25% ($p = 0.01$). In group II the initial glycosylated hemoglobin value was 4.83% ± 0.33%, which fell to 4.16% ± 0.28% ($p < 0.001$). The glycosylated hemoglobin values for the two groups were not different at the start ($p = 0.75$) but there was a significant difference ($p < 0.001$) after 6 weeks.

To determine the time course of the glucose changes in group I and group II, the daily blood glucose self-monitoring of fasting values for each group were evaluated (Fig. 1). By 4 weeks of the protocol, the mean fasting blood glucose for group II (75.8 ± 5.8 mg/dl) diverged significantly ($p < 0.001$) from that of group I (93.1 ± 6.5 mg/dl). The postprandial glucose levels on self blood glucose monitors in group I were higher than those of group II at the 6-week point (136 ± 10 mg/dl vs. 112 ± 10 mg/dl, $p < 0.01$).

The 10 women who trained (group II) showed a continuing and significant change in glucose response to exercise. On the first day of the training program, the mean glucose decrement during exercise expressed as a percent was 8.4% ± 3.0%. During the eighteenth and final training session, the mean decrement was 29.8% ± 8.0% ($p < 0.001$, initial vs. final percent decrease associated with a training session).

There were no episodes of fetal bradycardia or uterine activity noted during any exercise session. No episodes of hypoglycemia (blood glucose <60 mg/dl) occurred in the study population. None of the 19 women in group I or group II required insulin. Of note, the woman with gestational diabetes who was dropped from the study and who was placed on strict bed rest as a result of placenta previa, did require insulin. All 19 infants born to this study were healthy, term neonates with no signs of morbidity.

Comment

Our study documents that women with gestational diabetes can train with arm ergometry and that such a

program of cardiovascular conditioning exercise results in lower levels of glycemia than a program of diet alone. The effects of exercise on glucose metabolism became apparent after 4 weeks of training and appeared to impact hepatic glucose output (as reflected by fasting glucose levels), glucose clearance (as reflected by glucose values after a 50-gm oral glucose challenge), and mean blood glucose (as reflected by glycosylated hemoglobin values). These combined observations would be consistent with a long-term effect of exercise rather than a short-term effect from individual sessions. The important question of the duration of a training effect on glucose metabolism in women with gestational diabetes was not addressed in this study.

Our observations appear contrary to the results in the literature asserting that the natural progression of glucose intolerance during gestation is deterioration with increasing gestational week[22] and that walking exercise in type I insulin-dependent diabetic pregnant women is associated with no improvement.[23] However, neither of the above studies addressed the issue of gestational diabetes with its unique problems of glucose homeostasis.

The implications of our study for the health care of women with gestational diabetes remain to be determined. Compliance with the protocol was 100% and there was no maternal or infant morbidity associated with the training program in this small series. Our results also imply that a cardiovascular conditioning program might obviate insulin treatment in many women with gestational diabetes. Therefore the economic and health care implications of these observations warrant further testing.

REFERENCES

1. Summary and Recommendations of the Second International Workshop-Conference on Gestational Diabetes Mellitus. Diabetes 1985;34(suppl 2):123.
2. Freinkel N, Metzger BE, Phelps RL, et al. Heterogeneity of maternal age, weight, insulin secretion, HLA antigens, and islet cell antibodies, and the impact of maternal metabolism on pancreatic B cell and somatic development in the offspring. Diabetes 1985;34(suppl 2):1-7.
3. Peterson CM, Sims RV, Jones RL, Rieders F. Bioavailability of glipizide and its effect on blood glucose and insulin levels in patients with non-insulin dependent diabetes. Diabetes Care 1982;5:497-500.
4. Jovanovic L. In: Jovanovic L, ed. Controversies in diabetes and pregnancy. New York: Springer-Verlag, 1988:7.
5. Pedersen O, Beck-Nielsen M, Heding L. Increased insulin receptors after exercise in patients with insulin-dependent diabetes mellitus. N Engl J Med 1980;302:886-92.
6. American College of Obstetrics and Gynecology. Home exercise programs. Washington DC: American College of Obstetricians and Gynecologists, May 1986.
7. Artal R, Romem Y, Wiswell R. Fetal bradycardia induced by maternal exercise. Lancet 1984;2:258-60.
8. Hon EH, Wohlgemuth R. The electronic evaluation of fetal heart rate. IV. The effect of maternal exercise. AM J OBSTET GYNECOL 1961;81:361-71.
9. Pomerance JJ, Gluck L, Lynch VA. Maternal exercise as a screening test for uteroplacental insufficiency. Obstet Gynecol 1974;44:383-7.
10. Dale E, Mullinax K, Bryan D. Exercise during pregnancy: effect on the fetus. Can J Appl Sport Sci 1982;7:98-103.
11. Jovanovic L, Kessler A, Peterson CM. Human maternal and fetal response to graded exercise. J Appl Physiol 1985;58:1719-22.
12. Collings C, Curet LB. Fetal heart rate response to maternal exercise. AM J OBSTET GYNECOL 1985;151:498-501.
13. Hauth JC, Gilstrap LC, Widmer CK. Fetal heart rate reactivity before and after maternal jogging during the third trimester. AM J OBSTET GYNECOL 1982;142:545-7.
14. Edwards M, Metcalfe J, Dunham M, Paul M. Accelerated respiratory response to moderate exercise in late pregnancy. Respir Physiol 1981;45:229-41.
15. Dressendorfer E, Goodlin R. Fetal heart rate response to maternal exercise testing. Phys Sportsmed 1980;8:91-4.
16. Jovanovic L, Peterson CM, Board PJ, Dyer CR. Arm ergometry is a safe form of training for pregnant women. Clin Res 1987;35:289A.
17. Jovanovic L, Peterson CM. Optimal insulin delivery for the pregnant diabetic patient. Diabetes Care 1980;5(suppl I):24-37.
18. Jovanovic L, Peterson CM, Saxena BB, Dawood MY, Saudek CD. Feasibility of maintaining normal glucose profiles in insulin dependent pregnant women. Am J Med 1980; 68:105-112.
19. Fox EL, Bartels RL, Billings CE, O'Brien R, Bason R, Mathews D. Frequency and duration of interval training programs and changes in aerobic power. J Appl Physiol 1975;38(3):481-4.
20. Peterson CM, Jovanovic L, Raskin P, Goldstein DE. A comparative evaluation of glycosylated haemoglobin assays: feasibility of references and standards. Diabetologia 1984;26:214-7.
21. Heding L. Radioimmunoassay determination of human C-peptide in serum. Diabetologia 1975;11:541-8.
22. Jovanovic L, Peterson CM. Screening for gestational diabetes: optimal timing and criteria for retesting. Diabetes 1985;34(suppl 2):21-23.
23. Hollingsworth DR, Moore TR. Postprandial walking exercise in pregnant insulin-dependent (type I) diabetic women: reduction of plasma lipid levels but absence of a significant effect on glycemic control. AM J OBSTET GYNECOL 1987;157:1359-63.

Thrombosis prophylaxis in pregnancy with use of subcutaneous heparin adjusted by monitoring heparin concentration in plasma

Thomas C. Dahlman, MD, Margareta S. E. Hellgren, MD, and Margareta Blombäck, MD
Stockholm, Sweden

Twenty-six pregnant women were given prophylactic heparin treatment because of previous thromboembolic complications. The mean duration of treatment was 25 weeks (range, 6 to 32 weeks). The amount of heparin was adjusted to a plasma concentration of 0.08 to 0.15 IU/ml, measured as anti–factor Xa activity. This effect was compared with that on activated partial thromboplastin time. The average dose was 16,400 IU/24 hours or 225 IU/kg of body weight per 24 hours. When the plasma concentration was within the stipulated range, the dose was 234 IU/kg of body weight/24 hours. No significant prolongation of activated partial thromboplastin time was found in two thirds of the samples. Blood coagulation was either not activated or only slightly activated, as verified by a low level of fibrinopeptide A. Platelet counts and antithrombin III levels were generally not depressed. No thromboembolic complications occurred during the pregnancies or puerperium. Bleeding during delivery was not increased. No fractures of the spine caused by osteoporosis were found on radiologic examination post partum. (Am J Obstet Gynecol 1989;161:420-5.)

Key words: Pregnancy, thrombosis prophylaxis, heparin concentration, activated partial thromboplastin time, antithrombin III, fibrinopeptide A

The incidence of thromboembolic complications in pregnancy is about 1 in 1000.[1-3] About twice the incidence is found in the puerperium.[1,2] A previous history of thromboembolic complications is considered to give a risk of new thromboembolic complications in pregnancy of about 1 in 10.[4,5] Today we treat such women with prophylactic heparin treatment during pregnancy and the puerperium.[2] This has also been the policy of others.[3,6-8] Osteoporosis is a rare but feared complication of long-term heparin treatment.[2,3,5] Whether the incidence of osteoporosis is related to the amount of heparin given or to the duration of treatment is not fully understood. The effect of heparin on blood coagulation has to be measured in some way because the effect of a given dose varies among patients. This effect can be evaluated by measurement of the activated partial thromboplastin time, the heparin concentration as anti–factor Xa activity, and the level of fibrinopeptide A.[9,10] The sensitivity of activated partial thromboplastin time to small doses of heparin used as prophylaxis against thromboembolic complications is low. However, when heparin is measured as anti–factor Xa activity, an anticoagulant effect can be detected at a level as low as 0.02 IU/ml plasma.[11] During prophylactic heparin treatment, a concentration of heparin of approximately 0.1 IU/ml has been considered satisfactory, based on results obtained with subcutaneous low-dose heparin.

In this study the heparin dose has been adjusted by determining the anti–factor Xa activity as measured with chromogenic substrate S-2222. The fibrinopeptide A level was analyzed to detect abnormal activation of the blood coagulation. Antithrombin III levels and platelet counts previously have been reported to decrease during heparin treatment.[2,12,13] Therefore these variables were also monitored. Our purpose was to determine whether the heparin dosage could be kept low with a measurable anticoagulant effect of heparin, adjusted according to the anti–factor Xa activity, without activation of blood coagulation or rethrombosis.

Patients and methods

Twenty-six women with a previous history of thromboembolic complications were given prophylaxis against new thromboembolic complications in connection with pregnancy and the puerperium. The study was approved by the Ethics Committee of Karolinska Hospital. No patient with prophylaxis because of a markedly increased risk of thromboembolic complications (e.g., as a result of artificial heart valves or con-

From the Department of Obstetrics and Gynecology and the Department of Clinical Chemistry and Blood Coagulation, Karolinska Hospital.
Supported by the Swedish Medical Research Council (19X-520).
Received for publication October 29, 1987; revised December 13, 1988; accepted February 16, 1989.
Reprint requests: Thomas C. Dahlman, MD, Department of Obstetrics and Gynecology, Karolinska Hospital, S-104 01, Stockholm, Sweden.

Table I. Previous thromboembolic complications, contributing factors, and location

Location	*Contributing factor*					*Total*
	Pregnancy	*Puerperium*	*Oral contraceptives*	*Postoperatively*	*Idiopathic*	
Popliteal vein	1 (1)	—	4 (3)	—	1 (1)	6 (5)
Femoral vein	1 (1)	—	—	1 (0)	2 (2)	4 (3)
Iliac vein	4 (3)	1 (0)	2 (2)	—	—	7 (5)
Pulmonary embolism	—	2	1	1	—	4
Transient ischemic attacks	5	—	—	—	—	5
TOTAL	11	3	7	2	3	26

Figures within parentheses denote number of *left* venous thromboses (13/17).

genital antithrombin III deficiency) was included in the study. The mean age was 28 years (range, 19 to 42). The mean pregestational weight was 65 kg (range, 51 to 108), and the average increase of weight during pregnancy was 11 kg (range, 4 to 21). Eleven patients were nulliparous, 11 were primiparous, and four were secundiparous. Nine of the 26 women were smokers. Nineteen of the patients breast-fed their infants for longer than 6 weeks. One did not breast-feed. As shown in Table I, 11 women had thromboembolic complications during pregnancy, 3 in the puerperium, 7 when taking oral contraceptives, and 2 after operation. In three women no obvious reason for thromboembolic complications was found. The location of thromboembolic complications is also shown in Table I.

Thromboprophylaxis was provided in the form of unfractionated standard sodium heparin prepared from pig intestinal mucosa (Kabi AB). The patients injected themselves subcutaneously in the thigh or the hip every 12 hours during pregnancy. The plasma volume increases and hemostasis is changed during pregnancy in such a way that the need for heparin to achieve the same anticoagulant effect varies during different gestational periods.[14, 15] Furthermore, there is currently no ideal method for measuring antithrombotic effects of heparin, but there are sensitive methods for analyzing the anticoagulant effect. Therefore we chose to measure the anticoagulant effect to give the same treatment early and late during pregnancy, if possible. The dosage of heparin was adjusted to a plasma concentration (determined as the anti–factor Xa activity) corresponding to 0.08 to 0.15 IU/ml measured 3 hours after the morning dose of heparin because subcutaneous heparin reaches its maximum level in pregnant women 2 to 3 hours after injection.[14, 15] Heparin treatment was usually started early in pregnancy (mean, thirteenth week; range sixth to thirty-first week). During labor heparin was given in small doses (initially 5000 IU subcutaneously and then 2500 IU subcutaneously every 8 hours until parturition) in 20 patients. Dextran 40, 500 ml twice with 4-hour interval, was given after administration of a hapten to dextran (Promiten, Pharmacia, Uppsala) to the other six patients.[16] After delivery the amount of heparin was either 10,000 or 15,000 IU/24 hours, depending on the need for heparin before delivery. Heparin treatment was discontinued 6 weeks after delivery, when blood coagulation is generally normalized.[17] Blood loss was measured by volumetric measurements of shed blood and by grossly estimating the blood in gauzes.

On the fifth day post partum, all patients were examined by x-ray of the spine and hip to exclude osteopenia and compression fractures as a result of heparin-induced osteoporosis. Thirty matched normal women were examined at the same time and served as control subjects. The films were later mixed and judged by one experienced radiologist in this field without knowledge of the history of the individuals.

Blood samples were drawn by venipuncture approximately 3 hours after the morning dose of heparin. The sampling and analyses were performed approximately every fourth week until the thirtieth week of pregnancy, every second week until the thirty-sixth week, and thereafter every week until delivery. If the heparin dosage had to be changed, sampling was repeated within a week of the adjustment.

Platelet counts were measured by means of an automatic counter. The blood was centrifuged for the remainder of the analyses. Activated partial thromboplastin time was immediately analyzed according to the manufacturer's directions (Nyegaard, Oslo; reference value <35 seconds). The remaining plasma sample was kept frozen at −70° C until analyzed. The heparin concentration was measured within 3 to 4 days as anti–factor Xa activity with the chromogenic peptide substrate S-2222 (Kabi AB).[11] Fibrinopeptide A (a small peptide that splits from fibrinogen when attacked by thrombin to form fibrin) was measured by radioimmunoassay.[18] The reference value for fibrinopeptide in our laboratory for nonpregnant women is <2 ng/ml, and in early and late pregnancy 1.7 ± 0.8 and 2.5 ± 1.4 ng/ml respectively.[15] Antithrombin III was measured with the chromogenic peptide substrate S-

Table II. Amount of heparin administered, APTT, and FPA when heparin concentration is within stipulated range (0.08 to 0.15 IU/ml plasma) during pregnancy

Week of pregnancy	*Patients within range (n of total)*	*Heparin amount (IU/kg/24 hr) (mean ± SD)*	*Heparin concentration (IU/ml) (mean ± SD)*	*APTT (sec) (mean ± SD)*	*FPA (ng/ml) (mean ± SD)*
<16	6/15	228 ± 28	0.10 ± 0.01	36 ± 3	1.6 ± 0.8
16-20	10/22	238 ± 26	0.11 ± 0.02	38 ± 5	2.2 ± 1.2
21-25	22/25	245 ± 35	0.11 ± 0.02	36 ± 5	2.4 ± 1.1
26-30	20/25	245 ± 40	0.11 ± 0.02	37 ± 5	2.7 ± 1.4
31-35	20/26	230 ± 44	0.11 ± 0.02	38 ± 5	1.9 ± 0.8
36	11/17	214 ± 28	0.11 ± 0.01	37 ± 6	1.9 ± 0.8
37	9/20	234 ± 26	0.11 ± 0.02	40 ± 7	2.1 ± 1.1
38	7/14	233 ± 29	0.11 ± 0.03	38 ± 5	3.5 ± 2.6
39-40	4/11	191 ± 38	0.10 ± 0.02	39 ± 7	3.9 ± 2.4
Reference value (normal pregnancy)			0	<35	<4

**APTT*, Activated partial thromboplastin time; *FPA*, fibrinopeptide A.

Table III. Percentage of total number of patients within and outside stipulated range of heparin during different periods of pregnancy

Week of pregnancy	*Total no. of patients*	*Percentage of total patients (heparin concentration, IU/ml)*		
		<0.08	*0.08-0.15**	*>0.15*
<16	15	53	40	7
16-20	22	41	45	14
21-25	25	8	88	4
26-30	25	8	80	12
31-35	26	8	77	15
36	17	6	65	29
37	20	35	45	20
38	14	29	50	21
39-40	11	18	36	46

*Stipulated range.

2238 (Kabi AB).[19] The reference value (mean ± SD) for pregnant women does not differ from that of nonpregnant young women (103% ± 11% of normal; $n = 40$).[15] All analyses were performed in duplicate.

Statistical analysis. The variability in the amount of heparin needed to achieve an adequate anticoagulant effect during different periods of pregnancy was studied by analysis of variance. A p value <0.05 was considered significant.

Results

No clinical thromboembolic complications occurred during pregnancy, parturition, or the puerperium.

No case of vertebral fracture was found on radiologic examination on the fifth day post partum. Four of the 26 heparin-treated patients, but none of 30 controls, were considered osteopenic. These findings are included in a more extensive study, which is under compilation.

There was one intrauterine death at 38 weeks' gestation. No specific explanation was found. The other 26 infants (one pair of twins) were healthy. The birth weights of the infants were appropriate for gestational age. The mean weight was 3190 gm (range, 2280 to 5300 gm). Most infants were delivered in week 38 of pregnancy (range, 36 to 41 weeks). Labor was induced in 17 of 26 pregnancies with a ripe cervix to avoid spontaneous labor before heparin reduction. The mean week of induction was the thirty-ninth week (range, 37 to 41). In one case an elective cesarean section was performed because of placenta previa marginalis. All other women were delivered vaginally. Bleeding during delivery averaged 320 ± 230 ml (mean ± SD). Three patients lost >600 ml of blood. In one patient the blood loss was 1100 ml because of uterine atonia. The patient delivered by cesarean section had a blood loss of 800 ml. The third patient lost 700 ml of blood for unknown reasons. All three had normal activated partial thromboplastin times before delivery.

The average duration of heparin treatment was 25 weeks (range, 6 to 32). The total mean dose given per 24 hours was 16,400 IU (range, 13,000 to 23,000) or 225 IU/kg body weight per 24 hours (range, 190 to 250 IU/kg body weight per 24 hours). The average heparin dosage, the number of patients within the stipulated plasma concentration range during different weeks of pregnancy, the heparin concentration, the mean activated partial thromboplastin time, and the mean fibrinopeptide A level are shown in Table II. When the plasma heparin concentration was within the stipulated range, the mean dose was 234 IU/kg body weight/24 hours (range, 191 to 245 IU/kg body weight per 24 hours; Table II). In early pregnancy the average heparin dose needed to achieve the stipulated anticoagulant effect was 228 IU/kg body weight per 24 hours (Table II). Thereafter, the need for heparin increased to a maximum between weeks 21 and 30. The patients then required on average 245 IU/kg body weight per 24 hours (Table II). During the remaining weeks of pregnancy, the need of heparin decreased, but not significantly. The total changes in need for heparin in

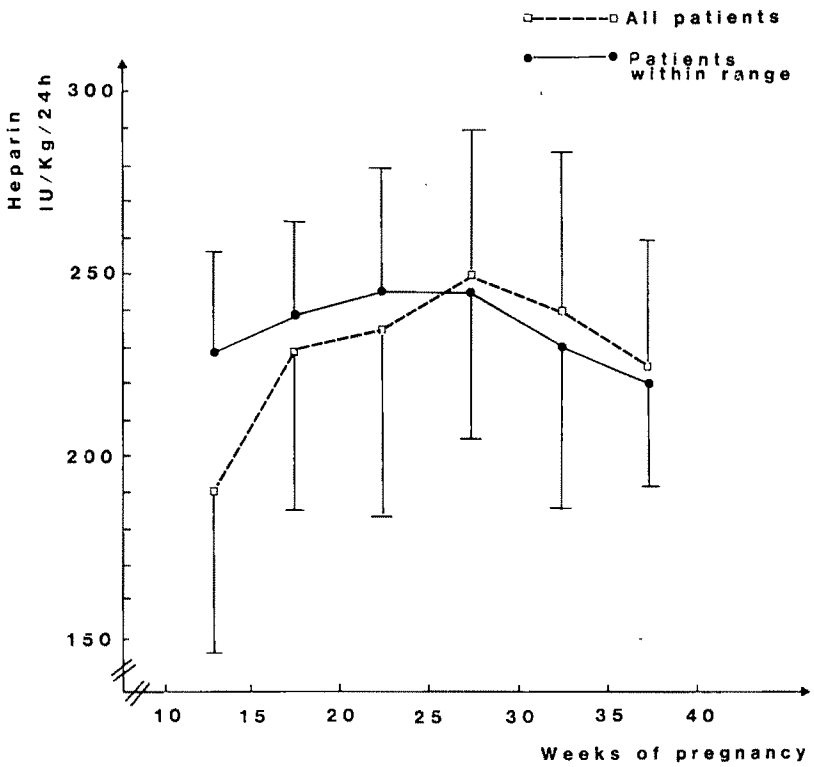


Fig. 1. Amount of heparin given during pregnancy (mean ± SD) during 5-week intervals. (For number of patients, see Table II.)

Table IV. Different ranges of APTT and the corresponding heparin concentrations

	Heparin concentration (IU/ml)					
APTT (sec)	*<0.05*	*0.05-0.07*	*0.08-0.15*	*0.16-0.18*	*>0.18*	*Total*
>49	0	0	5	10	10	25
40-49	1	10	43	11	17	82
36-39	6	7	30	5	1	49
31-35	11	29	59	2	1	102
<31	10	12	14	2	0	38
TOTAL	28	58	151	30	29	296

Figures denote number of samples.
APTT, Activated partial thromboplastin time (reference value, <35 seconds).

relation to gestational age were significant ($p < 0.025$, analysis of variance; Table II). The need for heparin is plotted and demonstrated in Fig. 1. A tendency toward longer activated partial thromboplastin times was noted as pregnancy proceeded, although the heparin concentration was kept constant. Table III shows that the most striking effects are insufficient treatment in the beginning of pregnancy (53% of the patients) and too high a dose at the end of pregnancy (46%). From the twenty-first to the thirty-fifth week of pregnancy, there was less need to change the heparin dose in the individual patient (Table III). The activated partial thromboplastin times and heparin concentrations are compared in Table IV. Of the 151 samples that were within the stipulated range (heparin concentration, 0.08 to 0.15 IU/ml plasma), 103 (68%) did not have a significantly prolonged activated partial thromboplastin time (i.e., >5 seconds longer than the upper normal limit).

The platelet count was monitored during the entire pregnancy and there was no depression during heparin prophylaxis. The mean level before heparin in the beginning of pregnancy was 189 ± 53 × 10^9/L, 1 week later the level was 263 ± 60 × 10^9/L, and at the last sampling (an average of 5 days before delivery), 252 ± 80 × 10^9/L. In no patient was temporary thrombocytopenia observed.

No depression of mean antithrombin III was found during heparin treatment. The mean level before the start of heparin treatment (mean, week 13) was 93% ±

10% of normal (n = 15) and the mean level before delivery was 94% ± 15% of normal (n = 26). Nevertheless, 32 of 289 samples were <80% of normal. Four patients had a single value <70% of normal. In three of 26 patients, low values (<80% of normal) were found in more than half of the samples. Two of the patients had values <70% of normal before delivery and had plasma substitution.

Comment

In an earlier study of pregnant women with previous thromboembolic complications, we used the activated partial thromboplastin time to monitor heparin administration. We then attempted a prolongation of 5 to 10 seconds above the upper normal range of activated partial thromboplastin time 3 hours after injection, during thrombosis prophylaxis. The mean amount of heparin given was then 25,000 IU/24 hours, divided into two doses. Because two of those patients had multiple fractures of the spine as a result of osteoporosis, we wanted to determine if a lower dose was sufficient to protect the women against thromboembolic complications. In this study we sought to keep the dose as low as possible and still have a measurable amount of heparin in plasma, without rethrombosis and concurrently reducing the risk for fractures of the spine. No thromboembolic or bleeding complications or fractures of the spine were observed in this study. The mean heparin dose was 16,400 IU/24 hours when the concentration of heparin was maintained at approximately 0.1 IU/ml. The results suggest that this low-dose heparin treatment is sufficient for protection against thromboembolic complications. Whether such a low dose might reduce the risk of heparin-induced osteoporosis will be evaluated in a more extensive study currently under compilation.

When a low dose of heparin is used, the activated partial thromboplastin time does not provide accurate monitoring of the heparin treatment. We therefore wanted to use a more sensitive and quantitative method in this study. The amount to be injected was therefore determined by the heparin level, measured as anti–factor Xa activity with a chromogenic substrate method, which has also been shown to reflect the antithrombotic effect.[20, 21] Our goal was to maintain the heparin peak level at approximately 0.1 IU/ml (for practical reasons, within the range 0.08 to 0.15 IU/ml). The heparin dose could be kept low because of measuring the maximal effect. If we had chosen some other time for the control of the anticoagulant effect, the heparin dose would probably have been higher.

The mean heparin dosage when the level was within the stipulated range was found to vary between 215 and 245 IU/kg body weight/24 hours during different periods of pregnancy. An increased need for heparin is observed in the second and early third trimester, with a tendency toward lower need at the end of pregnancy.

Variations among and within individuals in the anticoagulant effect of heparin are great. Two thirds of the samples within the stipulated range showed no prolongation of activated partial thromboplastin time (Table IV), which demonstrated that the activated partial thromboplastin time method is too insensitive for monitoring the anticoagulant effect of low-dose heparin treatment. Thus, if the activated partial thromboplastin time method had been used, larger amounts of heparin would have been required. However, activated partial thromboplastin time was used in connection with delivery since it reflects the total bleeding tendency and can be analyzed immediately.[22] When activated partial thromboplastin time was prolonged >10 seconds above the upper normal level, heparin was not given. Activated partial thromboplastin time was rechecked after 2 to 3 hours; 5000 IU of heparin subcutaneously or dextran was given if no prolongation was found.

No decrease in platelet count was demonstrated. Decreases in platelet count have been described in heparin treatment because of immunologic reactions[13] or could be suspected to occur because of activation and subsequent consumption. There was no mean decrease in antithrombin III, which could have occurred in the case of consumption caused by activation. Furthermore, the amount of heparin was apparently not high enough to cause a decrease in antithrombin III levels.[2, 12]

An increased fibrinopeptide A-level suggests an activation of coagulation. A slight elevation of fibrinopeptide A can be seen during the last trimester even in normal pregnancy.[23, 24] In our study, the heparin treatment seems to have been adequate since the mean fibrinopeptide A level was not higher even at the end of pregnancy compared to normal pregnancy. However, in some samples the fibrinopeptide A levels were somewhat increased, which might indicate that the heparin dosage was inadequate or that an activation had taken place during blood sampling. The latter seems possible because there were no thromboembolic complications. There was no correlation between high fibrinopeptide A levels (>4 ng/ml) and heparin concentration. A correlation could not have been expected because the heparin level reaches its maximum peak at 2 to 3 hours after injection whereas fibrinopeptide A (with a half-life of 2 to 3 minutes) could have greatly decreased during these hours. There were no samples taken for fibrinopeptide A or heparin measurements before injection. The fibrinopeptide A levels for these patients, if they had not been treated with heparin, are unknown.

The presence of lupus anticoagulant and/or cardiolipin antibodies, deficiencies of antithrombin III, and

proteins C and S should be ruled out before heparin prophylaxis is started. Experience with these abnormalities is limited. They require special consideration because of the increased risk of thromboembolic complications; thus higher doses of heparin might be necessary. In addition to anti–factor Xa activity, the platelet count, activated partial thromboplastin time, and antithrombin III should be checked shortly after the start of prophylaxis. Thereafter, it seems sufficient to control the platelet count and anti–factor Xa activity. Activated partial thromboplastin time and the platelet count should be measured just before delivery to exclude an increased bleeding tendency. Antithrombin III levels should also be analyzed; if decreased, plasma transfusions should be considered to reduce the risk for thromboembolic complications when heparin is reduced or withdrawn.

Our findings suggest that in most women without known hereditary blood coagulation defects a heparin dosage of approximately 240 IU/kg body weight per 24 hours, divided into two doses (generally 7500-10,000 IU twice daily), would be sufficient as thromboprophylaxis throughout the pregnancy. This dosage might be used if the anti–factor Xa method is not available. A recent retrospective Swedish study has shown that thrombosis prophylaxis with subcutaneous heparin during pregnancy resulted in a recurrence of thrombosis equal to that which occurred during no thromboprophylaxis. The patients with recurrence of thromboembolic complications during heparin prophylaxis were treated with subcutaneous heparin, 5000 IU twice daily.[25] Because the need for heparin varies among different patients, an individualized treatment would be ideal. We suggest the anti–factor Xa activity method for monitoring because it is the most sensitive current method of heparin analysis. On the basis of our experience, the patients should be checked once a month after the correct dose has been established. More frequent controls are needed during the third trimester. Whether this heparin regimen will further minimize the risk of fractures as a result of heparin-induced osteoporoses is unknown.

We thank Dr. Ulf Hansson for assistance with the statistical analysis and Mrs. Hjördis Johannesson for performing the heparin determinations.

REFERENCES

1. Rudigoz RC, Arnaud MF, Dargent D, Margin P. Le risque Thrombo-embolique pendant la grossesse et le post partum. J Gynecol Obstet Biol Reprod (Paris) 1981;10: 155-61.
2. Hellgren M, Nygård E-B. Long-term therapy with subcutaneous heparin during pregnancy. Gynecol Obstet Invest 1982;13:76-89.
3. Letsky EA. Thrombo-embolism during pregnancy. In: Coagulation problems during pregnancy. Current reviews in obstetrics and gynecology. London: Churchill Livingstone, 1985 vol 10:29-61.
4. Badaracco MA, Vessey MP. Recurrence of venous thromboembolic disease and use of oral contraceptives. Br Med J 1974;1:215-7.
5. Howell R, Fidlu J, Letsky E. The risks of antenatal subcutaneous heparin prophylaxis, a controlled trial. Br J Obstet Gynaecol 1983;90:1124-8.
6. Bonnar J. Venous thromboembolism and pregnancy. Clin Obstet Gynecol 1981;8:455-73.
7. Spearing G, Fraser J, Turner G, et al. Long-term self-administered subcutaneous heparin in pregnancy. Br Med J 1978;12:1457-8.
8. Hahn L. Pulsatile heparin administration in pregnancy: a new approach. Am J Obstet Gynecol 1986;155:283-7.
9. Teien A. Heparin. Studies on anticoagulant effects and eliminations from the blood [Thesis]. University of Oslo, Norway, 1978.
10. Nossel HL. Radioimmunoassay of fibrinopeptides in relation to intravascular coagulation and thrombosis. N Engl J Med 1976;295:428-32.
11. Teien AN, Lie M. Evaluation of an amidolytic heparin assay method. Increased sensitivity by adding purified antithrombin. Thromb Res 1977;10:399-410.
12. Conard J, LeCompte T, Horellon MH, et al. Antithrombin III in patients treated with subcutaneous or intravenous heparin. Thromb Res 1981;22:507-11.
13. Chong BH, Pitney WR, Castaldi PA. Heparin induced thrombocytopenia: association of thrombotic complications with heparin dependent IgG antibody that induces thromboxane synthesis and platelet aggregation. Lancet 1982;4:1246-9.
14. Bonnar J. Haemostasis and coagulation disorders in pregnancy. In: Bloom AL, Thomas DP, eds. Haemostasis and thrombosis. London: Churchill Livingstone, 1987:570-84.
15. Hellgren M. Thromboembolism and pregnancy [Thesis]. Karolinska Institutet, Stockholm, Sweden, 1981.
16. Åstedt B. Trombosbehandling under graviditet. Symposium Hematologiska graviditetsproblem. Lakartidningen 1977;74:1829-30.
17. Dahlman T, Hellgren M, Blombäck M. Changes in blood coagulation and fibrinolysis in the normal puerperium. Gynecol Obstet Invest 1985;20:37-44.
18. Kockum C, Frebelius S. Rapid radioimmunoassay of human fibrinopeptide A—removal of cross-reacting fibrinogen with bentonite. Thromb Res 1980;19:589-98.
19. Abildgaard U, Lie M, Ødegaard OR. Antithrombin (heparin cofactor) assay with "new" chromogenic substrates (S 2238 and chromozym TH). Thromb Res 1977;11:549-53.
20. Holm HA, Abildgaard U, Kalvenes S, et al. The antithrombotic effect of heparin in deep venous thrombosis: relation to four heparin assays. Acta Med Scand 1984; 216:287-93.
21. Ockelford PA, Carter CJ, Mitchell L, et al. Discordance between the anti-Xa activity and the antithrombotic activity of an ultralow molecular weight heparin fraction. Thromb Res 1982;28:401-9.
22. Holm HA, Abildgaard U, Kalvenes S. Heparin assays and bleeding complications in treatment of deep venous thrombosis with particular reference to retroperitoneal bleeding. Thromb Haemost 1985;53:278-81.
23. van Royen EA, ten Cate JW. Generation of a thrombin-like activity in late pregnancy. Thromb Res 1976;8:487-91.
24. Wallmo L, Karlsson K, Teger-Nilsson A-C. Fibrinopeptide A and intravascular coagulation in normotensive and hypertensive pregnancy and parturition. Acta Obstet Gynecol Scand 1984;63:637-40.
25. Tengborn L, Bergqvist D, Mätzsch T, Bergqvist A, Hedner U. Recurrent thromboembolism in pregnancy and puerperium. Am J Obstet Gynecol 1989;160:90-4.

Early pregnancy glycosylated hemoglobin, severity of diabetes, and fetal malformations

Michael J. Lucas, MD, Kenneth J. Leveno, MD, M. Lynne Williams, RN, Philip Raskin, MD, and Peggy J. Whalley, MD

Dallas, Texas

Total percent glycosylated hemoglobin (A_{1a+b+c}) was measured before 16 weeks' gestation in 105 insulin-treated diabetic women enrolled for prenatal care at Parkland Memorial Hospital. Seventy-three of the infants were normal, 14 had malformations, and there were 18 spontaneous abortions. The mean glycosylated hemoglobin level for the entire study group was 9.2%, compared with 9.4% for those pregnancies ending in abortion, 8.9% for those resulting in normal infants, and 10.3% when malformations occurred. The mean glycosylated hemoglobin value for women delivered of normal infants was significantly lower than the mean of those with malformed infants. Ten of the 14 malformations occurred in mothers whose early pregnancy values exceeded the mean of the entire study group. There was also an association between malformations and White classification of maternal diabetes since 10 of the 14 fetal anomalies occurred in women assigned to White Classes C, D, F, H, and R. When the distribution of malformations was analyzed according to both glycosylated hemoglobin level and White Class, there was evidence of an interaction to suggest that hyperglycemia increases the relative risk of fetal malformations when associated with maternal diabetes of longer duration and/or with vascular complications. (AM J OBSTET GYNECOL 1989;161:426-31.)

Key words: Glycosylated hemoglobin, diabetic pregnancy, malformations

A heterogeneous group of skeletal, cardiovascular, neurologic, and urogenital malformations has been estimated to occur two to five times more frequently in pregnancies complicated by diabetes. The cause has been attributed to a variety of factors that include hyperglycemia, diabetic microangiopathy and vascular insufficiency, hypoglycemia, genetic predisposition, and even use of insulin.[1]

In 1978, Leslie et al.[2] reported an association between hyperglycemia as reflected by elevated glycosylated hemoglobin levels at the initial visit in pregnancy and fetal malformations. Miller et al.[3] subsequently reported measurement of hemoglobin A_{1c} before 14 weeks' gestation in 113 diabetic women and found it significantly elevated in the 14 pregnancies that resulted in malformed infants.

A number of descriptive studies of malformations linked to maternal diabetes have also suggested a relationship to White's classification.[4, 5] The observation of increased malformation rates related to advanced White's classification may indicate that the variables used in this classification that include age at onset of diabetes, duration of disease, and microvascular complications also impact normal embryonic development. Alternatively, it is also possible that diabetic women with advanced disease may have higher average glycemia, and that relative hyperglycemia is the only causal factor in fetal malformations.

In this report we describe a prospective investigation of total glycosylated hemoglobin measured before 16 weeks' gestation in 105 insulin-treated women. Early pregnancy maternal hyperglycemia as reflected by elevated glycosylated hemoglobin levels was associated with fetal malformations. However, this effect of maternal hyperglycemia was also related to White's Class, an index of the duration and severity of diabetes. This suggests an interaction between hyperglycemia and these factors in the cause of fetal malformations in pregnancies complicated by diabetes.

Material and methods

Total glycosylated hemoglobin was measured in all pregnant insulin-treated diabetic women initially seeking prenatal care at Parkland Memorial Hospital before 16 weeks' gestation. Women with diabetes antedating pregnancy were classified according to White during in-hospital evaluations that included assessment of renal function and retinal examinations. Those women not diagnosed as diabetic before the index pregnancy, but who were treated with insulin because of fasting

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, and the Department of Internal Medicine, University of Texas Southwestern Medical School.

Received for publication April 6, 1988; revised November 21, 1988; accepted February 16, 1989.

Reprint requests: Michael J. Lucas, MD, Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of Texas Southwestern Medical School, 5323 Harry Hines Blvd., Dallas, TX 75235-9032.

hyperglycemia discovered before enrollment in the study, were separately classified as having gestational diabetes.

Hemoglobin A_{1a+b+c} was determined by ion exchange chromatography with the use of prepacked microcolumns (Quick-column, Helena Laboratory, Beaumont, Tex.) after twice washing the erythrocytes and overnight incubation at 23° C to remove the labile fractions.[6] Hemoglobin A_{1c} was also measured in 58 of the 105 women. This subfraction of glycosylated hemoglobin is frequently reported as an index of prior glycemia in diabetic patients, but was not available early in our study. However, the Pearson's correlation coefficient between the two glycohemoglobin measurements was 0.965. Preprandial plasma glucose levels were measured at 7 AM, 11 AM, 4 PM, and 9 PM during the day of glycosylated hemoglobin measurement and the mean plasma glucose concentration was calculated for each woman.

Infants of insulin-treated diabetic women were admitted to the Special Care Nursery for careful glucose monitoring. Malformations included those clinically diagnosed before discharge of the infant. To reduce the possibility of ascertainment bias, newborn examinations were performed by the pediatric staff without knowledge of the patient's enrollment into the study cohort. Assessment of the spontaneous abortions for malformations was precluded by maceration or incomplete availability of tissue for examination. Chromosomal analysis of aborted tissues was not performed.

Statistical analyses included Fisher's exact test of probability, log-likelihood χ^2, and Student's t test or analysis of variance when appropriate.

Results

Between February 1980 and July 1985, 105 consecutive insulin-treated women were enrolled for prenatal care before 16 weeks' gestation and had glycosylated hemoglobin levels measured. The range of gestational age at entry into the study was from 6 to 16 completed weeks' gestation. There was no apparent relationship between disease classification and gestational age when they were first seen. Twenty-three (22%) of these women were classified as having gestational diabetes because fasting hyperglycemia (fasting plasma glucose level >105 mg/dl; mean, 145 mg/dl) was first diagnosed during the index pregnancy at the time of presentation for prenatal care. The remainder of the women with diabetes antedating pregnancy were classified according to White as follows: Class A (n = 4), Class B (n = 27), Class C (n = 25), Class D (n = 18), and Classes F, H, and R (n = 8).

Total glycosylated hemoglobin (A_{1a+b+c}) was also measured in 14 nondiabetic normal women at a similar gestational age; their mean value was 6.0% (range, 5.0% to 6.9%). In contrast, the mean hemoglobin A_1 of the 105 study patients was 9.2% (range, 5.2% to 14.2%). The mean preprandial plasma glucose concentration on the day of hemoglobin A_1 determination for this group was 151 ± 51 mg/dl (SD). The correlation coefficient between the initial glycosylated hemoglobin and mean preprandial plasma glucose was 0.59 and is similar to other reports on the correlation between same-day measures.[7]

Table I. Pregnancy outcomes of 105 insulin-treated diabetic women according to glycosylated hemoglobin level determined before 16 weeks' gestation

Pregnancy outcome	*Pregnancies*		*Glycosylated hemoglobin (%) (mean ± SD)*
	No.	*%*	
Spontaneous abortion	18	17	9.4 ± 2.3
Malformed infant	14	13	10.3 ± 1.9*
Normal infant	73	70	8.9 ± 2.3
TOTAL	105	100	9.2 ± 2.3

*Comparison of mean A_1 levels between malformed and normal infants, p = 0.05 (Student's t test).

The 105 pregnancies studied were divided into three major outcome groups as shown in Table I. Eighteen women spontaneously aborted. Their mean hemoglobin A_1 level was 9.4% ± 2.3% (SD), which was not significantly different from the mean A_1 hemoglobin values of those pregnancies resulting in malformed (10.3% ± 1.9%) or normal (8.9% + 2.3%) infants (analysis of variance, F probability = 0.125). The mean ± SD gestational age at glycosylated hemoglobin determination for those pregnancies that ended in abortion was 9.8 ± 2.8 weeks compared with 11.4 ± 2 weeks for pregnancies that resulted in malformed infants and 11.6 ± 2.7 weeks for women who were delivered of normal infants. There was no statistical difference in the latter two groups in gestational age at the time of initial examination. Maternal age, race, and previous pregnancy outcomes for the three outcome groups did not differ significantly.

The 18 pregnancies that resulted in abortions were equally divided between missed and spontaneous abortions. There was no significant difference in glycosylated hemoglobin or maternal class of diabetes between these two subgroups. Analysis of the distribution of the abortions according to glycosylated hemoglobin levels by arbitrarily dividing the A_1 range into thirds revealed no association of this outcome with the degree of early pregnancy glycemia. Specifically, six (16%) spontaneous abortions occurred with glycosylated hemoglobin levels ≤8.0%, 8 (17%) with levels from 8.1 to 11.0%, and 4 (20%) in pregnancies with levels >11%. Similarly, analysis of the pregnancies that ended in abortion ac-

Table II. Distribution of 87 pregnancies not complicated by spontaneous abortion in relation to maternal glycosylated hemoglobin level and delivery of malformed infants

Hemoglobin A_1 level (%)	*No. of malformations per No. of pregnancies*	
5.2- 7.1	0/17 (0%)	4/45 (9%)*
7.2- 9.1	4/28 (14%)	
9.2-11.1	6/26 (23%)	10/42 (25%)*
≥11.2	4/16 (25%)	
TOTAL	14/87 (16%)	

*Comparison of malformation occurrence above versus below A_1 level of 9.2%, $p = 0.05$ (Fisher's exact test).

Table III. Incidence of fetal malformations according to White classification of maternal diabetes

Classification of diabetes	*No. of malformations per No. of pregnancies*	
Gestational or Class A	1/24 (4%)	4/46 (9%)*
Class B	3/22 (13%)	
Class C, D	8/34 (24%)	10/41 (24%)*
Classes F, H, R	2/7 (29%)	
TOTAL	14/87 (16%)	

*Comparison of malformation occurrence in Classes A, B, and G versus Classes C, D, F, H, and R; $p = 0.050$ (Fisher's exact test).

cording to classification of maternal diabetes showed no significant correlation.

Excluding those pregnancies that ended in abortion as a group of undetermined malformation outcome, 14 (16%) of the remaining 87 diabetic women were delivered of malformed infants. Eight infants had major malformations that included anencephaly ($n = 2$), hydrocephalus ($n = 1$), encephalocele ($n = 1$), sacral agenesis ($n = 1$), micropenis-hypospadias ($n = 1$), and ventricular septal defects ($n = 2$), one of which was associated with pulmonic stenosis. Minor malformations were diagnosed in six infants and included polydactyly ($n = 2$), partial limb atrophy ($n = 2$), supernumerary nipples ($n = 1$), and preauricular skin tags ($n = 2$). Two infants with testicular hydroceles and one with undescended testes were considered normal variants. The mean glycosylated hemoglobin levels of women delivered of infants with either major or minor malformations were not significantly different (i.e., 10.29% ± 1.8% vs. 10.27% ± 2.7%, respectively). The mean ± SD glycosylated hemoglobin level of the 73 women whose pregnancies resulted in normal infants was 8.9% ± 2.3% compared with 10.3% ± 1.9% for those delivered of infants with major or minor malformations ($p = 0.05$). Table II shows a stratification of pregnancies that progressed beyond abortion according to maternal glycosylated hemoglobin level with the malformation rate for each level indicated. There was a statistically significant distribution ($p = 0.05$) of malformation above the A_1 mean of 9.2%; 10 of the 14 malformations occurred in 42 women whose levels exceeded the mean, compared with four malformations in the 45 women with levels below the mean. The distribution of fetal malformations according to classification of maternal diabetes in the same 87 women is provided in Table III. A significant ($p < 0.05$) increase in malformations was observed in women whose diabetes was diagnosed at ≤19 years of age, those who had diabetes for ≥10 years, and those with vascular complications (White's Classes C, D, F, H, and R).

The relationship between maternal glycosylated hemoglobin level and classification of diabetes was analyzed independently of outcome. Women assigned to White's Classes C, D, F, H, and R were found to have significantly higher hemoglobin A_1 levels early in pregnancy. The mean ± SD glycosylated hemoglobin was 8.8% ± 2.3% for those women who were classified as gestational diabetics or assigned to Classes A and B compared with 9.7% ± 2.1% for those in Class C or greater ($p = 0.05$). Because of this correlation between glycosylated hemoglobin and class of diabetes, the distribution of malformations was tabulated according to both variables; the results are shown in Table IV. There was a tendency for the malformation rate to increase according to both class of maternal diabetes and glycosylated hemoglobin level. The effect of higher A_1 levels was more pronounced in women with advanced diabetes. Log-likelihood χ^2 analysis revealed that the statistically significant distribution of malformations according to A_1 level was attributable primarily to the strength of the association in those women with diabetes of longer duration and/or increased severity. This finding indicates an interaction between the two indices (A_1 and class) in association with the rate of malformation. Major malformations accounted for most of this observed interaction.

The major malformation rate in those diabetic pregnancies that progressed beyond abortion was roughly equivalent according to either the severity of diabetes or glycosylated hemoglobin level when considered separately. Specifically, there was a 17% major malformation rate in women of more advanced diabetes class ($p = 0.02$) and a 14% major malformation rate in those with glycosylated hemoglobin levels above the mean ($p = 0.125$). The rate remained 14% if the upper two thirds of the glycosylated hemoglobin range were considered. An improved discrimination resulted when both disease severity and glycosylated hemoglobin level were considered simultaneously. The major malformation rate was 25% (6 of 24) in those with both ad-

Table IV. Interaction of White classification of maternal diabetes, glycosylated hemoglobin level, and fetal malformations

Hemoglobin A_1	*Glasses A, B, G* (No. of malformations per No. of pregnancies)*	*Classes C, D, F, H, R† (No. of malformations per No. of pregnancies)*	*Total† (No. of malformations per No. of pregnancies)*
≤ 8.0%	1/23 (4%)	0/9 (0%)	1/32 (3%)
8.0-11.0%	2/14 (14%)	7/25 (28%)	9/39 (23%)
≥11.1%	1/9 (11%)	3/7 (43%)	4/16 (25%)
TOTAL	4/46 (9%)	10/41 (24%)	

*Statistical evaluation of distribution of malformations according to A_1 level for class grouping: Log-likelihood χ^2 distribution of malformations × A_1 (NS, $p > 0.5$).

†Statistical evaluation of distribution of malformations according to A_1 level for class grouping: Log-likelihood χ^2 distribution of malformations × A_1 ($p < 0.05$).

vanced diabetes and an A_1 level above the mean of 9.2%. In contrast, there were no major malformations in the 18 pregnancies with an A_1 level >9.2% within the less severe classes of diabetes. Log-likelihood χ^2 analysis of the distribution of major malformations within these four groups (two class categories and two A_1 strata) was also statistically significant ($p < 0.02$, Table V). The finding of a gradient rate of both major and all malformations according to A_1 level in the advanced diabetes group supports the specificity of the interaction.

Table V. Major malformations

Hemoglobin A_1	*Class of diabetes*	*No. of malformations per No. of pregnancies**
$A_1 < 9.2\%$	Classes A, B, G	1/28 (4%)
	Class ≥ C	1/17 (6%)
$A_1 \geq 9.2\%$	Classes A, B, G	0/18 (0%)
	Class ≥ C	6/24 (25%)

*Analysis of the distribution of major malformations within the four categories shown: $p = 0.017$ (log-likelihood χ^2).

Comment

The increased risk of malformations in infants of insulin-treated diabetic mothers is evident in this series, with an overall malformation rate of 16% in those pregnancies that progressed beyond spontaneous abortion. Analysis for possible teratogenic mechanisms suggests that maternal hyperglycemia during organogenesis as reflected by glycosylated hemoglobin levels is clearly a factor. However, increasing severity of diabetes, as indicated by the White classification of diabetes during pregnancy, appears to be at least a cofactor in the increased rate of malformations.

Glycohemoglobin has been established as a reliable index of prior glycemia.[7] The finding of increased malformation rate in association with elevated glycosylated hemoglobin implicates early pregnancy hyperglycemia as a causative factor. Miller et al.[3] demonstrated a relationship between early pregnancy maternal hyperglycemia and fetal malformations. They observed that women who were delivered of infants with major anomalies had significantly higher levels of hemoglobin A_{1c} during the first trimester. Similar results have been reported by other investigators.[8] Importantly, Mills et al.[9] have shown that organogenesis for all the sites in which congenital anomalies linked to diabetes are most common are essentially complete within the first 6 weeks of gestation. These studies provide circumstantial evidence that hyperglycemia during critical weeks of organogenesis plays a teratogenic role in the excess malformations characteristic of pregnancies complicated by diabetes.

Experimental studies support the concept of hyperglycemia as a teratogenic factor. Hyperglycemia induced in laboratory animals during the developmental period of gestation has been shown to increase malformation rates. Conversely, glycemic normalization has been shown to obviate that risk.[10] In a large interventional study in a well-defined human population of pregnant diabetics, Fuhrmann et al.[11] have demonstrated that strict periconceptual glycemic control abolishes the increase in malformations otherwise observed. Jovanovic and Jovanovic[12] also observed no malformations in infants of a group of diabetic mothers with microvascular complications in whom strict pregestational glycemic control was established; they used glycosylated hemoglobin "normalcy" to confirm adequate control.

Our finding of a relationship between malformation rate and the White classification of severity of diabetes during pregnancy as an independent variable is also consistent with previous reports.[4, 11, 13] In a study of 1452 pregnancies in Copenhagen between 1926 and 1972, Pedersen[5] reported a 4.9% incidence of malformations in White Classes A, B, and C compared with 13.7% in Classes D and F. Similarly, Soler et al.[4] reported that among 701 infants born to diabetic women in Bir-

mingham, England, mothers whose diabetes began before 20 years of age had a higher incidence of fetal malformations compared with those whose diabetes was diagnosed at an older age. In a more recent study from Copenhagen, the risk of fetal malformations among 99 insulin-dependent diabetic women examined by ultrasonographic scanning in the seventh and fourteenth weeks of pregnancy was significantly higher in White's Classes D and F compared with the less severe classes of A, B, and C.[14] Comess et al.[15] also observed a small subpopulation among Pima diabetic mothers characterized by onset of diabetes at an early age who were at particular risk for malformed infants. However, not all investigators have found severity or duration of diabetes to significantly influence malformation rates.[3]

It must also be considered that a confounding relationship may exist between hyperglycemia and severity of diabetes, with fetal malformations resulting from greater hyperglycemia in those with more severe disease. Indeed, a correlation between glycosylated hemoglobin and severity of diabetes during pregnancy has been reported.[7] Our finding of a significant glycosylated hemoglobin difference between the two class groups used in our analysis further suggests the presence of such a confounding relationship. However, the interaction we observed between class and A_1 level in association with the relative malformation rate implies that both variables are related to risk, and this has further etiologic implications.

We can only speculate as to why hyperglycemia would have a more potent effect on malformation occurrence in women with disease of longer duration and/or increased severity. A model with hyperglycemia as a "necessary but insufficient" teratogen might best fit the data. For instance, it might be that a metabolic consequence of hyperglycemia is the etiologic culprit, perhaps more likely produced in those with prolonged, advanced diabetes. This would explain the lack of major malformations observed in our 18 cases with high indices of early hyperglycemia but with recent onset of uncomplicated diabetes. Although this model might seem to contradict aforementioned studies, it has been difficult to experimentally isolate hyperglycemia from other variables.[1] Specific products or indirect effects of hyperglycemia such as disturbed glycolysis and arachidonic acid metabolism,[16] specific ketones,[17] and disturbances of insulin-like growth factors[18] have been implicated as embryopathic effectors. Identification of a specific metabolic disturbance associated with hyperglycemia that accurately predicts malformation occurrence would certainly be the ideal marker of risk.

The specific risk factor associated with disease classification is also unidentified. For instance, it is possible that ketosis-prone diabetics (type I) are those more susceptible to the teratogenic effects of hyperglycemia. Type I disease is very likely associated with the advanced class group used in this analysis. The multifactorial nature of malformation risk in association with diabetes is emphasized in the recent report by Mills et al.[19] that describes a lack of relationship between early measures of glycemia and malformation outcome in prospectively followed "insulin-dependent" diabetics. Unfortunately, no analysis by disease classification was reported; because the early enrollment groups had a lower malformation rate than those enrolled late, they nonetheless concluded that relative glycemia cannot be discounted as a risk factor.[19]

If hyperglycemia is a necessary cofactor as a teratogen in diabetes, then it is tempting to use glycohemoglobin in individual risk-factor analysis. However, a direct application of study data can result in some confusion, as exemplified in the use by Jovanovic and Jovanovic of the median A_{1c} value of 8.5% (as determined by Miller et al.[3]) as both a threshold of risk and a limit of "normalcy."[12] Our method of analysis was similar to that of Miller et al., yet our mean A_1 value of 9.2% projects to an A_{1c} value of 7.2% on our regression line between the two measures. This difference might be a result of laboratory technique, study population, or both, but in any case should prompt caution in the use of a specific glycosylated hemoglobin level as an absolute threshold for risk estimation. Furthermore, if "normalcy" is the glycohemoglobin range in pregnant women without diabetes, the highest measure in our study was the equivalent of an A_{1c} value of 5.5%. Although there were no malformations in our series in the 15 diabetic women with glycohemoglobin within this normal range, a clear threshold for malformation occurrence above this point is not apparent in the data. The tendency of malformation rate to increase with rising glycohemoglobin measure was attributable to the distribution within the more severe class group. However, we caution that relatively small numbers occur in each subcategory in this analysis. Therefore the results cannot be reasonably extrapolated except to suggest general trends in relative risk. For example, it would be a mistake to conclude from our results that we are implying that a Class B diabetic is safe from the occurrence of malformation.

In conclusion, it is clear that teratogenic mechanisms are operative during pregnancies that are complicated by diabetes but the mechanism(s) may be more complex than a simple relationship to relative glycemia. Awareness of the interaction between severity of diabetes and early glycohemoglobin measure might improve relative risk estimates in pregnant diabetics, but we emphasize appropriate caution in such clinical applications.

REFERENCES

1. Kalter H, Warkany J. Congenital malformations: etiologic factors and their role in prevention. N Engl J Med 1983;308:424, 491.

2. Leslie RDG, Pyke DA, John PN, White JM. Hemoglobin A_1 in diabetic pregnancy. Lancet 1978;2:958.
3. Miller E, Hare JW, Cloherty JP, et al. Elevated maternal hemoglobin A_{1c} in early pregnancy and major congenital anomalies in infants of diabetic mothers. N Engl J Med 1981;304:1331.
4. Soler NG, Walsh CH, Malins JM. Congenital malformations in infants of diabetic mothers. Q J Med 1976;45:303.
5. Pedersen J. The pregnant diabetic and her newborn. 2nd ed. Baltimore: Williams & Wilkins, 1977:191.
6. Mayer TK, Freedman ZR. Protein glycosylation in diabetes mellitus: a review of laboratory measurements and of their clinical utility. Clin Chim Acta 1983;127;147.
7. O'Shaughnessy R, Russ J, Zuspan FP. Glycosylated hemoglobins and diabetes mellitus in pregnancy. Am J Obstet Gynecol 1979;135:783.
8. Ylinen K, Aula P, Sterman U-H, Kesaniemi-Kuokkanen T, Teramo K. Risk of minor and major fetal malformations in diabetics with high haemoglobin A_{1c} values in early pregnancy. Br Med J 1984;289:345.
9. Mills JL, Baker L, Goldman AS. Malformations in infants of diabetic mothers occur before the seventh gestational week. Diabetes 1979;28:292.
10. Baker L, Egler JM, Klein SH, Goldman AS. Meticulous control of diabetes during organogenesis prevents congenital lumbosacral defects in rats. Diabetes 1981;30:955.
11. Fuhrmann K, Reiher H, Semmler K, Glockner E. The effect of intensified conventional insulin therapy before and during pregnancy on the malformation rate in offspring of diabetic mothers. Exp Clin Endocrinol 1984; 83:173.
12. Jovanovic R, Jovanovic L. Obstetric management when normoglycemia is maintained in diabetic pregnant women with vascular compromise. Am J Obstet Gynecol 1984; 149:617.
13. Molsted-Pedersen L, Tygstrup I, Pedersen J. Congenital malformations in newborn infants of diabetic women. Correlation with maternal diabetic vascular complications. Lancet 1964;1:1124.
14. Pedersen JF, Molsted-Pedersen L. Early fetal growth delay detected by ultrasound marks increased risk of congenital malformation in diabetic pregnancy. Br Med J 1981; 283:269.
15. Comess LJ, Bennett PH, Marr MG, et al. Congenital anomalies and diabetes in the Puma Indians of Arizona. Diabetes 1969;18:471.
16. Pinter E, Reece A, Leranth CZ, et al. Arachidonic acid prevents hyperglycemia associated with yolk sac damage and embryopathy. Am J Obstet Gynecol 1986;155:691.
17. Horton WE, Sadler TW. Effects of maternal diabetes on early embryogenesis: alterations in morphogenesis produced by the ketone body, β-hydroxybutyrate. Diabetes 1983;32:610.
18. Phillips LS, Belosky DC, Reichard LA. Nutrition and somatomedin. V. Action and measurement of somatomedin inhibitor(s) in serum from diabetic rats. VI. Somatomedin activity and somatomedin inhibitory activity in sera from normal and diabetic rats. Endocrinology1979;104:1513.
19. Mills JL, Knopp RH, Simpson JL, et al. Lack of relation of increased malformation rates in infants of diabetic mothers to glycemic control during organogenesis. N Engl J Med 1988;318:671.

Predictive value of increased plasma levels of fibronectin in gestational hypertension

Véronique Ballegeer, MD,[a] Bernard Spitz, MD,[b] Lena Kieckens,[a] Huberte Moreau,[a] André Van Assche, MD,[b] and Désiré Collen, MD, PhD[a]

Leuven, Belgium

Blood pressure, proteinuria, and plasma fibronectin and plasminogen activator inhibitor-1 levels were measured in 120 apparently healthy normotensive primigravid women during the first, second, and third trimesters of pregnancy and 2 days post partum. Thirty-two women developed hypertension (diastolic blood pressure ≥90 mm Hg) that in 17 women was associated with proteinuria (>0.3 gm/day). Fibronectin levels were 83% ± 22% of normal (mean ± SD) during the first trimester and 75% ± 20% at term in the healthy women but increased from 94% ± 36% to 187% ± 36% in the women who developed gestational hypertension (with or without proteinuria) ($p < 0.0001$). Plasminogen activator inhibitor-1 levels increased from 26 ± 19 ng/ml to 110 ± 86 ng/ml in healthy women and from 32 ± 35 ng/ml to 290 ± 90 ng/ml in hypertensive women ($p < 0.0001$). Increased levels of fibronectin at 25 to 36 weeks of pregnancy (≥mean + 2 SD of the healthy women, or >140%) were found in 31 of the 32 women with gestational hypertension with or without proteinuria and in 5 of the 88 healthy women (sensitivity 96%, specificity 94%). Fibronectin levels increased 3.6 ± 1.9 weeks earlier than the onset of hypertension and/or proteinuria. Increased levels of plasminogen activator inhibitor-1 at 25 to 32 weeks (≥280 ng/ml) were found in 16 of the 32 women who developed gestational hypertension with or without proteinuria and in 4 of the 88 healthy women (sensitivity 50%, specificity 95%). We conclude that increased fibronectin levels are the best predictor of gestational hypertension with or without proteinuria and that its level in plasma increases several weeks before the development of hypertension. (Am J Obstet Gynecol 1989;161: 432-6.)

Key words: Fibronectin, plasminogen activator inhibitor-1, preeclampsia, gestational hypertension

Fibronectins are large adhesive glycoproteins (440 kilodaltons) present in many tissues and in most body fluids.[1] The soluble form, usually referred to as plasma fibronectin, is present at a rather high concentration (300 μg/ml) in plasma. The insoluble form, called cellular fibronectin, is produced by fibroblasts, endothelial cells, macrophages, and blood platelets and is a widespread component of connective tissue and basement membranes. A variety of functions have been ascribed to fibronectin, including roles in opsonization, cell adhesion, cell motility, tissue repair, and coagulation.[1-4] Increased levels of fibronectin in plasma have been observed in established gestational proteinuric hypertension.[5-8]

The decreased fibrinolytic activity in patients with gestational proteinuric hypertension[9] has been attributed to an increased plasma level of both plasminogen activator inhibitor-1 (PAI-1) and plasminogen activator inhibitor-2 (PAI-2).[10-12] PAI-1 is secreted by endothelial cells, fibroblasts, blood platelets, and hepatocytes. PAI-1 levels increase steadily after the twentieth week of pregnancy.[11] PAI-2 is produced by placental tissue, it is undetectable in normal plasma but increases markedly during pregnancy.[11]

In this study we investigated the time course of changes in plasma levels during pregnancy of several plasma proteins including fibronectin and PAI-1 in an effort to correlate changes in their concentration with the development of gestational hypertension with or without proteinuria.

Material and methods

Patients. A total of 120 apparently healthy normotensive primigravid women selected from 129 consecutive primigravid women seen at the outpatient clinic of the Department of Obstetrics by B. S. between September 1987 and January 1988 were prospectively included in the study after giving informed consent. The nature of the study was explained in detail. None of the subjects who were requested to participate in the study declined. The blood pressure was measured in

From the Center for Thrombosis and Vascular Research[a] and the Department of Obstetrics and Gynaecology,[b] University of Leuven.
Supported by the Geconcerteerde Onderzoeksacties (project 85-90/78).
Presented in part at the Ninth Congress of the International Committee on Fibrinolysis, Amsterdam, The Netherlands, July 2-6, 1988.
Received for publication June 29, 1988; revised December 13, 1988; accepted February 16, 1989.
Reprint requests: Désiré Collen, MD, PhD, Center for Thrombosis and Vascular Research, Campus Gasthuisberg, Onderwijs en Navorsing, Herestraat 49, B-3000 Leuven, Belgium.

Table I. Blood pressure, uric acid, and plasma protein determinations in primigravid women

Parameter	*Group*	*Weeks of pregnancy* <20	*21-30*	*31-35*	*36-38*	*Day 2 post partum*
Systolic pressure (mm Hg)	N	120 ± 10	119 ± 12	125 ± 9	127 ± 11	125 ± 10
	H	117 ± 10	123 ± 10	130 ± 15	138 ± 10	135 ± 8
Diastolic pressure (mm Hg)	N	70 ± 6	72 ± 5	71 ± 9	72 ± 7	83 ± 4
	H	68 ± 9	75 ± 7 (2)	77 ± 10 (1)	87 ± 8 (11)	87 ± 7
Uric acid (mg/dl)	N	2.7 ± 0.5	3.0 ± 0.6	3.7 ± 0.4	3.7 ± 0.6	4.6 ± 0.8
	H	2.7 ± 0.6	3.2 ± 1.2	4.6 ± 1.5	4.7 ± 1.1	5.7 ± 1.1
Fibronectin (%)	N	83 ± 23	74 ± 26	81 ± 30	75 ± 20	90 ± 31
	H	94 ± 36	130 ± 71 (2)	170 ± 65 (13)	190 ± 36 (16)	170 ± 50
von Willebrand factor (%)	N	130 ± 62	160 ± 90	180 ± 45	210 ± 73	220 ± 60
	H	120 ± 57	180 ± 78	200 ± 53	270 ± 60	280 ± 87
PAI-1 (ng/ml)	N	26 ± 19	68 ± 53	140 ± 105	110 ± 86	30 ± 35
	H	32 ± 35	88 ± 70	200 ± 120	290 ± 91	60 ± 41
PAI-2 (ng/ml)	N	140 ± 47	330 ± 180	540 ± 220	470 ± 240	190 ± 62
	H	150 ± 50	400 ± 230	420 ± 260	700 ± 230	230 ± 140
t-PA (ng/ml)	N	5.6 ± 4.9	5.0 ± 2	6.1 ± 4.6	5.0 ± 1.8	5.2 ± 3.8
	H	8.2 ± 10	5.4 ± 2.4	9.9 ± 8.4	8.2 ± 3.5	9.0 ± 6.0
AT-III (%)	N	86 ± 13	90 ± 13	100 ± 12	98 ± 13	93 ± 12
	H	86 ± 12	96 ± 10	94 ± 11	94 ± 13	92 ± 11
D-Dimer (ng/ml)	N	250 ± 170	450 ± 210	600 ± 300	600 ± 300	730 ± 440
	H	300 ± 180	550 ± 250	630 ± 400	690 ± 230	1000 ± 790

N, Normotensive women (n = 88); *H*, hypertensive women (n = 32); *AT-III*, antithrombin III. Data represent mean ± SD. Numbers within parentheses indicate number of subjects with values >mean + 2 SD of 88 normotensive primigravid women for fibronectin and >90 mm Hg for diastolic blood pressure.

the sitting position. The diastolic pressure was defined as the pressure at which a Korotkoff phase V was obtained. Nine patients with a diastolic blood pressure >90 mm Hg were not entered into the study. Blood pressure was measured before 20 weeks of pregnancy, at 4-week intervals between 20 and 32 weeks, at 2-week intervals between 32 and 36 weeks, and at weekly intervals between 36 weeks and delivery.

Gestational hypertension was defined as a diastolic blood pressure of ≥90 mm Hg on at least two occasions during the second half of pregnancy in a previously normotensive woman. Gestational proteinuric hypertension (or preeclampsia) was defined as increased diastolic blood pressure and ≥0.3 gm proteinuria/day (as defined by the International Society of Study for Hypertension in Pregnancy).[13]

Management of the subjects was determined on the basis of clinical signs and blood pressure only and was not altered on the basis of results of blood analyses. The subjects were asked to refrain from aspirin intake during the study.

Methods. Blood samples were taken four times during pregnancy: before 20 weeks, between 20 and 30 weeks, either at 31 to 35 weeks or 36 to 40 weeks, and 2 days post partum. When increased levels of fibronectin were found, additional blood samples were taken at weekly intervals for monitoring its evolution, but the first positive sample was used in the calculations. Blood was obtained by venipuncture, anticoagulated with buffered citrate (nine parts of blood were mixed with one part 0.1 mol/L sodium citrate (citric acid, pH 4.5 and pH 7.5) and centrifuged for 15 minutes at 3000 *g* at 4° C. The plasma samples were stored at −20° C and analyzed within 14 days.

Fibronectin and von Willebrand factor antigen were measured by Laurell immunoelectrophoresis,[14] with commercial antisera from Behringwerke (Marburg/Lahn, West Germany) and Nordic (Tilburg, The Netherlands), respectively. Concentrations were expressed in percentage of a normal plasma pool. PAI-1,[15] tissue plasminogen activator (t-PA),[16] and fibrin fragment D-dimer[17] levels in plasma were determined with specific enzyme-linked immunosorbent assays. PAI-2 levels were determined with an immunoradiometric assay[18] with reagents provided by Dr. E. K. O. Kruithof (Centre Hospitalier Universitaire du Vaudois, Lausanne, Switzerland). Antithrombin III was determined with a chromogenic substrate assay (Kabi-Vitrum, Brussels, Belgium). Statistical analysis was performed by analysis of variance to assess the changes over time of the various parameters using the SAS program ANOVA (SAS Institute GmbH, Heidelberg, Federal Republic of Germany).

Results

Thirty-two of the 120 healthy initially normotensive primigravid women developed gestational hypertension, which in 17 was associated with proteinuria. In two women the first diastolic blood pressure ≥90 mm Hg was observed between 21 and 30 weeks of preg-

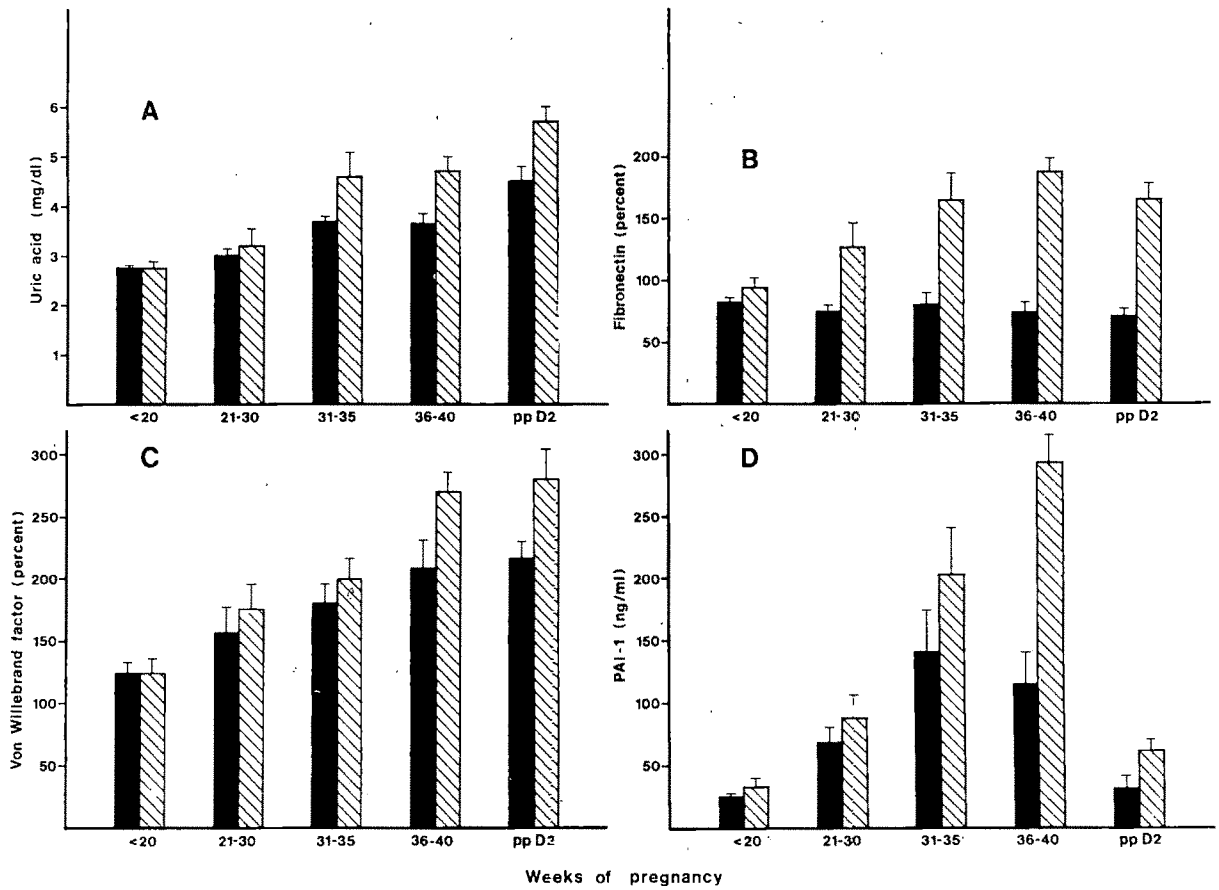


Fig. 1. Plasma levels of uric acid **(A)**, fibronectin **(B)**, von Willebrand factor **(C)**, and PAI-1 **(D)** during pregnancy in 88 normotensive women *(solid bars)* and in 32 women with gestational hypertension *(hatched bars)*. Results in *columns* represent means and *bars* are SEM.

nancy, in 1 between 31 and 35 weeks, in 11 women between 36 and 38 weeks, and in 18 women between 39 weeks and delivery. The mean diastolic blood pressure of the 32 primigravid women with gestational hypertension at the onset of labor was 150 ± 7 mm Hg systolic and 96 ± 6 mm Hg diastolic. Table I summarizes the results of blood pressure, uric acid, and plasma protein determinations in the 32 women who developed gestational hypertension with or without proteinuria, compared with the 88 women who remained normotensive.

In both the normotensive and hypertensive groups PAI-1, PAI-2, von Willebrand factor, and fragment D-dimer levels increased significantly during pregnancy. Significant differences between the normotensive and hypertensive groups were observed for uric acid, fibronectin, von Willebrand factor, t-PA, and PAI-1, which all increased more in the hypertensive group. The p values for all these differences were <0.01. The results of uric acid, fibronectin, von Willebrand factor, and PAI-1 are summarized in Fig. 1. No significant differences between the groups or changes during pregnancy were observed for antithrombin III levels. Multivariate analysis of results obtained at 36 to 40 weeks in the hypertensive group revealed significant correlations ($p < 0.01$) between fibronectin level and diastolic blood pressure ($r = 0.66$), uric acid ($r = 0.50$), von Willebrand factor ($r = 0.49$), and PAI-1 ($r = 0.60$). PAI-1 levels correlated with diastolic blood pressure ($r = 0.82$) and t-PA levels ($r = 0.60$).

The most significant differences between the normotensive and hypertensive groups were observed for the fibronectin and PAI-1 levels, which were already significantly more increased in the hypertensive group at 21 to 30 weeks' gestation ($p < 0.0001$), whereas at that time the differences in von Willebrand factor, t-PA, and uric acid levels were less pronounced. When increases in the fibronectin and PAI-1 levels were used as predictors of the development of gestational hypertension, and an increase of more than 2 SDs above the mean of the normotensive group (≥140% and 280 ng/ml, respectively) was used as the cutoff point, the results summarized in Table II were obtained. None of the 13 subjects with an increased fibronectin level at 31 to 35 weeks had returned to levels <140% when mea-

Table II. Frequency of increased levels of fibronectin and PAI-1 in normal and hypertensive primigravid women

Group	n	Fibronectin		PAI-1	
		Increased	Normal	Increased	Normal
Gestational hypertension	32	31	1	16	16
Control subjects	88	5	83	4	84

Upper limit of normal was defined as mean value + 2 SD of control group. This corresponded to 140% of normal for fibronectin and 280 ng/ml for PAI-1.

sured at 36 to 38 weeks. Increased fibronectin levels predicted the development of gestational hypertension with a sensitivity of 96% and a specificity of 94%. Increased PAI-1 levels predicted the developed of gestational hypertension with a sensitivity of only 50% and a specificity of 95%.

With use of an alternative definition of gestatinoal hypertension as an increase in systolic blood pressure of >30 mm Hg and of diastolic blood pressure of >15 mm Hg, two of the four subjects with an elevated fibronectin level but a diastolic blood pressure of <90 mm Hg would have been allocated to the group with gestational hypertension instead of the normal group. When the onset of the increase in fibronectin level was correlated with the first observation of hypertension, results were obtained as illustrated in Fig. 2. The increase in fibronectin level consistently preceded the increase of the diastolic blood pressure by an average of 3.6 ± 1.9 weeks.

Table III summarizes the results of diastolic blood pressure and fibronectin levels in the 32 women with gestational hypertension, subdivided in groups with or without proteinuria. No significant differences between the subgroups were observed.

Comment

Gestational proteinuric hypertension (preeclampsia) is a primary cause of perinatal mortality and morbidity. Early recognition and treatment with bed rest and aspirin favorably influence outcome.[19] The availability of simple predictors of the subsequent development of gestational hypertension might allow preventive treatment and further improve outcome. A close correlation between increased fibronectin levels and gestational proteinuric hypertension (preeclampsia) has been reported by several authors.[5-8]

Therefore we performed a prospective study in 120 apparently healthy initially normotensive primigravid women to correlate changes in blood pressure and proteinuria with uric acid and with several plasma proteins. As expected, several proteins increased during pregnancy in both groups, including von Willebrand factor, PAI-1, PAI-2, and fibrin fragment D-dimer, whereas before the thirtieth week fibronectin and PAI-1 increased significantly more in the hypertensive group than in the normotensive group. If an increase of fibronectin >140% of normal was used as the cutoff point, subsequent development of gestational hypertension within an average of 3.6 ± 1.9 weeks could be predicted with a sensitivity and specificity of approximately 95%. PAI-1 levels also increased early and more extensively in women with subsequent gestational hypertension, but this index had less predictive value, because PAI-1 levels also increased in the normotensive group.

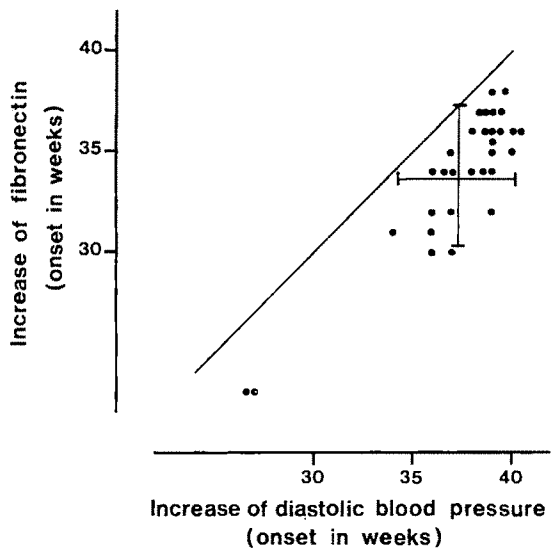


Fig. 2. Correlation between time of onset of increase in fibronectin level (>140%) and of increase of diastolic blood pressure (>90 mm Hg). Data represent duration of pregnancy at time when the first increase in fibronectin (>140%) or in diastolic blood pressure (>90 mm Hg), respectively, was observed in the subjects developing gestational hypertension. Increase in fibronectin level preceded increase in blood pressure by average of 3.6 ± 1.9 weeks (mean ± SD). Horizontal and vertical *bars* represent interval from mean − SD to mean + SD of duration of pregnancy at which increased values were first obtained.

Fibronectin levels may change in association with several disease states. Levels decrease in cases of liver insufficiency, disseminated intravascular coagulation, respiratory distress syndrome, sepsis, and polytrauma and increase in patients with rheumatoid arthritis.[20, 21] None of these diseases occurred in the subjects studied.

We also studied eight pregnant women with chronic hypertension at 31 to 35 weeks of pregnancy and found a normal fibronectin level (data not shown). This suggests that hypertension per se is not associated with increased fibronectin levels during pregnancy.

In conclusion, our results suggest that increased plasma fibronectin levels may be a highly sensitive and

Table III. Diastolic blood pressure and fibronectin levels in 32 primigravid women with gestational hypertension, subdivided according to presence or absence of proteinuria

Parameter	*Proteinuria*	*n*	*Weeks of pregnancy*				*At delivery*
			<20	*21-30*	*31-35*	*36-38*	
Diastolic pressure (mm Hg)	Yes	17	66 ± 8	76 ± 7	79 ± 13	91 ± 5	96 ± 6
	No	15	68 ± 6	71 ± 5	70 ± 8	83 ± 5	92 ± 2
Fibronectin (%)	Yes	17	101 ± 36	150 ± 93	170 ± 96	190 ± 32	—
	No	15	84 ± 22	106 ± 32	150 ± 26	180 ± 52	—

specific predictive test for (proteinuric) gestational hypertension. The index (cutoff point of ≥140% of normal) used in this study was derived from and reapplied to the same patient population and set at a level that allowed maximal separation between normal and hypertensive patients. Therefore prospective studies with the use of this same index are required to confirm its high sensitivity and specificity.

We thank Mr. De Wel (Department of Medische Informatica, K.U., Leuven, Belgium) for his help with the statistical analysis and Dr. E. K. O. Kruithof (Department of Haematology, Centre Hospitalier Universitaire du Vaudois, Lausanne, Switzerland) for providing all essential reagents for the PAI-2 assay.

REFERENCES

1. Mosher DF. Fibronectin. Relevance to haemostasis and thrombosis. In: Colman RW, Hirsh J, Marder VJ, Salzman EW, eds. Hemostasis and thrombosis. Philadelphia: Lippincott, 1987:210.
2. Russell BA. Human fibronectin metabolism. J Clin Invest 1985;76:143-8.
3. Gilboa N, Kaplan JE. Plasma fibronectin enhances fibrinolytic system in vitro. Thromb Haemost 1985;54:639-44.
4. De Groot PG, Sixma JJ. Role of von Willebrand factor in the vessel wall. Semin Thromb Hemost 1987;13:416-24.
5. Stubbs TM, Lazarchick J. Plasma fibronectin levels in preeclampsia: a possible biochemical marker for vascular endothelial damage. AM J OBSTET GYNECOL 1984;150: 885-7.
6. Lazarchick J, Stubbs TM, Romein L, et al. Predictive value of fibronectin levels in normotensive gravid women destined to become preeclamptic. AM J OBSTET GYNECOL 1986;154:1050-2.
7. Saleh AA, Bottoms SF, Welch RA, et al. Preeclampsia, delivery, and the hemostatic system. AM J OBSTET GYNECOL 1987;157:331-6.
8. Graninger W, Tatra G, Pirich K, et al. Low antithrombin III and high plasma fibronectin in preeclampsia. Eur J Obstet Gynecol Reprod Biol 1985;19:223-9.
9. Bonnar J, McNicol GP. Coagulation and fibrinolytic systems in preeclampsia and eclampsia. Br Med J 1971; 2:12-6.
10. Sprengers ED, Kluft C. Plasminogen activator inhibitors. Blood 1987;69:;381-7.
11. Kruithof EKO. Fibrinolysis in pregnancy: a study of plasminogen activator inhibitors. Blood 1987;69:460-6.
12. Wiman B, Csemiczsky G, Marsk L, Robbe H. The fast inhibitor of tissue plasminogen activator in plasma during pregnancy. Thromb Haemost 1984;52:124-6.
13. Davey DA, MacGillivray I. The classification of the hypertensive disorders of pregnancy. Clin Exp Hypertens 1986; B5:97-133.
14. Laurell CB. Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. Anal Biochem 1966;15:45-52.
15. Declerck PJ, Alessi M-C, Verstreken M, Kruithof EKO, Juhan-Vague I, Collen D. Measurement of plasminogen activator inhibitor 1 in biologic fluids with a murine monoclonal antibody-based enzyme-linked immunosorbent assay. Blood 1988;71:220-5.
16. Holvoet P, Cleemput H, Collen D. Assay of human tissue-type plasminogen activator (t-PA) with an enzyme-linked immunosorbent assay (ELISA) based on three murine monoclonal antibodies to t-PA. Thromb Haemost 1985; 54:684-7.
17. Declerck PJ, Mombaerts P, Holvoet P, De Mol M, Collen D. Fibrinolytic response and fibrin D-dimer levels in patients with deep vein thrombosis. Thromb Haemost 1987;58:1024-9.
18. Kruithof EKO, Nicolosa G, Bachmann F. Plasminogen activator inhibitor 1: development of a radioimmunoassay and observations on its plasma concentrations during venous occlusion and after platelet aggregation. Blood 1987;170:1645-53.
19. Lubbe WF. Low-dose aspirin in prevention of toxaemia of pregnancy. Does it have a place? Drugs 1987; 34:515-8.
20. Van de Water III L. Phagocytosis. In: McDonagh J, ed. Plasma fibronectin. Structure and function. Hematology. New York: Marcel Dekker, 1985 vol 5:175.
21. Connolly K, Stecher U, Kaplan JC, Mielens Z, Rostami J, Salens J. The effect of anti-inflammatory drugs on plasma fibronectin. J Rheumatol 1985;12:758-62.

Zinc, cadmium, and hypertension in parturient women

Noam Lazebnik, MD, Betty R. Kuhnert, PhD, and Paul M. Kuhnert, PhD, MD
Cleveland, Ohio

Zinc deficiency and cadmium toxicity have both been implicated in hypertension during pregnancy. The goals of this study were twofold: first, to assess the different zinc indices (plasma, red blood cell zinc, heat-labile alkaline phosphatase, and placental zinc) in normotensive and hypertensive parturients to determine whether they are altered in the different types of hypertension that occur during pregnancy; second, to assess whole-blood cadmium and placental cadmium with regard to hypertension and zinc status. Patients were diagnosec as having chronic hypertension or preeclamptic toxemia and were then further divided into groups on the basis of smoking status. Each patient was matched with a normal control subject based on age, parity, and smoking status. Forty-three hypertensive patients and their matched control subjects were studied. No differences were found in the various zinc indices between chronic hypertensive parturients and normal control subjects. However, in parturients with preeclamptic toxemia, the plasma zinc level was 19% lower than in control subjects ($p < 0.02$); these patients had the lowest plasma zinc level of the three groups. Placental zinc was also 12% lower in patients with preeclamptic toxemia than in control subjects ($p < 0.04$). Whole-blood cadmium and placental cadmium levels did not differ between control subjects or hypertensive patients. However, a significant positive correlation was found between whole-blood cadmium and plasma zinc levels in preeclamptic toxemia ($r = 0.53$; $p < 0.05$). The results support a marginal zinc deficiency in parturients with preeclamptic toxemia but not in those with chronic hypertension. The role of cadmium in the cause of preeclamptic toxemia remains unclear. (Am J Obstet Gynecol 1989;161:437-40.)

Key words: Zinc, cadmium, hypertension, pregnancy

Several previous studies have related abnormally low zinc levels to hypertension during pregnancy.[1-4] Some of these studies included only those patients with preeclamptic toxemia,[2-4] whereas others did not specify the type of hypertensive disorder.[1] Most studies used the maternal plasma zinc level as the sole indicator of zinc status. Recently, Adeniyi[4] reported lowered maternal plasma zinc and leukocyte zinc levels in some patients with preeclamptic toxemia.

Lazebnik et al.[5] and Kuhnert et al.[6,7] have shown that some indices of zinc status depend on smoking, age, or parity. These important factors were not taken into consideration in the early studies of zinc and hypertension. Moreover, other indices of zinc status such as red blood cell zinc, placental zinc, or zinc-dependent enzymes have not been adequately studied in hypertensive parturients.

There is also a need to study the possible role of cadmium in hypertension during pregnancy.[8] The strongest evidence to link cadmium with hypertension is derived from animal studies[2] and tissues at autopsy from hypertensive patients.[9] Cadmium toxicity has been hypothesized as being responsible for preeclamptic toxemia during pregnancy.[2] The question of whether pregnant patients are more sensitive to the toxic effect of cadmium or whether there are higher levels of cadmium in the blood or placenta of these patients has not been answered. Recent reports of interactions between cadmium and zinc in the placenta of normal patients[6,7] suggest that these questions need to be addressed in hypertensive patients.

Our study had two goals. First, we wanted to assess the different zinc indices (plasma zinc, red blood cell zinc, heat-labile alkaline phosphatase, and placental zinc) in normotensive and hypertensive parturient women to determine whether they are altered in the different types of hypertension in pregnancy. Second, we wanted to assess whole-blood cadmium and placental cadmium levels with regard to hypertension and zinc status.

Material and methods

Patients and collection of samples. Eighty-six women were studied at the time of delivery. All were given a full explanation of the purpose, potential benefits, and hazards of the study in order to obtain appropriate informed consent. The study was approved by the human investigation committee of Cleveland Metropolitan General Hospital.

From the Department of Obstetrics and Gynecology of Cleveland Metropolitan General Hospital and the Perinatal Clinical Research Center of Case Western Reserve University.
Supported in part by United States Public Health Service grants 5M01-RR-00210 and R01-HD-17015.
Received for publication August 19, 1988; revised January 3, 1989; accepted February 23, 1989.
Reprint requests: Noam Lazebnik, MD, Department of Obstetrics and Gynecology, Cleveland Metropolitan General Hospital, 3395 Scranton Road, Cleveland, OH 44109.

Table I. Indices of zinc status with regard to type of hypertension and smoking

	Plasma zinc (μg/dl) (mean ± SD)	*Red blood cell zinc (μg/dl) (mean ± SD)*	*Alkaline phosphatase (units/100 ml) (mean ± SD)*	*Placental zinc (μg/gm) (mean ± SD)*
Nonsmokers				
Preeclamptic toxemia (n = 17)	42 ± 10*	1231 ± 125	5.9 ± 2	9.7 ± 1.5*
Matched controls (n = 17)	52 ± 13	1213 ± 120	6.1 ± 2	10.9 ± 1.9
Chronic hypertension (n = 14)	53 ± 11	1307 ± 211	5.5 ± 2	10.3 ± 2.2
Matched controls (n = 14)	52 ± 11	1216 ± 198	5.3 ± 1	10.2 ± 1.7
Smokers				
Preeclamptic toxemia (n = 3)	43 ± 11	1251 ± 83	6.2 ± 1	9.7 ± 1.7
Matched controls (n = 3)	58 ± 7	1306 ± 266	5.5 ± 2	12.1 ± 1.4
Chronic hypertension (n = 9)	63 ± 9	1288 ± 207	5.5 ± 3	11.5 ± 2.8
Matched controls (n = 9)	64 ± 6	1116 ± 142	5.5 ± 2	11.5 ± 2.1

*Significantly different from matched controls, $p < 0.05$.

Patients were classified according to the following criteria. For the diagnosis of preeclamptic toxemia, the patient had to have a blood pressure of 140/90 mm Hg or an increase above baseline of 30 mm Hg in the systolic pressure or 15 mm Hg in the diastolic pressure. Generalized edema and/or proteinuria were used as additional diagnostic criteria. For the diagnosis of chronic hypertension, the patient had to have a blood pressure >140/90 mm Hg detected earlier than 20 weeks of gestation or dating to the prepregnancy period. Patients with diabetes, chronic urinary tract infection, liver diseases, chorioamnionitis, and chronic hypertension with superimposed preeclamptic toxemia were excluded from the study.

An aliquot of 20 ml of venous blood was obtained within an hour of delivery by means of a plastic syringe and stainless steel needle to assess the patient's zinc status. Samples were analyzed for plasma zinc, red blood cell zinc, and heat-labile alkaline phosphatase, a zinc-requiring enzyme. The samples were also analyzed for whole-blood cadmium. The procedures for sample collection and analysis have been reported previously.[5] Smoking status was determined by analysis of plasma thiocyanate, by the clinical record, and by questionnaires. Patients with thiocyanate values <60 μm/L were defined as nonsmokers and those with values >59 μm/L were defined as smokers.

Samples of placental tissue were obtained after several cotyledons had been perfused with 2 L of cold 0.9% saline solution to remove excess blood; the membranes and blood vessels were removed. Each sample was analyzed for placental cadmium and placental zinc.

The patient population was divided into two groups, those parturients with chronic hypertension and parturients with preeclamptic toxemia. The groups were further subdivided into smokers and nonsmokers. Each patient was then matched with a normal control subject based on age, parity, and smoking status.

Data analysis. Statistical analyses were performed with the BMDP statistical software package[10] and a Dec Vax 11/750 computer with a Unix operating system. Data analysis consisted of descriptive statistics, t tests, and simple correlations to obtain an overview of significant differences between the groups (smokers and nonsmokers) and interrelationships between the various biochemical indices. One-way analysis of variance was done to assess significance for a given trace element between the normal parturients, parturients with chronic hypertensive disorder, and pregnancy-induced hypertension. Significance was accepted at $p < 0.05$.

Results

Forty-three hypertensive patients and their matched control subjects were included in the study. Since there were only three parturients with preeclamptic toxemia who smoked, they were not included in the statistical analyses. However, they were included in the tables.

Maternal and placental zinc status, and whole blood and placental cadmium levels are presented in Tables I and II, respectively. The results in Table I suggest that there were no differences in the various zinc indices between chronic hypertensive parturients and normal control subjects in either of the smoking groups. However, in parturients with preeclamptic toxemia, the plasma zinc level was 19% lower than in normal subjects. This was true for nonsmokers and smokers. Patients with preeclamptic toxemia had the lowest plasma zinc level of any group. Neither the red blood cell zinc nor heat-labile alkaline phosphatase levels were different.

Table II. Whole-blood and placental cadmium with regard to type of hypertension and smoking

	Whole-blood cadmium (ng/gm) (mean ± SD)	*Placental cadmium (ng/gm) (mean ± SD)*
Nonsmokers		
Preeclamptic toxemia (n = 17)	0.63 ± 0.3	5.95 ± 2.3
Matched controls (n = 17)	0.60 ± 0.3	5.89 ± 2.3
Chronic hypertension (n = 14)	0.64 ± 0.3	5.66 ± 3.3
Matched controls (n = 14)	0.63 ± 0.3	6.41 ± 2.6
Smokers		
Preeclamptic toxemia (n = 3)	1.98 ± 1.2	10.53 ± 1.5
Matched controls (n = 3)	0.99 ± 0.2	6.86 ± 1.2
Chronic hypertension (n = 9)	1.29 ± 0.4	14.1 ± 8.4
Matched controls (n = 9)	1.22 ± 0.8	11.7 ± 4.0

None of the values were significantly different from matched controls at $p < 0.05$.

There were no significant differences in placental zinc levels between parturients with chronic hypertension and their controls; this was true whether or not they smoked. The level of placental zinc was 12% lower in nonsmoking parturients with preeclamptic toxemia; there was a similar trend in the three smokers with preeclamptic toxemia.

The whole-blood cadmium level, which is shown on Table II, was not different between control patients and those with chronic hypertension or preeclamptic toxemia. When smoking status was taken into account, normal smokers had higher levels of cadmium than normal nonsmokers ($p < 0.01$). Chronic hypertensive patients who smoke also have higher whole blood cadmium levels than chronic hypertensive patients who do not smoke. There were very few parturients with preeclamptic toxemia who smoked. Their cadmium levels were similar to normal and chronic hypertensive smokers.

Placental cadmium levels were not statistically different between normal subjects and hypertensive parturients regardless of the type of hypertensive disorder. However, chronic hypertensive patients who smoked had the highest placental cadmium levels. Smoking significantly increased placental cadmium levels regardless of hypertensive status.

Relationships between levels of maternal whole blood cadmium and the different zinc indices in maternal blood were tested with regression analysis in smokers and nonsmokers. A significant correlation was found between levels of whole blood cadmium and plasma zinc in nonsmoking parturients with preeclamptic toxemia ($r = 0.53$, $p < 0.05$). No correlations with the other zinc indices were found in either normal subjects or chronic hypertensive patients.

Comment

Several studies have been published in the past that suggested a relationship between zinc status and hypertensive disorders during pregnancy.[1-5] Other authors have hypothesized a role for cadmium.[2, 9] The purpose of our study was to comprehensively assess various indices of zinc status and examine potential interactions with cadmium in a carefully diagnosed and matched population. The major findings are that patients with preeclamptic toxemia, but not chronic hypertensive patients, have altered zinc indices and an abnormal relationship between whole blood cadmium and plasma zinc levels. The results support a marginal zinc deficiency in patients with preeclamptic toxemia but not in those with chronic hypertension. The role of cadmium in the cause of preeclamptic toxemia remains unclear.

Until recently, plasma zinc was the only zinc index studied.[1-3] Adeniyi[4] recently reported a relationship between plasma zinc, maternal leukocyte zinc, and preeclamptic toxemia. Compared with normal subjects, pregnant patients who developed preeclamptic toxemia and were delivered of small for gestational age infants had significantly lower levels of maternal leukocyte zinc. No difference was found in patients with preeclamptic toxemia who gave birth to appropriate for gestational age infants. The author did not control for maternal age, parity, or smoking status. Our results also support altered plasma zinc in pregnant patients wtih preeclamptic toxemia. Regardless of smoking status, the level of plasma zinc was 19% lower than in the matched control subjects. Mean maternal plasma zinc levels of 42 μg/dl at term were well below the mean value of 57 μg/dl for plasma zinc previously reported for our population.[5]

In view of the potential role of zinc deficiency in the cause of pregnancy-induced hypertension, the effect of zinc supplementation during pregnancy was studied by Hunt et al.[11] Zinc supplementation reduced the occurrence of pregnancy-induced hypertension in Mexican-American women, but the occurrence of hypertension was not related to serum zinc concentrations.

Placental zinc levels in patients with preeclamptic toxemia were also significantly lower than in matched control subjects. This finding has not been previously reported. In contrast, levels were not different in par-

turients with chronic hypertension. If it is assumed that placental zinc is representative of zinc levels in other organs in the body, then it may be speculated that preeclamptic toxemia is associated with depletion of zinc stores in other body tissues and in the plasma. This supports the hypothesis that suboptimal zinc stores may be the cause of the hypertensive disorder. However, preeclamptic toxemia is primarily a disease of primigravid women, whereas depleted zinc levels are more common in multiparous patients.[7] Nevertheless, there are some clinical and laboratory manifestations common to preeclamptic toxemia and zinc deficiency in laboratory animals.[2] These include abnormal water balance, elevated hematocrit, altered prostaglandin synthesis, bleeding tendency, abnormal platelet aggregation, and growth retardation of the fetus.

Regression analyses were done to determine whether the different zinc indices in blood are influenced by whole-blood cadmium levels. A significant correlation was found between the level of whole-blood cadmium and plasma zinc in nonsmoking parturients with preeclamptic toxemia. This has not been reported previously. No relationships were found in normal parturients or in those with chronic hypertension. Because of these findings, we cannot reject the hypothesis that preeclamptic toxemia results from increased sensitivity to cadmium or to an unexplained cadmium-zinc interaction. However, higher levels of whole-blood or placental cadmium were not found and may not contribute directly to the hypertension disorder.

There are several theories that attempt to explain an association with zinc and cadmium in preeclamptic toxemia. Mukherjee et al.[12] suggested that lowered plasma zinc levels in preeclamptic toxemia are related to the concurrent hypoalbuminemia that occurs in preeclamptic toxemia. Others have suggested that marginal zinc deficiency states associated with pregnancy and a low-protein diet might confer an increased sensitivity to cadmium toxicity.[2] This condition may in turn result in vasoconstriction, sodium retention, altered catecholamine metabolism, increased renin activity, and resultant hypertension. However, if this were true, one would expect an increased incidence of preeclamptic toxemia in smokers who have higher cadmium levels. In contrast, fewer smokers develop preeclamptic toxemia.[13] Our study does not strongly support the cadmium hypothesis because neither whole-blood nor placental cadmium levels differed among normal controls, chronic hypertensive patients, or parturients with preeclamptic toxemia. This was true regardless of smoking status.

There is further support for the role of cadmium in hypertension. It has been suggested that cadmium could influence sodium and calcium metabolism in experimental hypertension.[14] Application of moderate cadmium caused a marked elevation in blood pressure and an increase in sodium retention. Further investigation suggested an increase of intracellular calcium concentrations in rats with cadmium-induced hypertension. The increased intracellular sodium concentrations caused an increase in intracellular calcium concentrations, which in turn induced an increased vascular tone and resulted in an elevation of blood pressure.[14]

We conclude that maternal plasma zinc, placental zinc, and the relationship between whole-blood cadmium and plasma zinc is altered in parturients with preeclamptic toxemia, but not in those with chronic hypertension. Maternal red blood cell zinc and heat-labile alkaline phosphatase are not changed. The results suggest a marginal zinc deficiency in patients with preeclamptic toxemia but not in those with chronic hypertension. The role of cadmium in the cause of preeclamptic toxemia remains unclear.

This study suggests a need for additional well-controlled studies of the relationship between zinc supplementation, tissue and plasma zinc, cadmium levels, and the incidence of preeclamptic toxemia.

REFERENCES

1. Apgar J. Zinc and reproduction. Annu Rev Nutr 1985; 5:43.
2. Chisolm JC, Handorf CR. Zinc, cadmium, metallothionein and progesterone: do they participate in the etiology of pregnancy-induced hypertension? Med Hypotheses 1985;17:231.
3. Cherry FF, Bennet EA, Bazzano GS, Johnson LK, Forsmire GJ, Batson HK. Plasma zinc in hypertension/toxemia and other reproductive variables in adolescent pregnancy. Am J Clin Nutr 1981;34:2367.
4. Adeniyi AAF. The implications of hypozincemia in pregnancy. Acta Obstet Gynecol Scand 1987;66:579.
5. Lazebnik N, Kuhnert BR, Kuhnert PM, Thompson KL. Zinc status, pregnancy complications, and labor abnormalities. Am J Obstet Gynecol 1988;158:161.
6. Kuhnert PM, Kuhnert BR, Erhard P, Brashear WT, Groh-Wargo SL, Webster S. The effect of smoking on placental and fetal zinc status. Am J Obstet Gynecol 1987; 157:1241.
7. Kuhnert BR, Kuhnert PM, Zarlingo TJ. Associations between placental cadmium and zinc and age and parity in pregnant women who smoke. Obstet Gynecol 1988;71:67.
8. Schroeder HA. Cadmium as a factor in hypertension. J Chronic Dis 1965;18:647.
9. Karlicek V, Topolican O. Cadmium in kidneys of patients with essential hypertension and renal hypertension. Cas Lek Cesk 1974;113:41.
10. Dixon WJ, ed. BMDP statistical software. Berkeley: University of California Press, 1983.
11. Hunt IF, Murphy NJ, Cleaver AE, et al. Zinc supplementation during pregnancy: effects on selected blood constituents and on progress and outcome of pregnancy in low-income women of Mexican descent. Am J Clin Nutr 1984;40:508.
12. Mukherjee MD, Sandstead HH, Ratnaparkhi MV, Johnson LK, Milne DB, Stelling HP. Maternal zinc, iron, folic acid and protein nutrition and outcome of pregnancy. Am J Clin Nutr 1984;40:496.
13. Underwood PB, Kesler KF, O'Lane JM, Callagan DW. Parental smoking empirically related to pregnancy outcome. Obstet Gynecol 1967;29:1.
14. Zumkley H, Richter KD, Bartram HP, et al. Influence of low and high cadmium diet in normotensive and hypertensive SH rats. Klin Wochenschr 1985;63:70.

Erythrocyte cation metabolism in preeclampsia

James R. Sowers, MD, Michael B. Zemel, PhD, Richard A. Bronsteen, MD, Paula C. Zemel, PhD, Mary F. Walsh, PhD, Paul R. Standley, MS, and Robert J. Sokol, MD
Detroit, Michigan

To determine if there are abnormalities in cellular cation regulation in pregnancy-induced hypertension, erythrocyte intracellular levels of calcium, magnesium, sodium, and potassium and circulating parathyroid hormone and "endoxin" were examined in 13 women with pregnancy-induced hypertension and 34 control subjects matched for gestational age (≥35 weeks). Both endoxin and parathyroid hormone levels were higher in patients with pregnancy-induced hypertension than in control subjects (endoxin, 294 ± 34 vs. 210 ± 19 pg/ml, $p < 0.05$; parathyroid hormone, 0.65 ± 0.05 vs. 0.60 ± 0.03 ng/ml); the increase was significant only for endoxin. Intracellular calcium was higher in the patients with pregnancy-induced hypertension (0.033 ± 0.010 vs. 0.015 ± 0.001 mEq/L, $p < 0.05$, in the patients with pregnancy-induced hypertension and control patients, respectively) but intracellular sodium, potassium, and magnesium levels were not different. This intracellular calcium elevation may be caused directly by the increase in parathyroid hormone or indirectly by the observed elevation in endoxin. Our data indicate that the observed effect is specific because no changes in intracellular sodium, potassium, or magnesium levels were found. (AM J OBSTET GYNECOL 1989;161:441-5.)

Key words: Preeclampsia, cation metabolism, calcium, endoxin

Studies of the cause and pathogenesis of pregnancy-induced hypertension (PIH) and preeclampsia have reported primary abnormalities related to immunologic, hemodynamic, dietary, endocrine, and uteroplacental blood flow function.[1] However, cellular abnormalities in PIH remain poorly understood. Recently some attention has been directed to the possibility that altered cellular cation metabolism may play a role in the pathogenesis of PIH and preeclampsia.[2-6]

Hypertension is more common in blacks than in whites. Although the mechanisms for these racial differences are not clear, there is accumulating evidence to indicate that differences in cellular cation metabolism may partially be responsible for the racial differences in hypertension.[7] Cooper et al.[7] recently reported that untreated black patients with hypertension have elevations in both erythrocyte sodium and platelet ionized calcium levels. It has been suggested that concomitance of elevated cellular sodium and calcium may result from the elaboration of a natriuretic substance, "endoxin," that inhibits sodium-potassium adenosine triphosphatase activity and thereby elevates intracellular sodium levels.[8] Accordingly, elevations in sodium would then result in decreased sodium-calcium exchange and elevations in cellular calcium.[8] Another possible mechanism for elevated intracellular calcium is suggested by recent observations regarding parathyroid hormone, a known calcium ionophore, in several tissues, including vascular smooth muscle.[9] Our investigative group has recently reported that increasing dietary salt in black patients with hypertension results in significant increases in levels of both parathyroid hormone and intracellular calcium.[10] We have recently found that parathyroid hormone inhibits erythrocyte sodium-potassium adenosine triphosphatase activity (unpublished data). These data suggest that parathyroid hormone may raise levels of intracellular calcium both directly, as a calcium ionophore, and/or indirectly, by inhibiting sodium-potassium adenosine triphosphatase activity, thus reducing sodium-calcium exchange.

This study was undertaken to evaluate erythrocyte cation concentrations and those circulating factors that may regulate intracellular cations in black patients with preeclampsia and normotensive control subjects of similar gestational age.

Material and methods

We evaluated 47 primigravid black women with gestational age ≥35 weeks. Patients with preexisting intrinsic renal disease, diabetes mellitus, or hypertension were excluded from the study. Preeclampsia was defined by standard American College of Obstetricians and Gynecologists criteria for hypertension and proteinuria. Specifically, hypertension was defined as the presence of a blood pressure of 140/90 mm Hg. Proteinuria was defined as excretion of >300 mg of urinary protein/24 hours. None of the patients studied took antihypertensive medication at the time of study. Table

From the Departments of Internal Medicine and Obstetrics and Gynecology, Wayne State University School of Medicine.
Supported in part by National Institutes of Health grant HL40006-01.
Received for publication July 11, 1988; revised December 13, 1988; accepted February 16, 1989.
Reprint requests: James R. Sowers, MD, Wayne State University School of Medicine, UHC 4H, 4201 St. Antoine, Detroit, MI 48201.

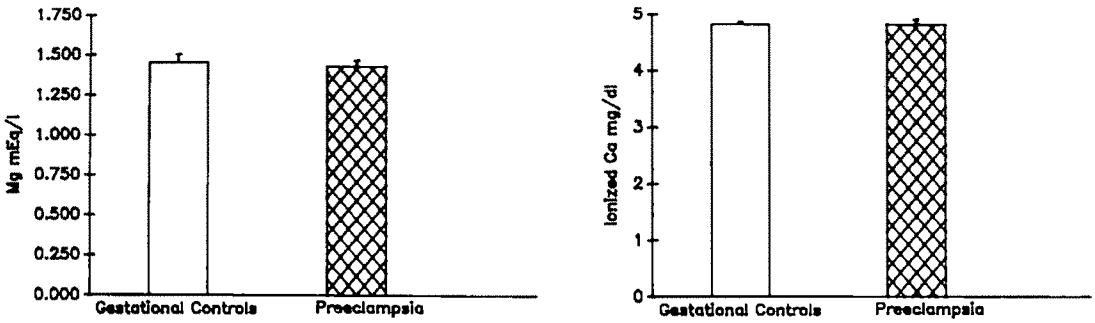


Fig. 1. Serum magnesium and ionized calcium in patients with preeclampsia and normotensive gestational control subjects (mean ± SE).

Table I. Demographic characteristics of study populations

Group	*N*	*Age (yr)*	*Mean gestational age (wk)*	*Mean arterial pressure (mm Hg)*
Control subjects	34	25.2 ± 2.2	38.0 ± 0.5	79.2 ± 1.8
Women with preeclampsia	13	23.7 ± 1.1	38.7 ± 0.6	98.5 ± 3.5*

All data expressed as mean ± SEM.
*$p < 0.001$ versus control subjects.

I describes the key characteristics of the two study groups. Informed consent was received from all patients. This study was approved by our institutional review board.

Blood samples were obtained by venipuncture with the patients in the lateral decubitus position. The ionized calcium level was measured by a calcium-specific electrode standardized to pH 7.40 (model I CAI, Radiometer, Copenhagen, Denmark) in serum obtained under anaerobic conditions and analyzed in duplicate either immediately or in serum frozen up to 24 hours. Serum sodium, potassium, and magnesium levels were measured by atomic absorption spectrophotometry. Heparinized blood for determination of erythrocyte intracellular cation levels was collected in prechilled tubes and prepared as follows. The sample was centrifuged at 1500 *g* for 10 minutes at 4° C, and the plasma and buffy coat were removed by aspiration. The remaining erythrocytes were then washed three times in ice-cold 140 mmol/L choline chloride with 10-minute centrifugations at 1500 *g* and 4° C between washings. After the last washing, the hematocrit and cell count for each sample preparation were determined and aliquots were prepared for determination of cation levels by lysing in one volume of distilled-deionized water. Samples were frozen for subsequent analysis.

Sodium and potassium levels were determined in the aliquots prepared for intracellular cation analysis by flame atomic absorption after dilution in the appropriate diluent to suppress ionization interference (26 mmol/L potassium chloride for sodium determination and 43.5 mmol/L sodium chloride for potassium determination). Erythrocyte calcium and magnesium levels were determined by atomic absorption after dilution of the washed sample in 0.5% lanthanum chloride and 0.001% Triton X-100.

Serum levels of parathyroid hormone were measured by a radioimmunoassay that uses an antibody directed to the midportion of the human parathyroid molecule.[10] Endoxin was assayed by a radioimmunoassay procedure with a Rainin digoxin antibody. All data were evaluated for normality of distribution and equality of variance before determination of differences between sample means by *t* test. All data are expressed as mean ± SEM.

Results

Mean age and gestational age were not different for the patients with preeclampsia and the control groups (Table I). Mean arterial blood pressure was obviously greater in the preeclamptic group. The young black women were all from similar socioeconomic backgrounds and resided in urban Detroit.

Fig. 1 shows that the serum magnesium and plasma ionized calcium levels in the two groups were comparable. Similarly, intracellular levels of sodium, potassium, and magnesium in erythrocyte were not different in the two groups (Fig. 2). However, intracellular calcium levels were twice as high ($p < 0.01$) in the erythrocytes from patients with preeclampsia (0.033 ± 0.010 mEq/L) as in the normotensive control patients (0.015 ± 0.001 mEq/L) (Fig. 2).

Fig. 3 indicates that plasma levels of endoxin were greater ($p < 0.05$) in the patients with preeclampsia

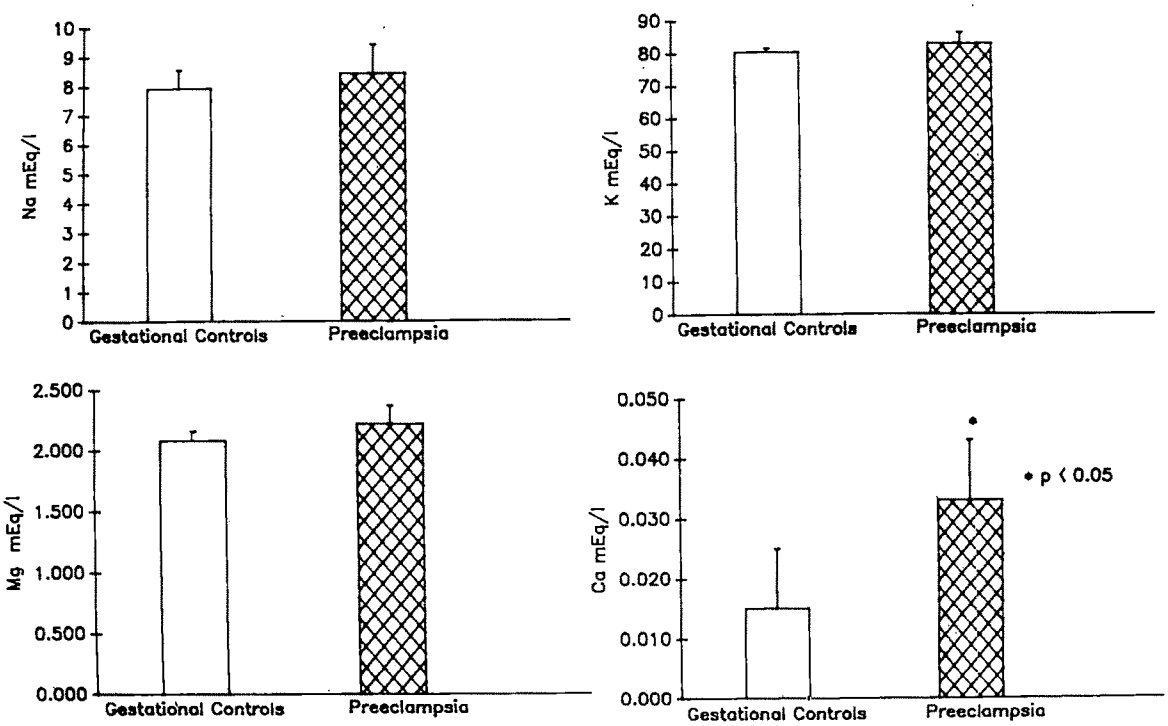


Fig. 2. Erythrocyte cation levels in patients with preeclampsia and gestational control subjects (mean ± SE).

(294 ± 34 pg/ml) than in the gestational control subjects (210 ± 19 pg/ml). Serum parathyroid hormone levels were higher in the patients with preeclampsia (0.65 ± 0.05 ng/ml) than in the normotensive gestational control subjects (0.60 ± 0.03 ng/ml), but this difference only approached statistical significance ($p < 0.1$, Fig. 4).

Comment

This study demonstrates that erythrocyte calcium levels are clearly elevated in young black patients with preeclampsia when compared with normotensive gestational control subjects. This increase was specific for calcium because intracellular concentrations of sodium, potassium, and magnesium were not different for the two study groups. This increase in erythrocyte calcium also appears to be independent of serum ionized calcium levels, which were not altered in the group of patients with preeclampsia.

The increase in erythrocyte calcium levels is similar to the twofold elevation in platelet calcium in preeclampsia that was recently reported.[11] Increased calcium concentrations also have been observed in placental tissue from patients with preeclampsia.[12] If these observations in erythrocyte, platelet, and placental tissue extend to maternal vascular smooth muscle, then increased cellular calcium may be causally related to the enhanced vascular reactivity observed in preeclampsia.[13] Increased vascular constriction related to elevated smooth muscle calcium levels may be a factor in the increased incidence of uteroplacental hypoperfusion seen in preeclampsia. Nitrendipine, a calcium channel blocker, has been shown to markedly inhibit vasoconstrictor responses in human chorionic arteries.[14]

Increased intracellular calcium has been reported in erythrocytes[10] and in platelets[7] of patients with essential hypertension. This increase has been attributed both to intrinsic cellular defects and alterations in circulating factors (e.g., natriuretic factor (endoxin) and parathyroid hormone), which may then directly or indirectly affect increases in intracellular calcium. In our study, levels of endoxin, a digoxin-like inhibitor of sodium-potassium adenosine triphosphatase, were higher in women with preeclampsia than in normotensive gestational control subjects. This factor may increase peripheral vascular resistance through inhibition of sodium-potassium adenosine triphosphatase activity.[8] However, if this were an important operative mechanism for increased cellular calcium levels, then intracellular sodium levels should also be elevated.[8] In this study erythrocyte sodium levels were slightly (not significantly) higher in patients with preeclampsia than in gestational subjects. This observation is in agreement with previous reports that erythrocyte sodium levels are not elevated in PIH[4] or in preeclampsia.[5] Although short-term exposure of erythrocytes to digoxin results in a decrease in sodium-potassium adenosine triphos-

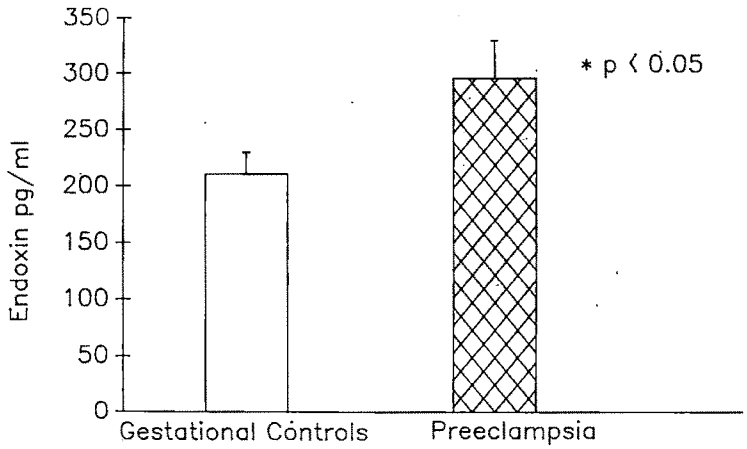


Fig. 3. Plasma endoxin levels in patients with preeclampsia and gestational control subjects (mean ± SE).

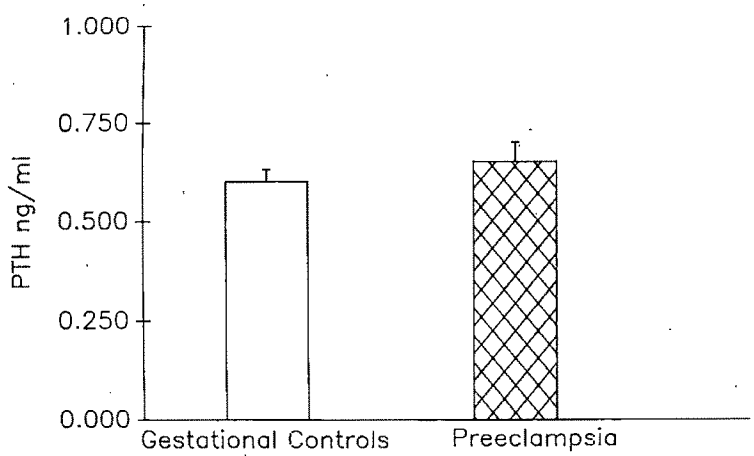


Fig. 4. Serum parathyroid hormone *(PTH)* levels in patients with preeclampsia and gestational control subjects (mean ± SE).

phatase activity and an accompanying increase in cell sodium,[15] long-term exposure is associated with compensatory increases in sodium-potassium adenosine triphosphatase activity and a return to pretreatment cell sodium levels.[15] Accordingly, these observations may explain the minimal increases in erythrocyte sodium levels despite significant elevations in endoxin in preeclampsia.

In this study circulating parathyroid hormone levels were elevated and approached statistical significance in patients with preeclampsia despite the fact that serum concentrations of ionized calcium and magnesium were not different in the two study groups. Our observation of similar calcium levels in patients with preeclampsia and controls is in agreement with previous observations by Richards et al.[16] Normal ionized calcium levels with a tendency toward increased parathyroid hormone levels could reflect decreased calcium intake and/or absorption, or increased urinary excretion of calcium. Regardless of the cause, elevated parathyroid hormone levels may be an important factor with regard to elevated erythrocyte calcium levels. Indeed, parathyroid hormone is a well-defined calcium ionophore in a variety of cell types, including erythrocytes and vascular smooth muscle.[9] Recent data from our laboratory suggest that parathyroid hormone elevations in salt-sensitive hypertensive black patients may be causally related to salt-induced elevations in blood pressure,[10] possibly through the action of parathyroid hormone as a calcium ionophore.[9]

Thus, to the extent that data from erythrocytes may be extrapolated to vascular smooth muscle, our data suggest a potential role for parathyroid hormone in increased cellular uptake of calcium in vascular smooth muscle, thereby increasing vascular reactivity in preeclampsia.

We thank Doris King for her assistance in manuscript preparation. We also thank Barbara Bedford for technical assistance.

REFERENCES

1. Lindheimer MD, Katz AI. Current concepts: hypertension in pregnancy. N Engl J Med 1985;313:675.
2. Kuhnert BR, Kuhnert PM, Murray BA, Sokol RJ. Na/K-Mg-ATPase activity in the placenta and maternal and cord erythrocytes of preeclamptic patients. Am J Obstet Gynecol 1977;127:56.
3. Worley RJ, Heutschel WM, Cormier C, et al. Increased sodium-lithium countertransport in erythrocytes of pregnant women. N Engl J Med 1982;307:412.
4. Weissberg PL, Weaver J, Woods JL, West MJ, Beevers DG. Pregnancy induced hypertension: evidence for increased cell membrane permeability to sodium. Br Med J 1983; 287:709.
5. Aronson JK, Moore MP, Redman CWG, Harper C. Erythrocytic cation transport receptor number and activity in pregnancies complicated by essential hypertension and preeclampsia. Br Med J 1984;288:50.
6. Gallery EDM, Esber RP, Brown MA, Hawkins MR, Ross M. Alterations in erythrocyte Na^+,K^+-cotransport in normal and hypertensive human pregnancy. J Hypertens 1988;6:153.
7. Cooper RS, Shamsi N, Katz S. Intracellular calcium and sodium in hypertensive patients. Hypertension 1987;9: 224-9.
8. Friedman SM. Cellular ionic perturbations in hypertension. J Hypertens 1983;1:109-14.
9. Schleiffer R, Berthelot A, Gairard A. Effects of parathyroid extract on blood pressure and arterial contraction and on Ca exchange in isolated aorta in the rat. Blood Vessels 1979;16:220-1.
10. Zemel MB, Kraniak J, Standley P, Sowers JR. Erythrocyte cation metabolism in salt-sensitive blacks as affected by dietary sodium and calcium. Am J Hypertens 1988;1:386-92.
11. Haller H, Oeney T, Hauck U, Destler A, Philipp T. Increased intracellular free calcium and altered sensitivity to angiotensin II in platelets of preeclamptic women. Montreal: International Society for the Study of Hypertension in Pregnancy, 1988:101.
12. Pitkin RM. Calcium metabolism in pregnancy and the perinatal period: a review. Am J Obstet Gynecol 1985; 151:99.
13. Gant NF, Daley GL, Chand S, Whalley PJ, MacDonald PC. A study of angiotensin II pressor response throughout primigravid pregnancy. J Clin Invest 1973;52:2682.
14. Pipkin FB, Chinnery E. The effect of a calcium antagonist, nitredipine, on the responses of isolated strips of human chorionic plate artery to prostaglandins E_2 and E_1. Br J Pharmacol 1985;86:705.
15. Cumberbatch M, Zarcian K, Davidson C, Morgan DB, Swamini S. The early and late effects of digoxin treatment on the sodium content and Na^+,K^+-ATPase of erythrocytes. Br J Clin Pharmacol 1981;11:565.
16. Richards SR, Nelson DM, Zuspan FP. Calcium levels in normal and hypertensive pregnant patients. Am J Obstet Gynecol 1984;149:168.

Immunoregulatory activity in supernatants from cultures of normal human trophoblast cells of the first trimester

Mrinal K. Sanyal, PhD,[a] Charles J. Brami, MD, Paul Bischof, PhD,[b] Edwina Simmons, MD, Eytan R. Barnea, MD, John M. Dwyer, MD, PhD, and Frederick Naftolin, MD, DPhil
New Haven, Connecticut, and Geneva, Switzerland

Supernatants from isolated trophoblast cell cultures (trophoblastic fluid) derived from first-trimester human placentas were assessed for immunoregulatory activity. Trophoblastic fluid at different days of culture consistently inhibited the blast transformation of allogenic lymphocytes. This suppressor effect had no apparent correlation with biosynthesis of human chorionic gonadotropin by trophoblast cells, since this hormone was secreted into the culture fluid only for the initial 3 days. However, the culture fluids of such purified trophoblast cells contained an immunosuppressive factor, pregnancy-associated plasma protein A, which was measurable throughout the culture period of 8 days. The presence of pregnancy-associated plasma protein A in significant amounts in trophoblastic fluid collected at daily intervals indicated a continuous secretion ability of pregnancy-associated plasma protein A by trophoblast cells in culture parallel to the suppressive immunoregulatory effect of the fluid. Such immunosuppressive effect was absent in the culture fluids of control BeWo malignant trophoblast cells; the BeWo cell culture fluids had markedly reduced levels of pregnancy-associated plasma protein A. The culture supernatant of normal trophoblast cells of placentas from first-trimester pregnancy activated in vitro the generation of a population of suppressor lymphocytes. This effect is generally considered responsible for immunologic tolerance. Therefore demonstration of immunosuppressive effects and the presence of relatively high levels of pregnancy-associated plasma protein A in trophoblastic fluid indicate that such proteins secreted by the trophoblast cells may be important in the local immunoregulatory processes of the fetal allograft. (Am J Obstet Gynecol 1989;161:446-53.)

Key words: Pregnancy-associated plasma protein A, first trimester, immunosuppression, suppressor cell activation, trophoblast cell culture

As an intervening layer of cells between fetal and maternal tissue, the placental trophoblast is likely to play a crucial role in the regulation of maternal immune responses to the fetus (allograft) during pregnancy. The absence of expression of major histocompatibility complex antigens on the syncytiotrophoblast, the ability of the fetal placenta to form complexes with maternal-blocking antibodies, the existence of strong suppressor cell activity in the fetus, and the production of molecules by the trophoblast or maternal decidua that induce immunosuppression in the microenvironment of the placenta are among the suggested mechanisms of immunoregulation that are of considerable current interest.[1, 2]

In this regard, Bischof et al.[3, 4] and Barnea et al.[5] have shown that human decidua and trophoblast cells produce specific proteins, such as pregnancy-associated plasma protein A, which have the capability to inhibit in vitro lymphoblast transformation. It has also been reported that placental steroid and protein hormones could regulate the local immune responses.[6] In addition, immunoglobulins eluted from murine placentas specifically enhanced the tumor allografts of paternal strain placed on a maternal recipient. IgG fractions isolated from human placental eluates prevented the production of a macrophage-inhibiting factor by maternal lymphocytes responding to paternal antigens.[7] Thus it is likely that the microenvironment of the trophoblast interface is an important element in the main-

From the Department of Obstetrics and Gynecology and Internal Medicine,[a] Yale University School of Medicine, and the Department of Obstetrics and Gynecology,[b] University of Geneva.
Supported in part by grants from the National Institutes of Health (BRSG RR 5358 and HD 14587) to M. K. S., a fellowship grant to C. J. B. (DGRST No. 813 ST/BF, Government of France), and the Swiss National Fund for Scientific Research (3.309.082).
Received for publication February 17, 1986; accepted March 10, 1989.
Reprint requests: Mrinal K. Sanyal, PhD, Department of Obstetrics and Gynecology, Yale University School of Medicine, 333 Cedar St., New Haven, CT 06510.

tenance of the fetal allograft. The factor produced by the trophoblast cells may be involved in the regulation of this process during gestation.

Therefore in the present study we examined the immunosuppressive activity on mononuclear cells and also the suppressor T cell activation capability of culture supernatants of isolated and purified first-trimester human placental trophoblast cells (trophoblastic fluid). In addition, we simultaneously assayed concentrations of human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein A in the same trophoblast cell culture supernatant to determine whether trophoblastic cells may produce immunoregulatory factors that may be important for the maintenance of the fetal allograft.

Material and methods

Trophoblast cell culture. Human trophoblast cells of first-trimester pregnancies were isolated and cultured as primary monolayer cultures by the procedures previously described.[8, 9] In this procedure first-trimester (8 to 12 weeks' gestation), normal human placentas of elective termination of pregnancy by suction procedure were washed in ice-cold normal saline solution, and placental villi were dissected under a binocular microscope (magnification ×50). These villus tissues were then exposed for 10 to 20 minutes to a solution of tissue culture medium 199 (Gibco Laboratories, Grand Island, N.Y.) containing 0.2% trypsin, 0.02% ethylenediaminetetraacetic acid, and 2200 Kunitz units/ml of bovine pancreatic deoxyribonuclease (all from Sigma Chemical Co., St. Louis, Mo.). After constant agitation for 10 minutes, dislodged trophoblast cells were collected by centrifugation at 400 × *g* for 10 minutes. They were then purified by centrifugation through a Ficoll-Hypaque gradient and assessed for viability by the trypan blue exclusion test. Aliquots of trophoblast cells (0.5×10^6 cells/ml) were placed into 35 mm diameter Petri dishes containing tissue culture medium (Waymouth's MB 752/1 and Gey's basal salt solution, 55:45), 15% bovine fetal serum, and antibiotics (penicillin and streptomycin, 100 μg/ml). They were then cultured for various intervals ranging from 1 to 12 days at 37° C in 5% CO_2 and air. The culture medium for these trophoblast cells was changed daily.

BeWo malignant trophoblast cells (gift of Dr. Roland Pattillo, Department of Obstetrics and Gynecology, University of Wisconsin, Milwaukee, Wis.) were used as controls in the present study. These cells were dispersed with a solution of Ca^{2+}- and Mg^{2+}-free Earl's basal salt solution containing 0.05% trypsin and 0.02% ethylenediaminetetraacetic acid. They were then cultured with Waymouth's medium (MB 752/1) that was mixed with Gey's balanced salt solution and bovine fetal serum (50:40:10) in an atmosphere of 5% CO_2 and air at 37° C. The culture medium for BeWo cells was changed daily or at 2- to 4-day intervals.

The supernatants of normal trophoblast cell culture (trophoblastic fluid) or those of BeWo cell culture after centrifugation were frozen at −20° C until used for β-hCG and pregnancy-associated plasma protein A radioimmunoassays (RIA) and in vitro immunosuppressive activity assessment by lymphocytes. The fragments of normal villus trophoblasts adjacent to those used for disaggregation were frozen in liquid nitrogen. These frozen villi were sectioned in a cryostat (4 to 6 μm sections) and used for immunohistochemical studies. For histologic controls, paraffin sections of placental villi and cultured normal trophoblast cells or BeWo malignant cells attached to the chamber slides were stained with hematoxylin and eosin.

Biosynthesis of hCG by trophoblast cells. Biosynthesis of hCG by isolated trophoblast cells was ascertained by immunohistochemical tests and RIA measurement of trophoblast cell homogenates and culture fluids. Immunohistochemical studies were performed with rabbit anti-hCG serum (Cappel, Inc., Malvern, Pa.) to identify hCG-bearing cells.[8] All preparations were examined by indirect immunofluorescence binding with diluted antisera (1:100). The incubation period for diluted antisera with tissues and cells was for 4 to 8 hours at 4° C, which was previously found to be optimal. Conjugates of fluorescein isothiocyanate– or tetraethylrhodamine isothiocyanate–labeled sheep antirabbit IgG were used as a second antibody for localization of hCG. The concentration of β-hCG in the trophoblast cell homogenates (10^6 cells/ml in fresh medium) and the trophoblast culture supernatant was estimated by RIA methods with highly specific antisera to β-hCG.

Secretion of pregnancy-associated plasma protein A by trophoblast cells. Pregnancy-associated plasma protein A in homogenized cultured trophoblast cells and culture fluids was measured by an RIA procedure previously described.[10] The antisera used for RIA was produced in the rabbit against a purified preparation of pregnancy-associated plasma protein A. This antiserum was highly specific and had no apparent cross-reactivity with other similar serum proteins.

Responsiveness of human mononuclear cells. Mononuclear cells were isolated from blood donated by healthy adult volunteers by means of standardized techniques. They were recovered by ficoll-Hypaque gradient centrifugation and resuspended in tissue culture media (RPMI 1640) containing 15% bovine fetal serum and antibiotics. For mitogen stimulation of lymphocytes, phytohemagglutinin (Sigma Chemical Co., St. Louis, Mo.) was added to cells suspended at a concentration of 2×10^6 cells/ml. The three phytohemagglutinin doses were chosen in this study to provide the mitogenic stimulation for normal lymphocytes. The fi-

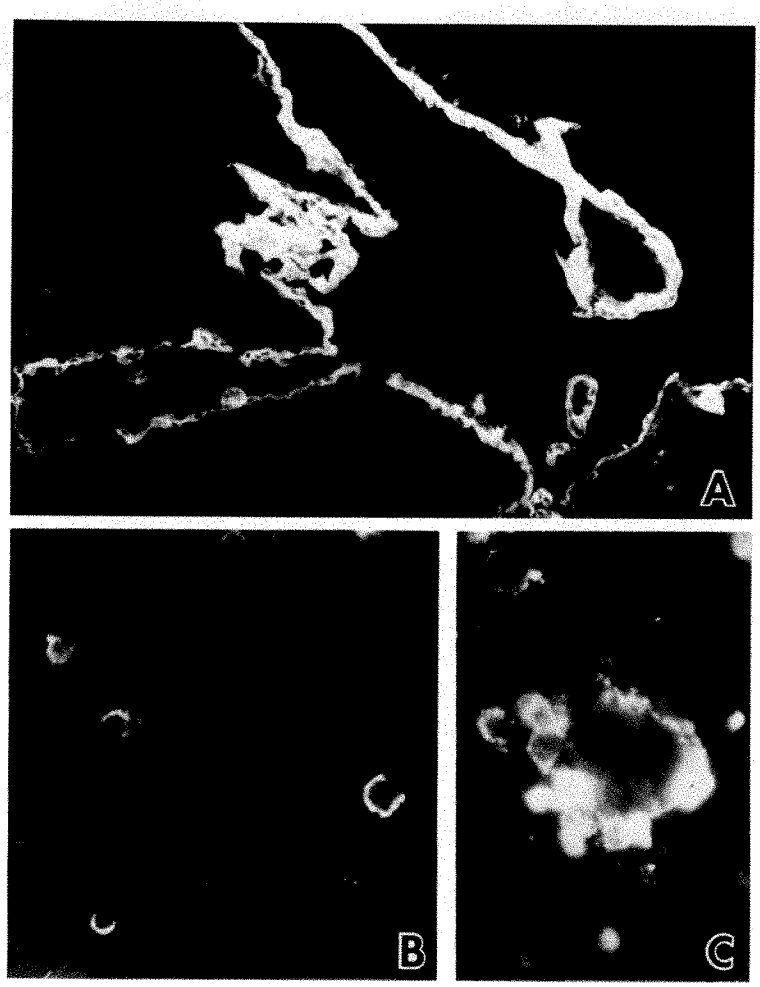


Fig. 1. A, Frozen section of placental villi stained for immunofluorescence localization of hCG by conjugates of anti-hCG and tetraethylrhodamine isothiocyanate. Intense positive staining for hCG could be seen on the outer villus syncytiotrophoblast layer. (×250.) **B,** Dislodged and purified polygonal trophoblast cells treated with conjugates of anti-hCG and tetraethylrhodamine isothiocyanate showed fluorescence staining, indicating presence of hCG in such cells. (×450.) **C,** Multinucleated trophoblast derived cell(s) also stained with anti-hCG and tetraethylrhodamine isothiocyanate conjugate showed localization of hCG. (×450.)

nal concentrations of these three phytohemagglutinin doses in the culture wells were 5 μg/ml (high), 1 μg/ml (medium), and 0.25 μg/ml (low). The culture supernatant from normal trophoblast cell culture (trophoblastic fluid) or that from BeWo cells (25 μl/well) or the same volume of culture medium as a control was added at the initiation of the mitogen stimulation.

After 3 days of culture under standard conditions, 0.5 μCi (tritiated methyl)-thymidine (New England Nuclear, Inc., Boston, Mass., SA 79 Ci/mmol/L) was added to each culture well. One day later the cultured cells were harvested and prepared for tritiated thymidine incorporation assays by means of a liquid scintillation counter. Background counts per minute (cpm) from control cultures without phytohemagglutinin were subtracted from those of phytohemagglutinin-stimulated cultures. The mean counts per minute and standard error of the mean (SEM) from triplicate values of each well were calculated. The suppression of thymidine incorporation into mononuclear cells is expressed as the percentage reduction in the response of phytohemagglutinin, which was calculated with the following formula[11]:

$$\% \text{ Suppression} = 1 - \frac{(\text{cpm responders} + \text{trophoblastic fluid}) \times 100}{(\text{cpm responders} + \text{culture medium})}$$

Generation of suppressor cell activity. To determine whether trophoblastic fluid could produce suppressor T cell activity for immunoregulation, lymphocytes treated with supernatant from normal trophoblast cell cultures were examined for their ability to reduce the

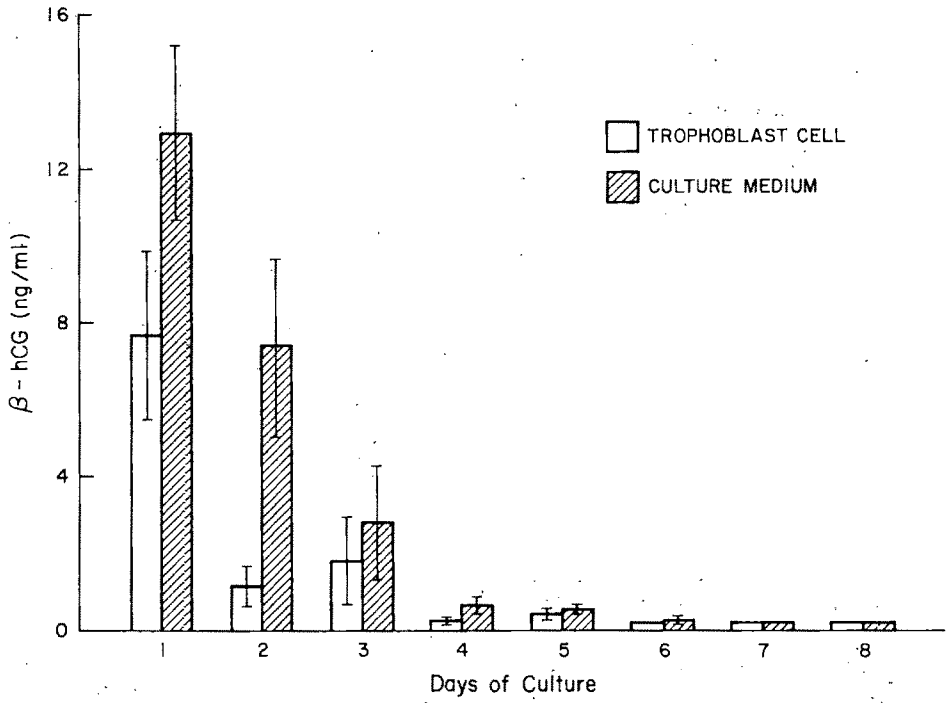


Fig. 2. Concentrations of β-hCG in cells and supernatants of trophoblast cell cultures collected at 24-hour intervals after change of medium. Approximately 10^6 cells/ml were homogenized with culture medium for assessment of β-hCG concentration in cells. Concentrations of this hormone declined steadily with prolongation of culture periods. Hormone concentration in trophoblastic fluid was reduced to a level undetectable by RIA procedures after 3 days of culture.

mitogenic response of mononuclear cells to phytohemagglutinin.[11] Lymphocytes (10×10^6/ml) were cultured with or without supernatants from normal trophoblast cell cultures. The culture volume consisted of 3 ml of suspended lymphocytes for every 1 ml of trophoblastic fluid. After 72 hours, these cells were removed, resuspended in fresh medium, and incubated with mitomycin (Sigma Chemical Co.) at 100 μg/ml concentration for 30 minutes at 37° C. These treated cells were washed four times with buffered salt solution and then assessed for viability by the eosin exclusion procedure. Approximately 85% of mitomycin-treated cells were viable, and these cells were then cocultured with responder mononuclear cells for suppressor cell assays. These assays were performed in microculture plates with wells containing a total of 10^5 normal fresh responder mononuclear cells and an equal number of mitomycin-treated control cells or mitomycin- and trophoblastic fluid–treated lymphocytes. After 72 hours of exposure to phytohemagglutinin (1 μg/ml), cell cultures were exposed to a pulse of tritiated thymidine and terminated as described earlier. Suppression was expressed as the percentage reduction in response to phytohemagglutinin as calculated by the following formula:

$$\% \text{ Suppression} = 1 - \frac{(\text{cpm responders} + \text{TF stimulated cells}) \times 100}{(\text{cpm responders} + \text{control cells})}$$

Results

Characterization of trophoblastic cells and secretion of pregnancy-associated plasma protein A in culture. As described in previous studies, three cell types were recognizable in the cultures of isolated normal trophoblast cells of first-trimester human placentas.[8,9] The most abundant cell type was a medium-sized round or polygonal epithelioid cell with a large nucleus. The second category consisted of larger cells containing many nuclei. The fibroblast cells were greatly reduced in number initially, but prolongation of the culture period tends to increase the population of such cells. The rabbit anti-hCG and tetraethylrhodamine isothiocyanate (or fluorescein isothiocyanate) conjugates clearly stained the intact villus trophoblastic layer of cells (Fig. 1, *A*) and the epithelioid and multinucleated cells during the first 3 days of culture (Fig. 1, *B* and *C*). The BeWo cells also exhibited similar immunostaining with anti-hCG and fluorescein isothiocyanate conjugates.

The levels of β-hCG in cultured normal trophoblast cell homogenates and in trophoblastic fluids during dif-

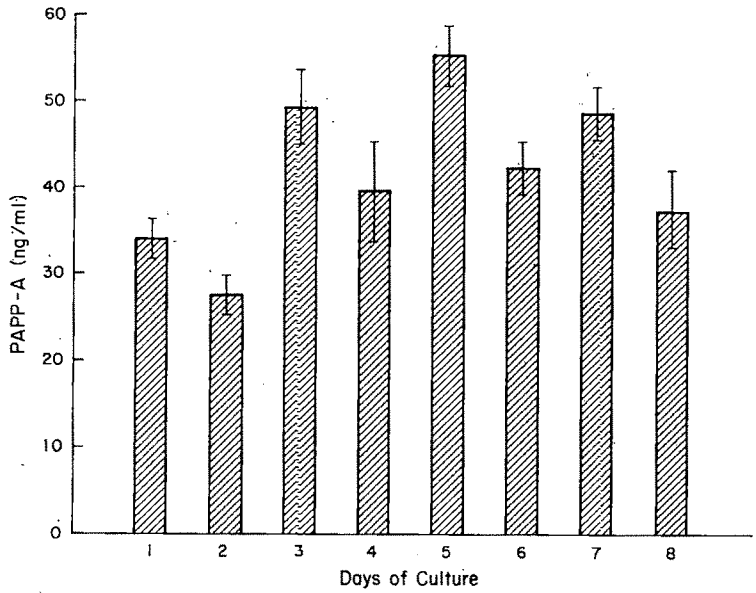


Fig. 3. Pregnancy-associated plasma protein A *(PAPP-A)* concentrations in supernatants of the same trophoblast cell cultures shown in Fig. 2. Undiminished concentration of pregnancy-associated plasma protein A during different days of cultures (24-hour intervals) indicates secretion capability by isolated trophoblast cells of such proteins during culture for prolonged periods.

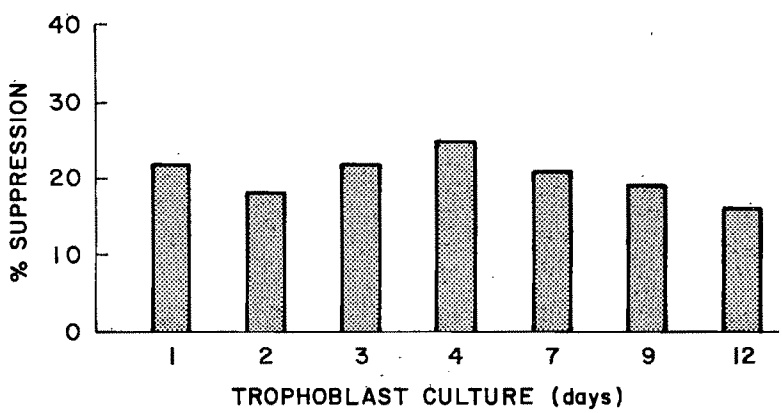


Fig. 4. Immunosuppressive activity of supernatants of trophoblast cell cultures at different days of culture. Immunosuppressive activity in 24-hour culture supernatants after change of medium is approximately 14% to 20% in all culture samples tested. This shows that immunosuppressive activity and pregnancy-associated plasma protein A values in trophoblast cell culture fluid show similar curves and persist during extended cultures as long as 12 days.

ferent days of culture measured by RIA are shown in Fig. 2. The secretion and biosynthetic ability of hCG progressively declined in such cells during culture, as shown by reduced levels of the hormone in trophoblastic fluid, especially after 3 days of culture. However, the increase in pregnancy-associated plasma protein A concentration in trophoblastic fluids, estimated at 24-hour intervals, was unchanged, showing undiminished secretion ability of the protein by such cells in culture for a prolonged period of 8 days (Fig. 3). The pattern of continuous pregnancy-associated plasma protein A secretion contrasted with the decline in hCG levels in the trophoblastic fluid during an extended period of culture.

The BeWo cell culture fluid with a cell population similar to that of normal trophoblast cells produced significant quantities of hCG continuously. However, the levels of pregnancy-associated plasma protein A were reduced in the BeWo cell culture fluids, and at confluency cultures pregnancy-associated plasma protein A concentrations were consistently <14 ng/ml in replicate cultures. The levels of pregnancy-associated

Table I. Effect of trophoblast cell culture supernatants on responsiveness to phytohemagglutinin stimulation of lymphocytes*

Responder cells alone†	*Responder cells + normal trophoblast culture fluid*	*% suppression*
38,997 ± 603	33,315 ± 1,696	15
39,607 ± 2,536	33,944 ± 1,905	15
25,267 ± 992	20,967 ± 717	18
27,640 ± 1,228	25,493 ± 193	12
31,417 ± 1,311	24,818 ± 2,688	22
30,532 ± 2,124	25,155 ± 372	18
27,854 ± 832	25,057 ± 254	11
19,135 ± 222	16,417 ± 1,734	15

*Mean counts per minute ± SEM (tritiated thymidine incorporation).

†Responder lymphocytes from eight different subjects were studied.

plasma protein A in the initial culture medium containing bovine fetal serum were low (<5 ng/ml).

Effects of trophoblastic culture fluids on allogenic lymphocytes. Immunosuppression studies with trophoblastic culture fluids and lymphocytes were performed using the following three experimental protocols: (1) supernatant samples of trophoblast cell cultured for 3 to 5 days from different placentas were combined and used for blast-transformation of one population of allogenic lymphocytes; (2) the effects of combined trophoblastic fluid from one placental trophoblast cell culture on lymphocytes from different subjects were assessed; and (3) immunosuppressive effects were measured in trophoblastic fluid collected on different days of culture, at 24-hour intervals in cultures for prolonged periods.

In the first series, trophoblastic fluid from nine different placentas were used for blast-transformation of one population of allogenic lymphocytes. Trophoblastic fluid from all nine placental tissues significantly inhibited blast-transformation. The mean counts per minute ± SEM for all control responder cells was 25,672 ± 992, whereas for trophoblastic fluid collected on the same days of culture, the overall response of the same cells was 21,337 ± 763. This represented a significant reduction in tritiated thymidine incorporation into allogenic lymphocytes after trophoblastic fluid treatment (16.1% ± 3.0% suppression, $p < 0.01$, t test). In the second series of experiments, the effects of combined trophoblastic fluid from each placental culture of 3 to 5 days assayed on different populations of phytohemagglutinin-stimulated lymphocytes isolated from eight different subjects are illustrated in Table I. Control cultures gave an average value of 30,056 ± 2258 cpm (mean ± SEM), and this was significantly reduced to 25,646 ± 1921 cpm by trophoblastic fluid ($p < 0.01$, paired t test), which represented an average of 15.0% ± 1.7% suppression. Finally, experiments that used samples of trophoblast cells and fluid at different periods of culture also showed that the immunosuppressive effects of the supernatants of primary trophoblast cultures evaluated by the same methods persisted throughout the culture period tested (days 1 to 12) at a level between 15% and 20% as shown in Fig. 4.

Table II. Effect of BeWo trophoblastic cell culture fluid on phytohemagglutinin stimulation of normal lymphocytes*

Responder cells alone†	*Responder cells + BeWo cell culture fluid*	*% suppression*
24,633 ± 1,018	23,760 ± 931	ND
29,173 ± 2,380	32,905 ± 533	ND
22,524 ± 1,953	22,053 ± 1,352	ND
25,834 ± 4,900	25,582 ± 2,402	ND
20,496 ± 1,235	21,951 ± 1,889	ND
24,638 ± 805	24,442 ± 1,992	ND

ND, None detectable.

*Mean counts per minute ± SEM.

†Responder lymphocytes from six different subjects.

Table III. Spontaneous suppressor cell activity of mononuclear cells stimulated by trophoblast cell culture supernatants*

Responder cells† + control cells	*Responder cells + TF-stimulated cells*	*% suppression*
15,405 ± 1,502	6,132 ± 209	61
15,721 ± 736	6,898 ± 323	57
15,664 ± 1,757	7,190 ± 1,067	55
14,972 ± 777	11,106 ± 644	26
17,095 ± 74	13,174 ± 588	23
11,504 ± 487	10,692 ± 1,235	8

TF, Trophoblast cell culture fluid.

*Mean counts per minute ± SEM (tritiated thymidine incorporation).

†Responder lymphocyte cells from six of 11 subjects studied with positive suppression.

In contrast to normal trophoblast cells of the first trimester, the BeWo malignant cell culture fluid did not show immunosuppression under identical experimental conditions (Table II). Responder lymphocytes from six different subjects were used, and no significant immunosuppression was demonstrated by tritiated thymidine incorporation procedures.

Induction of suppressor cell activity by trophoblastic fluid. In this study responder lymphocytes were collected from 11 different subjects at random. Preincubation of mononuclear cells with trophoblastic fluid from normal trophoblast culture enhanced suppressor activity in six of 11 subjects (Table III). Cells from the remaining five subjects in our study group were non-

responsive; lymphocytes exposed to the same trophoblastic fluid did not reduce the mitogen-induced transformation of allogenic responder cells. The degree of suppression in the six subjects also was variable and ranged from 8% to 61%. In cells from three of six subjects, more than 50% suppression could be achieved under the conditions used in our assay system. The overall average suppression in these six subjects was 38.33% ± 8.24%, and the mean ± SEM of tritiated thymidine incorporation of controls (15,060 ± 701 cpm) and treated subjects (9199 ± 1059 cpm) were significantly different in the paired t test ($p < 0.01$).

Comment

The effects of various pregnancy-related serum proteins and hormones on maternal immune response have been previously studied. Results have shown that they may play an important role in immunoregulatory processes during pregnancy. The specific factors involved and the mechanisms of immunoregulation at the cellular levels are still unclear. The suggestion that placental peptide factors, especially pregnancy-associated serum protein A, may significantly contribute in immunoregulation has been a compelling possibility.[3,4] The present study demonstrated the possible immunoregulation capabilities of trophoblast cells of the human first-trimester pregnancy by such factors. Isolated trophoblast cells from this period of pregnancy secreted pregnancy-associated plasma protein A in culture, and the same culture fluid has been found to be highly immunosuppressive. In addition, this study also showed that trophoblast cell-culture fluid could generate suppressor cells,which indicates the possibility of participation of trophoblast cells in local immunoregulation at the interface between fetal and maternal compartments in such processes.

In cultures of isolated trophoblast cells of the first trimester the polygonal epithelioid cells were presumably cytotrophoblasts, and the multinucleated cells were formed by aggregation of the trophoblast cells resembling syncytiotrophoblast cells. The trophoblastic origin of these cells in the present study has been ascertained by their morphology and hCG biosynthesis capability (Figs. 1 and 2). These cells also secreted substances in the culture fluid that consistently reduced the responsiveness of lymphocytes to phytohemagglutinin. This in vitro suppressor effect of trophoblastic fluid was observed in all the monolayer cultures, even for prolonged cultures for 12 days, when hCG hormone biosynthesis had ceased (Fig. 4). Since several trophoblast cell types were present in normal first-trimester trophoblastic cultures; which cell types were responsible for immunoregulatory effects remain uncertain.

The immunosuppressive effect was unrelated to hCG secretion capability, since both the cell types of our study; normal trophoblast cells and BeWo malignant cells synthesized this hormone (Fig. 2). The culture fluids of BeWo cells had no detectable immunosuppressive effect, and their pregnancy-associated plasma protein A levels were relatively low (Table II). In contrast, the immunosuppressive effect of trophoblastic fluid from normal trophoblast cells correlated with the pregnancy-associated plasma protein A secretion capability throughout the culture period (Figs. 3 and 4). Since pure pregnancy-associated plasma protein A preparations have also been shown to be immunosuppressive by the same allogenic lymphocyte tests,[3] it is therefore likely that pregnancy-associated plasma protein A in trophoblastic fluid may have contributed in part to the immunosuppressive effects of trophoblastic fluid. The data of the present study showing that the trophoblastic fluids consistently reduced phytohemagglutinin-stimulated proliferative response of lymphocyte samples support this suggestion. Trophoblastic fluid produced a 15% to 20% reduction in tritiated thymidine incorporation into phytohemagglutinin-stimulated lymphocytes, indicating significant overall immunosuppressive capabilities of trophoblastic fluid in all the samples tested (Table I). In these immunosuppression studies, both the suppressor and effector cells were from one patient and had identical human lymphocyte antigen (HLA) haplotypes.

In our subsequent studies of the ability of trophoblastic fluid to activate suppressor cell activity, the indicator cultures contain responder lymphocytes that were randomly selected and may have expressed human lymphocyte antigen haplotypes that were different from those of the suppressor cells (Table III). In this study the reduced responsiveness of lymphocytes to phytohemagglutinin in the presence of the trophoblastic fluid–treated cells was recorded in six of 11 subjects. This response measures suppressor cell activity that exceeds the normal spontaneous suppressor cell activity of the control cultures; no suppressor cell activity was generated by 3 days of allogenic coculture in these indicator systems.[11] It is possible that the variable degree of genetic differences between the human lymphocyte antigen haplotypes was responsible for the negative results in five study subjects. Nevertheless, the possibility of suppressor T-lymphocyte activation by secretory products derived from first-trimester trophoblast cells was clear from these data.

It is now established that major histocompatibility complex class 1 antigens (HLA-A, HLA-B, and HLA-C) are not expressed in the human syncytiotrophoblast cells but messenger ribonucleic acid for them could be expressed.[12,13] HLA-DR antigen was also not expressed normally in trophoblastic tissues, but under specific culture conditions or transformed neoplastic condition,

they may be expressed in such tissues.[8, 14] HLA-DR antigen is responsible for activation of T-lymphocytes,[15] and this process may be highly significant during early gestation when syncytiotrophoblasts were absent or reduced over the cytotrophoblast cell layer. The trophoblast and decidual cells produce immunoregulatory molecules that may generate suppressor cells to help embryo implantation and prevent rejection of the conceptus.[16] Presumably pregnancy-associated plasma protein A is one such factor produced by human trophoblast and decidual cells,[4] and combined effects of such factors were responsible for the suppressor effects of the trophoblast cells.

Thus it seems likely that human trophoblast cells secreted various factors that may be important in maintaining the fetal allograft. Local inhibition of immune mechanisms and activation of suppressor lymphocytes by placental factor(s) make an attractive hypothesis, since they would minimize the risk of general immunosuppression that could produce adverse effects on both the fetus and mother during pregnancy. Our present findings suggest that suppressor T cells are perhaps produced by factors present in the trophoblastic fluids, although the specific role of components in trophoblastic fluid was not ascertained for this process in this study. Suppressor T cells are responsible for immunologic tolerance in various cells and are now considered essential in the homeostatic mechanisms for responsiveness against self-components. The trophoblast cells are also the site of synthesis for various specific proteins and steroidal hormones that are immunosuppressive.[6] Therefore a combination of various factors generated by trophoblast cell interface between the maternal and fetal compartments may be critical in local immunoregulation for maintenance of the fetal allograft.

We appreciate critical appraisal of the study by Dr. Fred S. Kantor. We thank Ms. Charline Johnson for her invaluable technical assistance in this study. We also thank Ms. Diana Ruffner for her assistance in preparing this manuscript.

REFERENCES

1. Beer AE, Billingham RE. Maternal immunological recognition mechanisms during pregnancy. In: Maternal recognition of pregnancy. Amsterdam: Excerpta Medica, 1979:293-322.
2. Billington WD. Immunological aspects of implantation and fetal survival: the central role of trophoblast. Curr Top Dev Biol 1987;23:209-31.
3. Bischof P, Lauber K, deWustemberger B, Girard JP. Inhibition of lymphocyte transformation of pregnancy-associated plasma protein-A (PAPP-A). J Clin Lab Immunol 1982;7:61-5.
4. Bischof P, Duberg S, Sizonenko MT, et al. In vitro production of pregnancy-associated plasma protein A by human decidua and trophoblast. Am J Obstet Gynecol 1984;148:13-8.
5. Barnea ER, Sanyal MK, Brami CJ, Bischof P. In vitro production of pregnancy-associated plasma protein-A (PAPP-A) by trophoblastic cells. Arch Gynecol 1986; 237:187-90.
6. Siiteri PK, Stites DP. Immunologic and endocrine interrelationships in pregnancy. Biol Reprod 1982;26:1-14.
7. Rocklin RE, Kitzmiller JL, Garvoy MR. Further characterization of an immunological blocking factor that develops during pregnancy. Clin Immunol Immunopathol 1982;22:305-15.
8. Brami CJ, Sanyal MK, Dwyer JM, Johnson CC, Kohorn EI, Naftolin F. HLA-DR antigen on human trophoblast. Am J Reprod Immunol 1983;3:165-74.
9. Stromberg K, Azikhan JC, Speeg KV Jr. Isolation of functional human trophoblast cells and their partial characterization in primary cell culture. In Vitro 1978;14: 631-8.
10. Bischof P, Haenggeli L, Sizonenko MT, Herrmann WL, Sizonenko P. A radioimmunoassay for the measurement of pregnancy-associated plasma protein-A (PAPP-A) in humans. Biol Reprod 1981;24:1076-81.
11. Dwyer JM, Johnson C, Desuales M. Behavior of human immunoregulatory cells in culture. Clin Exp Immunol 1979;38:449-513.
12. Kabawat SW, Mostoufi-Zadeh M, Driscoll SG, Bhan AK. Implantation site in normal pregnancy. A study with monoclonal antibodies. Am J Pathol 1985;118:76-84.
13. Hunt JS, Fishback JL, Andrews GK, Wood GW. Expression of class I HLA genes by trophoblast cells. Analysis by in situ hybridization. J Immunol 1988;140:1293-99.
14. Takahashi H, Adachi S, Yoshiya N, Suzuki T, Kanazawa K, Takeuchi S. Expression of HLA-DR molecules in human gestational choriocarcinoma cell lines and malignant cell lines. Placenta 1987;8:293-8.
15. Palacios R. Mechanisms of T-cell activation: role and functional relationships of HLA-DR antigens and interleukins. Immunol Rev 1982;63:73-110.
16. Clark DA, Slapsys R, Chaput A, et al. Immunoregulatory molecules of trophoblast and decidual suppression cell origin at the maternofetal interface. Am J Reprod Immunol Microbiol 1986;10:100-4.

Increased myometrial responsiveness to oxytocin during term and preterm labor

R. E. Garfield, PhD, and S. Beier, PhD
Hamilton, Ontario, Canada, and Berlin, West Germany

The contractile effects of oxytocin on the longitudinal and circular muscle of rat uteri from animals undergoing term and preterm labor were studied in vitro to define the action of the hormone. The maximal tension and slopes of oxytocin dose contractile–response curves, but not the pD_2 values ($-\log ED_{50}$), were higher in both muscle layers at term delivery and also during preterm delivery induced with antiprogesterones compared with responses from tissues of nondelivering animals. These data indicate that the myometrium is hyperresponsive and hyperreactive to oxytocin during term and preterm birth but not hypersensitive. The increase in oxytocin responsiveness of the myometrium may be fundamental to the initiation of labor and regulated by the increases in oxytocin receptors and postreceptor events, including gap junctions. (Am J Obstet Gynecol 1989;161:454-61.)

Key words: Myometrium, oxytocin, labor and delivery, parturition, gap junctions

Oxytocin, an octapeptide from the posterior pituitary, is recognized as a potent uterine stimulant that is thought to be essential for the contractile events of parturition.[1] Clinically, oxytocin is used extensively in obstetrics to increase the frequency and force of myometrial contractility. The initiation of term and preterm labor has been ascribed to the augmentation of myometrial responses to circulating levels of oxytocin brought on by an increase in oxytocin receptors present on the muscle cells and decidua[2-5] and also to the increase in gap junctions between the muscle cells (i.e., improved electrical coupling).[8-9] Several,[9-11] but not all,[12-14] studies in vivo and in vitro demonstrate that the myometrium is moderately more sensitive to oxytocin near the end of pregnancy. Differences in response to oxytocin of the longitudinal versus circular muscle layers are also recognized.[12-14] However, these studies are incomplete, since there are no pharmacologic studies of the effects of oxytocin on the myometrium undergoing either term or preterm labor when the receptors and gap junctions are at the highest levels.

This study clarifies the contractile effects of oxytocin on uterine strips from rats during labor and delivery at term and also preterm. Uterine tissues were obtained from nondelivering rats (days 17, 18, and 21 of gestation and also 1 day post partum), rats delivering spontaneously at term (day 22), and during preterm delivery (days 17 and 18) after treatment with one of three different antiprogesterone compounds (ZK 98.299 and ZK 98.734, Schering, Berlin, West Germany; RU 486, Roussel Uclaf, Romainville, France). The tissues, longitudinal and circular muscle strips, were mounted in muscle baths and the isometric force was recorded. Oxytocin was added cumulatively to the bath every 5 to 10 minutes to give final concentrations ranging from 10^{-11} to 10^{-5} mol/L. Dose-response curves were computed from the force developed by the tissue cross-sectional area per unit time. The maximum response (E_{MAX}), pD_2 value (i.e., $-\log ED_{50}$), and the slope of the dose-response curve were calculated for each tissue, and means for each group and differences between groups were estimated.

Material and methods

Timed-pregnant Wistar rats (Woodlyn, Charles Rivers, St. Constaint, Quebec, Canada) weighing about 300 gm were used. Animals treated with antiprogesterone agents were injected on the morning of the sixteenth day of gestation with either vehicle (controls) or one of the antiprogesterones (10 mg/kg) suspended in oil. At 24 (day 17) or 48 hours (day 18) after the antiprogesterone treatments, when, respectively, 10% to 70% of the fetuses were delivered, the animals were killed by a blow to the head. Similarly, animals were killed on day 22 of gestation during spontaneous birth (one to three fetuses delivered) or 24 hours post partum. After death the uteri were removed, opened down the mesometrial side, and pinned out in dishes with Krebs solution. Tissue strips (about 2 mm by 2 cm), either parallel or perpendicular to the long axis (i.e., representing longitudinal or circular muscle), were cut from

From the Departments of Neurosciences and Obstetrics and Gynecology, McMaster University Health Sciences Centre, and Research Laboratories of Schering AG.
Supported by a grant from the Medical Research Council of Canada.
Received for publication May 25, 1988; revised February 14, 1989; accepted March 13, 1989.
Reprint requests: R. E. Garfield, PhD, Neurosciences Department, McMaster University Health Sciences Centre, 1200 Main St. W., Hamilton, Ontario, Canada L8N 3Z5.

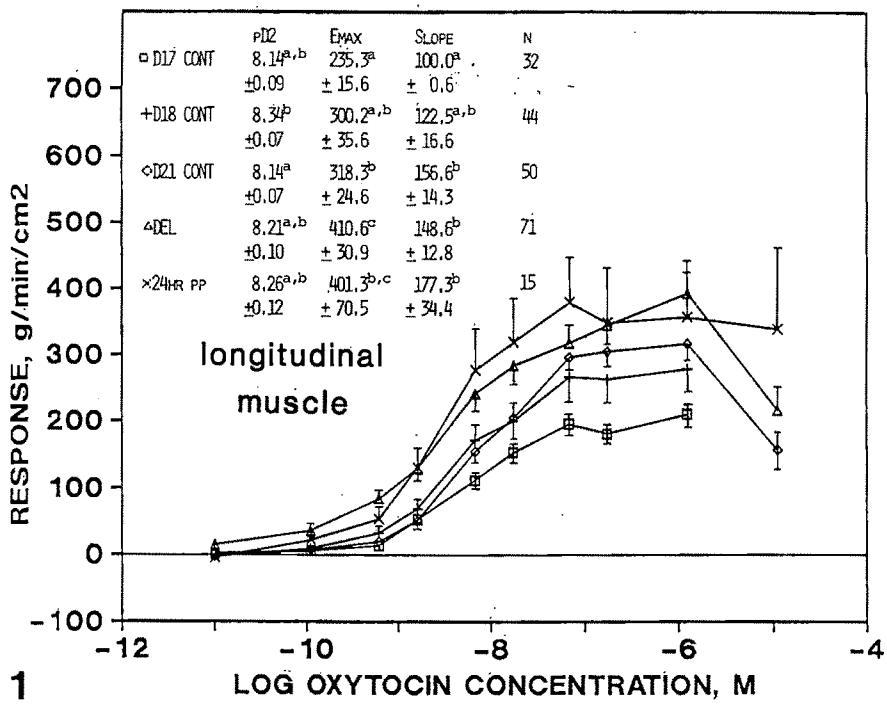


Fig. 1. Log oxytocin dose contractile–response curves for longitudinal uterine tissues from rats delivering spontaneously at term *(DEL)* and nondelivering at days 17, 18, and 21 *(D17, D18,* and *D21)* of gestation and postpartum *(pp)* (*bars* = SEM). Inserted values of pD_2, E_{MAX} (gm/min/cross-sectional area), and slope represent means for each group ± SEM (N = number of strips, four strips per rat). Values with different superscripts indicate significant differences ($p < 0.05$) between values.

the center of one uterine horn over fetal positions but not including the placenta regions.

The ends of the strips were tied with silk thread, and each strip was hung vertically in a 20 ml organ bath containing Krebs-Ringer solution oxygenated with 5% CO_2 and 95% O_2. The tissues were allowed to equilibrate for 20 minutes, and a resting force of 0.5 *g* was then placed on each strip. Force was monitored with Grass FT 30D isometric transducers connected to an eight-chananel Grass recorder. The mean force developed by the muscle strip per unit time was determined by measuring the area under the contraction curves with a microcomputer-linked digitizing tablet. The cross-sectional area of each tissue was estimated after the experiment from the wet weight of the strip (W), its length in the bath (L) and density (D) by means of the following formula:

$$\text{Area} = \frac{W}{L} \div D$$

The force developed by the tissue strip after adding the agonist minus the force of the spontaneous activity was taken as the tension developed in response to the agonist. The responses for each strip at each cumulative concentration of agonist were expressed as a percentage of the maximum response (E_{MAX}) and plotted against the log of the concentration of the agonist. Each dose-response curve was fitted to a third-order polynomial by microcomputer and the pD_2, ED_{20}, and ED_{50}, and ED_{80} values were obtained by interpolation. Mean estimates for E_{MAX} and pD_2 values were calculated for all strips within each group. The slope of the dose-response curve for each tissue was computed by measuring the slope of a line fitted to the ED_{20}, ED_{50}, and ED_{80} values. Means between groups were compared by a one-way analysis of variance or an unpaired Student *t* test. The mean forces generated/unit time/cross-sectional area for each dose of the agonist were plotted to produce the dose-response curves (Figs. 1 to 6).

Intact preparations of longitudinal myometrium in minimum essential medium (Gibco Laboratories, St. Lawrence, Mass.) with Hank's salts and 2 mmol/L Ca^{++} were pinned at the in vivo length and examined at 37° C with a Leitz-inverted microscope. Glass microelectrodes with resistances of 40 to 80 MΩ were used to record membrane potentials and measure input resistance (R_o) during smooth muscle cell impalements that were routinely maintained for 2 to 4 minutes. R_o is the point resistance to current flow from a cell during intracellular current injection and reflects the combined resistance of gap junctional and nonjunctional membrane, as well as (constant) myoplasmic resistance. R_o was determined during cell impalement from the voltage deflection of a previously bridge-balanced elec-

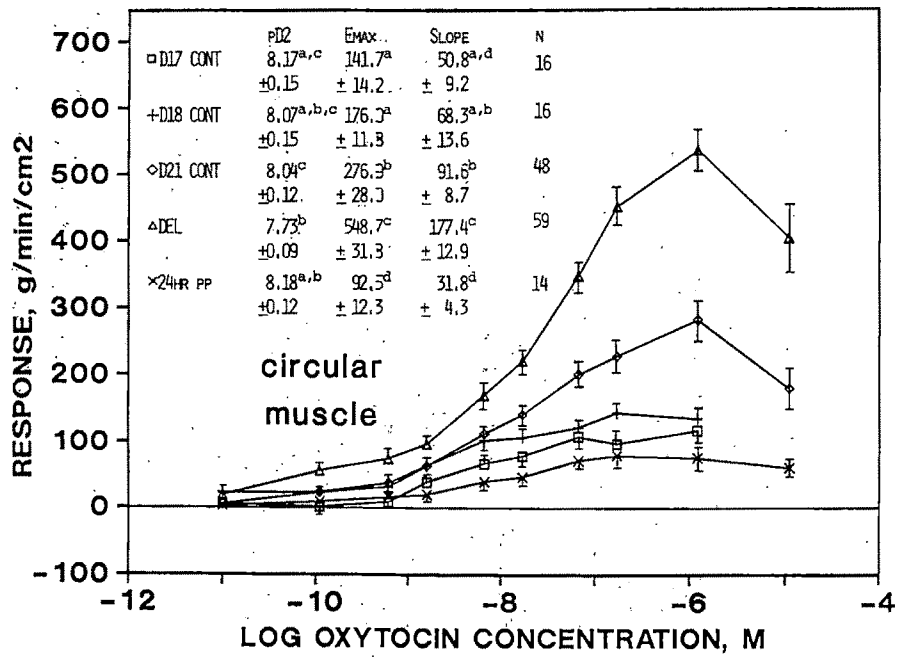


Fig. 2. Data as shown in Fig. 1 for circular uterine strips.

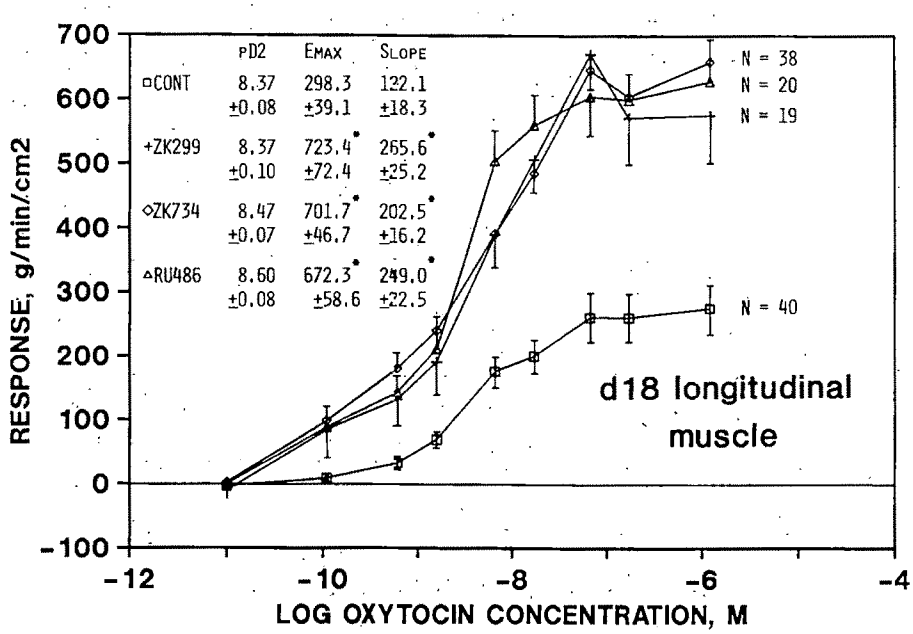


Fig. 3. Data as shown in Fig. 1 for longitudinal uterine tissues from control rats and animals delivering prematurely (about 70% of fetuses expelled) at day 18 of gestation after treatment with one of three different antiprogesterone compounds (ZK 299, ZK 734, and RU 486). *Significant differences between mean of control and treated.

trode to current injection (500 pA; 200 msec/sec). Values of membrane potential and R_o were retained only if the electrode returned to zero potential and bridge balance ± 1 MΩ upon removal from the cell.

Results

Oxytocin log dose contractile–response curves for longitudinal tissues from animals delivering at term and tissues from control animals at days 17, 18, and 21 of gestation and 1 day postpartum are shown in Fig. 1. Tissues from the delivering animals gave higher maximum responses compared with tissues from control nondelivering, preterm rats. The mean E_{MAX} values were significantly higher ($p < 0.05$) than those of the controls, but generally there were no differences in the pD_2 values or the slopes of the dose-response curves.

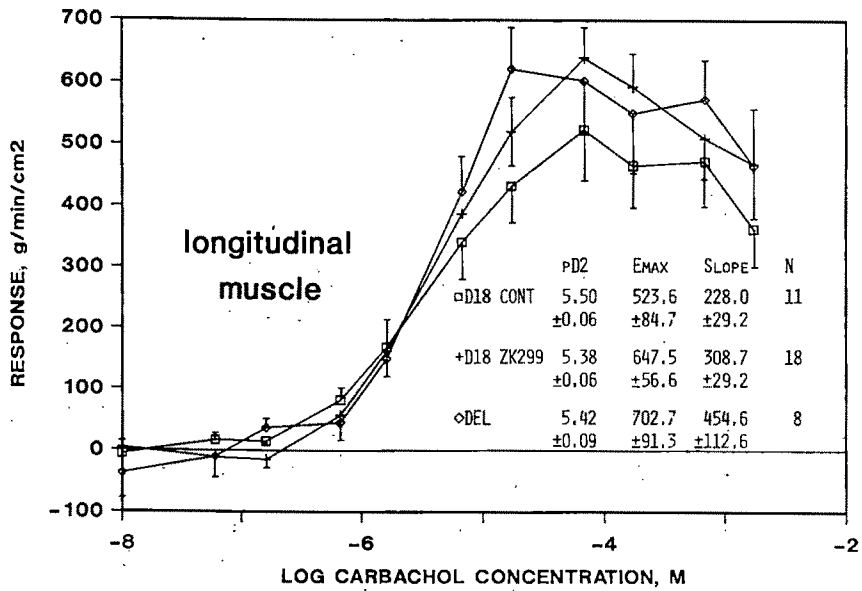


Fig. 4. Log carbachol dose contractile–response curves for longitudinal uterine strips from rats at day 18 nondelivering controls, day 18 delivering prematurely after ZK 299, and day 22 spontaneously delivering at term. There were no significant differences between means of pD_2, E_{MAX}, and slope values for the groups.

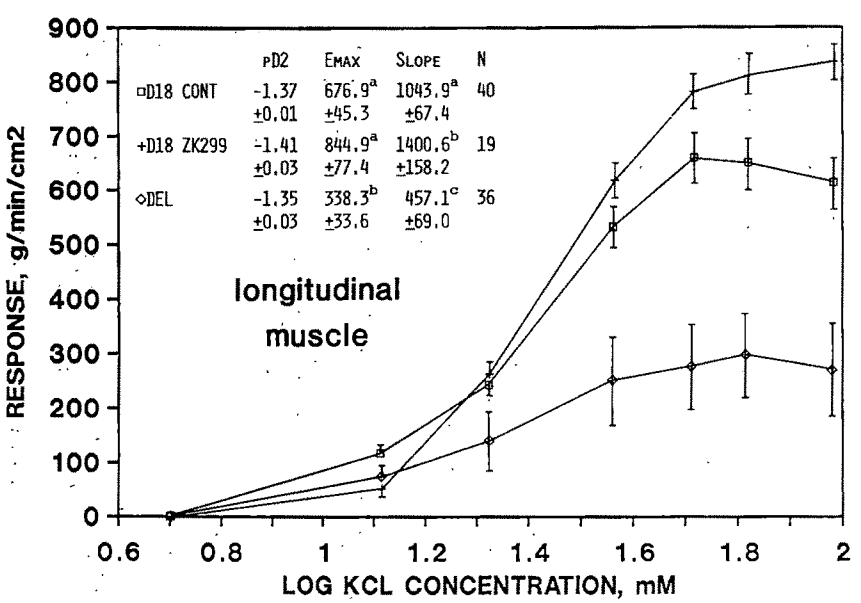


Fig. 5. Log KCl dose contractile–response curves for tissues from rats at day 18 nondelivering, delivering prematurely, and delivering at term. Mean values of pD_2, E_{MAX}, and slope with different superscripts show significant differences.

There were no differences between tissues from delivering versus postpartum animals. Fig. 2 shows the dose-response relationships for circularly cut myometrial strips from the same groups, as shown in Fig. 1. Again, there were no major changes in pD_2 values. However, the maximum responses (about two to four times as high) and also slopes of the dose-response curves were greater in tissues from term delivering rats than tissues from control rats at days 17, 18, and 21. In this case the mean values of E_{MAX} and slopes for tissues from postpartum animals were significantly reduced compared with those of delivering animals.

The dose-response relationships for longitudinal tissues from control nondelivering and preterm delivering rats at day 18 of gestation are illustrated in Fig. 3. The results from tissues of animals treated with each of the particular antihormones are comparable with those obtained from tissues during spontaneous delivery at term (Figs. 1 and 2). The maximum responses and slopes of the curves were significantly higher dur-

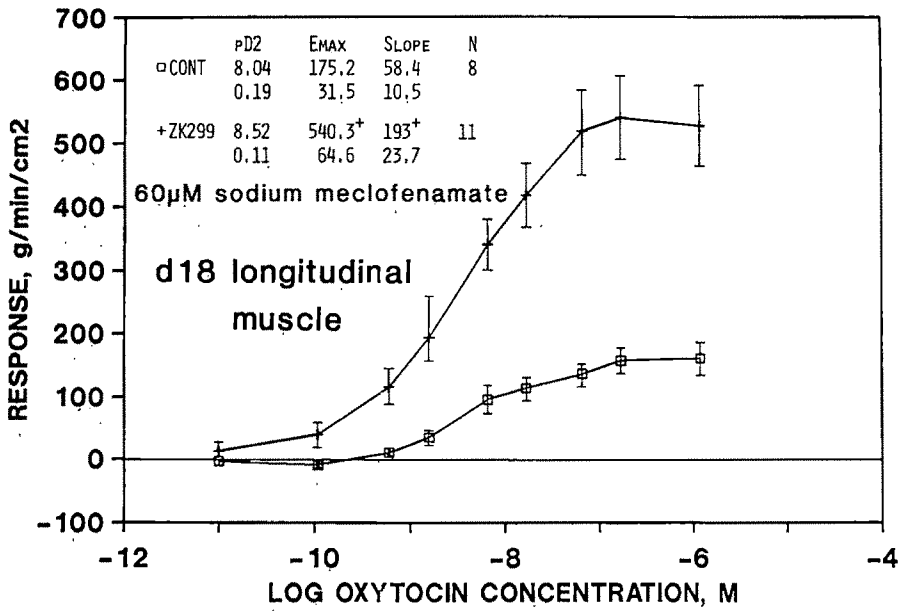


Fig. 6. Oxytocin dose-response curves from longitudinal uterine strips at day 18, nondelivering and delivering after ZK 299. All tissues were treated with 60 μmol/L sodium meclofenamate. Note that differences are similar to tissues not treated with the prostaglandin inhibitor (Fig. 3).

Table I. Resting membrane potential and input resistance in uterine longitudinal smooth muscle cells

	Membrane potential (mV ± SD) (n)	*Input resistance (R_o) (MΩ ± SD) (n)*
Delivering (term, day 22)		
Control	−40.5 ± 2.7 (101)	12.0 ± 4.8 (101)
1.2 mmol/L octanol	−39.8 ± 2.6 (90)	48.5 ± 15.2 (90)*
Wash (10 min)	−40.2 ± 1.9 (20)	11.6 ± 4.1 (20)
Preterm (nondelivering, day 21)		
Control	−42.6 ± 3.3	21.7 ± 11.10
Preterm (nondelivering, day 18)		
Control	−46.0 ± 3.0 (80)	24.1 ± 7.4 (80)
1 2 mmol/L octanol	−46.5 ± 2.4 (60)	49.0 ± 8.6 (60)*
Wash (10 min)	−46.9 ± 2.3 (20)	24.5 ± 4.9 (20)
Preterm (delivering, day 18), ZK 299 injected		
Control	−40.8 ± 2.7 (55)	13.8 ± 4.8 (55)
1.2 mmol/L octanol	−40.5 ± 2.4 (50)	44.3 ± 13.3 (50)*
Wash (10 min)	−41.3 ± 2.0 (15)	12.3 ± 5.3 (15)

n, Number of impalements in tissues from 10 to 15 animals. Shown are values before (control), during octanol treatment, and 10 minutes after washout.

*Significant differences ($p < 0.05$) from control values.

ing preterm birth induced with the antiprogesterones than in control tissues. Once more there were no significant differences in pD_2 values. Similar results were found in the circular muscle at day 18 and in both muscle layers at day 17 of gestation during the beginning of preterm birth in antiprogesterone-treated rats (data not shown).

The effects of carbachol and KCl on contractility of the myometrium were also studied to ascertain if the effects of other agents are also higher during term and preterm labor. Fig. 4 shows carbachol dose-responses for longitudinal tissues from day 18 nondelivering, day 18 delivering after ZK299, and day 22 spontaneous delivering rats. Unlike oxytocin, there were no significant differences in the carbachol dose-response curves. However, the maximum carbachol responses were similar to maximum values obtained with oxytocin during term and preterm delivery (Figs. 1 and 2). KCl responses, which were different from results with both oxytocin and carbachol, were lower in tissues from delivering term animals compared with tissues from nondelivering and preterm delivering animals at day 18 (Fig. 5).

To test if prostaglandin synthesis was involved in the

differences between delivering and nondelivering tissues in response to oxytocin, some tissues were treated with sodium meclofenamate (60 μmol/L) (Fig. 6). Again, the maximum responses and the slopes of the dose-response curves increased dramatically during preterm delivery after administration of ZK 98.299 compared with tissues from nondelivering animals. The responses were comparable with those obtained in the absence of the inhibition of prostaglandins (Fig. 3).

The effect of octanol, a specific gap junction uncoupling agent, on the contractile responses of oxytocin, KCl, and carbachol (not shown) was also studied. When added to the muscle baths, the alcohol eliminated spontaneous activity in a dose-dependent fashion and prevented the increase in contractility that is normally produced by the agonists. At 1.2 mmol/L octanol, even the highest concentrations of agonists failed to elicit a response (Fig. 6). When octanol was added to agonist-contracted tissue, relaxation occurred within about 3 minutes. The effect of the alcohol was immediately (about 5 minutes) reversible.

The resting membrane potential of longitudinal muscle tissues from delivering rats as measured with microelectrodes was -40.5 ± 2.7 mV from term animals and -40.8 ± 2.7 mV from preterm animals (Table I). These values were more depolarized than in tissues from nondelivering rats (-42.6 ± 3.3, day 21, and -46.0 ± 3.0, day 18), but the means of neither the delivering nor the nondelivering tissues were significantly different in the octanol-treated strips. However, input resistance was considerably higher after octanol compared with control values in tissues from both delivering and nondelivering animals. Oxytocin (10^{-8} mol/L) depolarized the resting membrane potential by a few millivolts both in the absence of octanol and also in its presence when membrane input resistance was elevated (data not shown).

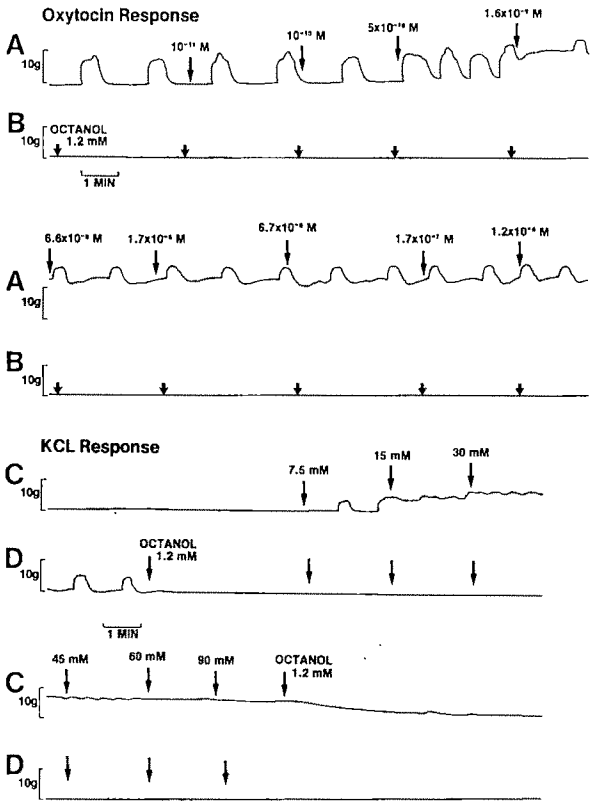


Fig. 7. Continuous tracings of longitudinal muscle (*strips A to D*) contractile responses to oxytocin (*top*) and KCl (*bottom*) in the absence (control) and presence of octanol.

Comment

The results of this study demonstrate unequivocally that the longitudinal and circular muscle layers of the myometrium are hyperresponsive (increased maximum response) and hyperreactive (increased slopes of dose-response curves) to oxytocin during term and preterm labor. During delivery both muscle layers develop two to four times or more tension in response to oxytocin than they do before delivery. The changes are particularly prominent in both muscle layers during preterm labor. However, there is no change in the sensitivity of the muscle to oxytocin, as would be reflected by a shift in dose-response curves and a change in pD_2 values. Moreover, the elevation in slopes and maximum responses is exclusively limited to oxytocin and not to the nonspecific stimulation of contractility observed with KCl or the effects of carbachol. These data suggest that the endocrine events that occur at term and those that arise from blocking the progesterone receptor with the antiprogesterones[15-17] increase either the effectiveness of the oxytocin-receptor interaction or a postreceptor mechanism to intensify the response. Such mechanisms might involve the number of receptors, the process by which receptor occupation influences contraction, or the extent of electrical coupling between cells. The increase in the ability of the myometrium to respond to oxytocin may be indispensable physiologically for the control of parturition. Furthermore, unless this change occurs before the pharmacologic application of the hormone, its effectiveness will be seriously restricted.

Numerous studies show that oxytocin receptor numbers in the uterus increase substantially at term in various animals and humans without a rise in plasma levels of the hormone.[1-5, 18, 19] These studies attribute the increase in receptors to changes in steroid hormone levels. An increase in the number of oxytocin receptors either at term or preterm after antiprogesterone treatment would increase either the sensitivity of the myometrium to oxytocin if spare receptors exist or intensify the maximum effects if there is no receptor reserve. Accordingly, the data in this study are consistent with

the supposition that there is little receptor reserve and that an increase in functional oxytocin receptors augments responsiveness to the hormone during term and preterm birth. Previous studies provide incomplete data on the effects of oxytocin on the myometrium during pregnancy, and none has shown dramatic increases in maximal effects as demonstrated in this study. Although in vivo studies indicate that the sensitivity to oxytocin may be raised at term, part of this may be attributable to the ability of oxytocin to stimulate the synthesis of excitatory prostaglandins[20, 21] and also the perception that the threshold dose (i.e., concentration that first initiates contractility above baseline) is lower during term (Fig. 1) and preterm birth (Fig. 3). The inhibition of prostaglandin synthesis with sodium meclofenamate (60 μmol/L) did not change the dose-response relationships in some experiments in this study (Fig. 6). Furthermore, none of the previous in vivo or in vitro studies has systematically examined the effects of oxytocin on tissues from delivering animals. Thus as shown by this study, the changes in the receptors or other events that occur during or immediately before delivery apparently contribute considerably to the ability of oxytocin to excite the muscle. These results add support to the hypothesis that the increase in oxytocin receptors may initiate labor not by raising the sensitivity to the hormone but by magnifying the force the muscle produces.

However, oxytocin receptor studies do not entirely agree with the information in this study. Oxytocin-binding sites to rat uterine tissues increase immediately before labor then decline to preterm levels within 1 day postpartum.[2] These data do not correlate with the action of oxytocin on the longitudinal muscle during term delivery and postpartum (Fig. 1: E_{MAX} gradually rises until delivery and does not fall postpartum), but they correspond to this study in regard to the circular muscle layer (Fig. 2: dramatic rise in oxytocin action occurs during delivery then falls postpartum). This may then indicate that the rise and fall in oxytocin receptor occur primarily in the circular muscle layer. Similar conclusions have been reached by Crankshaw[12] and Tuross et al.,[14] although they neither examined oxytocin effects during term delivery nor postpartum, and both studies did not determine oxytocin action during preterm delivery.

Since myometrial gap junctions (sites of electrical communication) are greatly increased during term parturition[6-8] and also during preterm birth after antiprogesterone treatment,[22] the increased responsiveness of the myometrium to oxytocin could be related in part to better electrical coupling between the muscle cells. The driving force for myometrial contractility is thought to be provided by propagated action potentials from pacemaker regions, and the frequency and intensity of contractions are, respectively, proportional to the regularity and duration of action potentials in each muscle cell and the total number of cells that are active.[23] Therefore an agonist that generates electrical activity or depolarizes the resting membrane potential may greatly augment the frequency of phasic contractile activity if the velocity of propagation of action potentials between cells is increased and amplify the contractile force by spreading further to recruit additional cells when gap junctions are increased. Similar increases in contractility to agonists have been observed in vas deferens after denervation and the appearance of gap junctions.[24] The fact that all responses to carbachol are similar (Fig. 4) to those of oxytocin during delivery (Figs. 1 to 3) when gap junctions are increased suggests either that this mechanism is not the sole system involved or that the two agonists have distinctly different actions. It is possible that either the location of agonist receptors (i.e., on all cells versus specific cells) is different or the pattern of contractility (i.e., phasic versus tonic contractions) varies with the agonist used.

However, like oxytocin receptors, gap junctions rise immediately before delivery and then decline rapidly within 1 day postpartum.[6, 7] Thus the data in this study (Fig. 1) are also not consistent with the hypothesis that the increase in gap junctions is responsible for the changes in responsiveness, at least in the longitudinal muscle layer during term delivery and postpartum. If so, during normal delivery at term, it is difficult to attribute changes in oxytocin receptors and gap junctions to the hyperresponsiveness of the longitudinal muscle during preterm delivery (Fig. 3). Nevertheless, there may be differences in the contractile significance of the presence of large numbers of gap junctions and oxytocin receptors between the longitudinal and circular muscle during term and early delivery. Furthermore, there may be distinctions in the functional states of the receptors (i.e., coupling to postreceptor events) and channels of the junctions (i.e., open or closed) between the two muscle layers at term and preterm.

The possible significant role of electrical coupling in the action of agonists was observed after the addition of octanol, an alcohol that closes pores in the gap junctions and electrically uncouples cells[25,26], it completely prevents contractions produced by oxytocin, KCl, and also carbachol (Fig. 7). Although a nonspecific inhibition of contractility by octanol cannot be excluded, it did not change the resting membrane potential but raised the input resistance (Table I). Large reproducible changes in input resistance were correlated with either the relative number of gap junctions at term and preterm[6, 22] or the presence of octanol, which is known to specifically increase junctional resistance.[25, 26] Therefore these changes in input resistance are thought to

reflect predominant changes in junctional coupling (i.e., a decrease in R_o when junctions develop during term and preterm delivery and an increase when the junctions reversibly close in response to octanol). These data are interpreted to mean that cell-to-cell communication is necessary for spontaneous and agonist-induced contractility.

The selective increase in responsiveness of both layers of the myometrium to oxytocin during term and preterm delivery may be essential for labor and is probably imparted by the specific increase in oxytocin receptors, changes in postreceptor mechanisms, and to enhanced electrical coupling. The absence of change in the maximum capacity of the myometrium to contract between preterm nondelivery and delivery at term or preterm, provided the tissue is challenged appropriately (i.e., carbachol at any time and oxytocin during delivery), suggests that no change occurs in the force-generating potential of the myometrium. The maximum response to KCl is diminished during term delivery and is lower than responses observed with oxytocin or carbachol. This result remains to be explained but may be the result of an inhibition of the muscle electrogenic Na^+ to K^+ pump by some system at term and the depolarization of the muscle (Table I) during delivery. Octanol inhibits spontaneous and agonist-stimulated contractions, indicating that electrical coupling may be required for contractions of the myometrium.

We thank Walter Elger, Schering, and D. Philibert, Roussel, for the antiprogesterone compounds, Ms. D. Merrett for technical assistance, and my colleagues, Drs. M. S. Kannan, D. J. Crankshaw, and L. Branda, for reading the manuscript, and Dr. M. Blennerhassett for help with the microelectrode measurements.

REFERENCES

1. Fuchs A-R. The role of oxytocin in parturition. In: Huszar G, ed. The physiology and biochemistry of the uterus in pregnancy and labor. Boca Raton: CRC Press, 1986;163-83.
2. Soloff MS, Alexandrova M, Fernstrom MJ. Oxytocin receptors: triggers for parturition and lactation. Science 1979;204:1313-5.
3. Fuchs A-R, Fuchs F, Husslein P, Soloff MS, Fernstrom MJ. Oxytocin receptors and human parturition: a dual role for oxytocin in the initiation of labor. Science 1982;215:1396-8.
4. Sheldrick EL, Flint APF. Endocrine control of uterine oxytocin receptors in the ewe. J Endocrinol 1985;106:249-58.
5. Reimer RK, Goldfien AC, Goldfien A, Roberts JM. Rabbit uterine oxytocin receptors and in vitro contractile response: abrupt changes at term and the role of eicosanoids. Endocrinology 1986;119:699-709.
6. Garfield RE, Sims S, Daniel EE. Gap junctions: their presence and necessity in the myometrium during parturition. Science 1977;198:958-60.
7. Garfield RE. Myometrial ultrastructure and uterine contractility. In: Bottari S, Thomas JP, Voker A, Vokaer R, eds. Uterine contractility. New York: Masson, 1984:81-109.
8. Garfield RE, Hayashi RH. Appearance of gap junctions in the myometrium of women during labor. AM J OBSTET GYNECOL 1981;140:254-60.
9. Caldeyro-Barcia R, Sereno JA. The response of the human uterus to oxytocin throughout pregnancy. In: Caldeyro-Barcia R, Heller H, eds. Oxytocin. London: Pergamon Press, 1959:177.
10. Fuchs A-R, Periyasamy S, Alexandrova M, Soloff MS. Correlation between oxytocin receptor concentration and responsiveness to oxytocin in pregnant rat myometrium: effect of ovarian steroids. Endocrinology 1983;113:742-49.
11. Kuriyama H, Suzuki H. Effects of prostaglandin E_2 and oxytocin on the electrical activity of hormone-treated and pregnant rat myometria. J Physiol (Lond) 1976;260:335-49.
12. Crankshaw DJ. The sensitivity of the longitudinal and circular muscle layer of the rats myometrium to oxytocin in vitro. Can J Physiol Pharmacol 1987;65:773-7.
13. Goren HJ, Geonzon RM, Hollenberg MD, Lederis K, Morgan DO. Oxytocin action; lack of correlation between receptor number and tissue responsiveness. J Supramolec Struct 1980;14:129-38.
14. Tuross N, Mahtanhi M, Marshall JM. Comparison of effects of oxytocin and prostaglandin $F_{2+\alpha}$ on circular and longitudinal myometrium from the pregnant rat. Biol Reprod 1987;37:348-55.
15. Baulieu EE. RU486: an antiprogestin steroid with contragestive activity in women. In: Baulieu EE, Segal SJ, eds. The antiprogestin steroid RU 486 and human fertility control. New York: Plenum Press, 1985:1-25.
16. Elger W, Fahnrich M, Beier S, Quig SS, Chawlisz K. Endometrial and myometrial effects of progesterone antagonists in pregnant guinea pigs. AM J OBSTET GYNECOL 1987;157:1065-74.
17. Elger W, Beier S, Chwalisz K, et al. Studies on the mechanisms of action of progesterone antagonists. J Steroid Biochem 1986;25:835-45.
18. Alexandrova M, Soloff MS. Oxytocin receptors and parturition. I. Control of oxytocin receptor concentration in the rat myometrium at term. Endocrinology 1980;106:730-5.
19. Dawood MY. Neurohypophyseal hormones. In: Fuchs F, Klopper A, eds. Endocrinology of pregnancy. Philadelphia Harper & Row, 1977:204-28.
20. Roberts JS, McCracken JA. Does prostaglandins $F^{2\alpha}$ released from the uterus by oxytocin mediate the oxytocin action of oxytocin? Biol Reprod 1976;15:457-63.
21. Husslein R, Fuchs A-R, Fuchs F. Oxytocin and the initiation of human parturition. I. Prostaglandin release during induction of labor with oxytocin. AM J OBSTET GYNECOL 1981;141:688-97.
22. Garfield RE, Gasc JM, Baulieu EE. Effects of the antiprogesterone RU 486 on preterm birth in the rat. AM J OBSTET GYNECOL 1987;157:1281-85.
23. Marshall JM. Regulation of activity in uterine smooth muscle. Physiol Rev 1962;42:213-27.
24. Westfall DP. Supersensitivity of smooth muscle. In: Bulbring E, Brading A, Jones AW, Tomita T. Smooth muscle: an assessment of current knowledge. London: Edward Arnold Ltd, 1981:285-309.
25. Spray DC, White RL, Carvalho AC, Harris AL, Bennett MVL. Gating of gap junctional conductance. Biophys J 1984;45:219-30.
26. Veenstra RD, Dehann RL. Measurement of single channel currents from cardiac gap junctions. Science 1986;233:972-4.

CA 125 in the epithelium closely related to the embryonic ectoderm: The periderm and the amnion

Yoshihiko Nanbu, MD, Shingo Fujii, MD, Ikuo Konishi, MD, Hirofumi Nonogaki, MD, and Takahide Mori, MD

Kyoto, Japan

The amnion is believed to be derived from either cytotrophoblastic cells or embryonic ectoderm. However, it produces and secretes CA 125, which is considered a differentiation antigen of fetal coelomic epithelium derived from the mesoderm of germ cells. To verify this, the immunohistochemical localization of CA 125 in human fetal tissues (between 7 and 23 weeks of gestation) derived from the ectoderm, endoderm, or mesoderm, and in the fetal membranes and placenta was studied. Among the mesoderm-derived tissues, only the fetal coelomic epithelium–related tissues were positive for anti-CA 125 from 15 weeks of gestation. The endoderm-derived tissues did not react with anti-CA 125. However, among the ectoderm-derived tissues, only the periderm reacted with anti-CA 125 from 7 weeks until it sloughed from the stratum intermedium by 23 weeks of gestation. Among the fetal membranes and placenta, only the amnion reacted with anti-CA 125 from 9 weeks to term. These findings indicate that the amnion and the periderm, both of which constitute the epithelia covering the amniotic cavity, in addition to the fetal coelomic epithelium-related tissues, produce CA 125. (AM J OBSTET GYNECOL 1989;161:462-7.)

Key words: CA 125, immunohistochemistry, amnion, periderm, ectoderm

CA 125 is an antigenic determinant recognized by a monoclonal antibody OC 125 against an ovarian cancer cell line (OVCA 433).[1] An assay of serum CA 125 has been shown to be useful for the diagnosis and monitoring the course of patients with ovarian cancer.[2] However, serum CA 125 levels increase not only in women with other gynecologic malignancies,[3] but also in women with benign conditions such as endometriosis,[4] pelvic inflammatory diseases,[5] and during pregnancy.[6] Immunohistochemically, the CA 125 antigen is detected in the coelomic epithelium–related tissues; pleura, pericardium, peritoneum, and müllerian ducts of the fetus[7]; and müllerian-derived epithelia of the adult such as tubal, endometrial and endocervical epithelium.[8] Therefore CA 125 is considered a differentiation antigen associated with fetal coelomic epithelium and its derivatives,[7] which are believed to be derived from the mesoderm of germ cells.

Amniotic fluid contains a large amount of CA 125.[9] We recently demonstrated by immunohistochemical and biochemical studies that both the decidua and the amnion can produce CA 125.[10] Nevertheless, the amniotic cavity, which develops by cavitation within the inner cell mass of germ cells, is formed with the floor of the embryonic ectoderm and the roof of the amnion, which is thought to be derived from cytotrophoblastic cells.[11] In addition, the flattened amniotic cells are continuous with the embryonic ectoderm, and are alternatively called the amniotic ectoderm.[11] Moreover, the amniotic cells are now thought to arise from the periphery of the embryonic ectoderm.[12, 13] Consequently, it is unclear why the amnion produces and secretes CA 125. Therefore we examined the immunohistochemical localization of CA 125 in human fetal tissues derived from the ectoderm, endoderm, or mesoderm and in the fetal membranes and placenta.

From the Department of Gynecology and Obstetrics, Faculty of Medicine, Kyoto University.

Supported in part by the Grant-in-aid for Scientific Research from the Ministry of Education (No. 62570752), Japan.

Received for publication December 21, 1988; revised March 8, 1989; accepted March 17, 1989.

Reprint requests: Shingo Fujii, MD, Department of Gynecology and Obstetrics, Faculty of Medicine, Kyoto University, Sakyoku, Kyoto, 606, Japan.

Material and methods

Fresh specimens of various fetal tissues were obtained from human fetuses (three female fetuses at 9, 17, and 23 weeks of gestation, four male fetuses between 15 and 17 weeks of gestation, and one fetus of unknown sex at 7 weeks of gestation) at legal elective abortions; the tissues listed in Table I were used for the study. Informed consent was obtained to study the fetuses. Tissues of the amnion, chorion, and decidua between 9 and 40 weeks of gestation obtained at either legal elective abortions or spontaneous deliveries were also used for this study.

All tissue were snap-frozen in Tissue-TEK OCT compound (Ames Co., Elkhart, Ind.) and stored at −70° C. Four-micrometer cryostat sections were prepared from each specimen and stored at −20° C until use. The immunohistochemical staining of CA 125 with primary antibody OC 125 was performed with a CIS His CA 125 staining kit (Oris, Saclay, France) according

Table I. Immunohistochemical reactivity of OC 125 with fetal tissues, fetal membranes, and placental tissues

Tissue examined	*Gestational age (wk) and sex of fetus*							
	7 (Unknown sex)	*9 (Female)*	*17 (Female)*	*23 (Female)*	*15 (Male)*	*16 (Male)*	*16 (Male)*	*17 (Male)*
Ectoderm-derived tissues								
Skin								
Periderm	+	+	+	−	+	+	+	+
Stratum intermedium	−	−	−	−	−	−	−	−
Others	−	−	−	−	−	−	−	−
Endoderm-derived tissues								
Small intestine		−			−	−	−	
Large intestine		−	−		−	−	−	−
Lung		−				−	−	−
Liver		−				−	−	
Bladder			−	−	−	−		−
Mesoderm-derived tissues								
Müllerian duct	−	−						
Uterine cervix			+	+				
Uterine corpus			+	+				
Fallopian tube			+	+				
Ovarian surface epithelium		−	−	−				
Coelomic epithelium	−	−						
Pleura						+		+
Peritoneium				+	+			+
Pericardium					+			
Others	−							
Heart		−			−	−	−	
Kidney		−				−	−	−
Testis					−	−	−	−
Fetal membranes	*3 cases (9-15 wk)*		*9 cases (16-27 wk)*		*5 cases (28-40 wk)*			
Amnion	+		+		+			
Chorionic epithelium	−		−		−			
Placenta								
Cytotrophoblast	−		−		−			
Syncytiotrophoblast	−		−		−			

+, Reactivity of OC 125 positive; −, reactivity of OC 125 negative.

to the manufacturer's instructions. The sections were treated with 3% hydrogen peroxide and incubated with antibody (diluted with 3 ml of phosphate-buffered saline solution according to the manufacturer's instructions) at 4° C overnight. The sections were then treated with anti-mouse IgG-biotin complex followed by avidin-peroxidase complex, and were stained with 3-amino-9-ethylcarbazole solution with 0.15% hydrogen peroxide. The sections were counterstained with hematoxylin. For the negative controls, normal mouse serum or phosphate-buffered saline solution was used instead of each primary antibody. For the positive controls, the sections of ovarian serous carcinoma with high levels of serum CA 125 were used.

Results

Ectoderm-derived tissues (Table I). The periderm of the fetuses at 7 and 9 weeks of gestation was moderately positive for anti-CA 125 in the cytoplasm (Fig. 1). The periderm of the fetuses at 15 (Fig. 2), 16, and 17 weeks of gestation were strongly positive for anti-CA 125 in the cytoplasm irrespective of sexual differentiation. However, the stratum intermedium beneath the periderm did not react with anti-CA 125. At 23 weeks of gestation, cells of the periderm were already sloughed from the straum intermedium, and the latter constituted the epidermal layer of the skin. Therefore at this stage the fetal skin was negative for anti-CA 125 (Fig. 3).

The other ectoderm-derived tissues such as the central nervous system and neural crest derivatives were negative for anti-CA 125.

Endoderm-derived tissues (Table I). The endoderm-derived tissues such as the lung, intestine, and urinary bladder did not react with anti-CA 125 in any fetus examined.

Mesoderm-derived tissues (Table I). In the female fetuses of both 17 and 23 weeks of gestation, epithelial

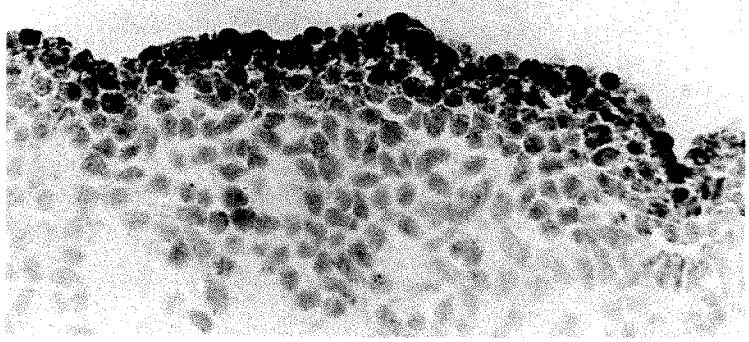


Fig. 1. Immunohistochemical staining is moderately positive with anti-CA 125 in the cytoplasm of the periderm at 9 weeks of gestation. (Original magnification ×400.)

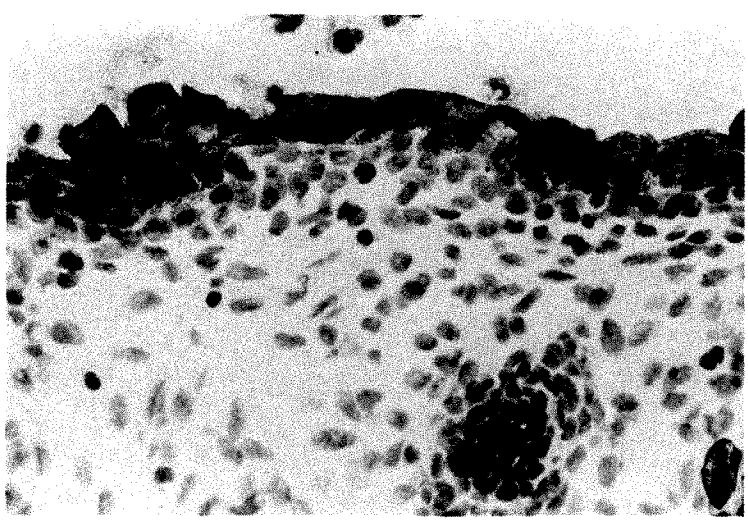


Fig. 2. The periderm at 15 weeks of gestation is strongly reactive with anti-CA 125 in the cytoplasm. (Original magnification ×400.)

cells of the uterine cervix, corpus, and fallopian tube (Fig. 4) were weakly positive for anti-CA 125, whereas the surface epithelium of the fetal ovary was negative. Fetal coelomic epithelium-derived epithelia such as the pleura, pericardium (Fig. 5), and peritoneum were faintly positive for anti-CA 125 in both female and male fetuses between 15 and 23 weeks of gestation. Reactivity was observed along the luminal surface and in the apical portion of the cytoplasm. However, these tissues at 7 and 9 weeks of gestation were negative for anti-CA 125.

The mesoderm-derived tissues, which were not associated with the fetal coelomic epithelium, were negative for anti-CA 125.

Fetal membranes (Table I). The amniotic epithelium of the fetal membranes at gestational ages between 9 and 40 weeks was strongly positive for anti-CA 125 (OC 125), not only along the luminal surface and in the apical portion of the cytoplasm, but also as accumulations of atypical coarse granules of chromogen deposits in the cytoplasm (Fig. 6).

The chorionic epithelium of the fetal membranes at any stage examined in this study was negative.

Placenta (Table I). Both cytotrophoblast and syncytiotrophoblast did not react with anti-CA 125 in all the placentas examined.

Comment

Our study demonstrated that CA 125 is present in fetal coelomic epithelium and its derivatives after 15 weeks of gestation. However, it is usually only weakly positive for anti-CA 125 until 23 weeks of gestation. In addition, we found that both the amnion and the per-

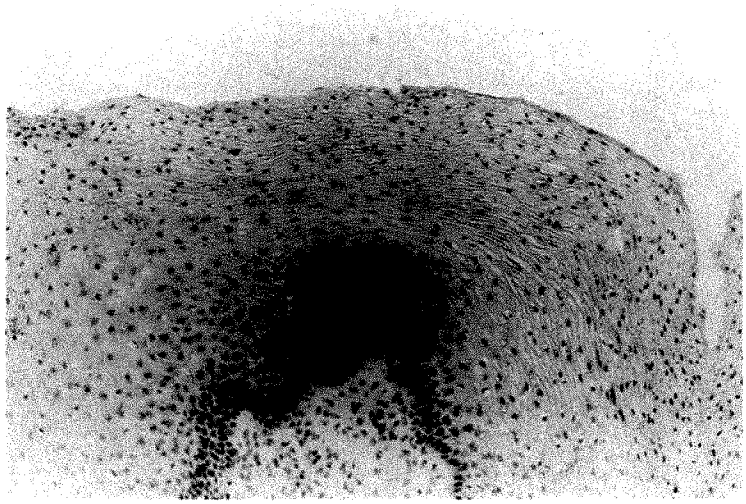


Fig. 3. Fetal skin at 23 weeks of gestation sloughs off the periderm, and the dermis shows negative staining for anti-CA 125. (Original magnification ×100.)

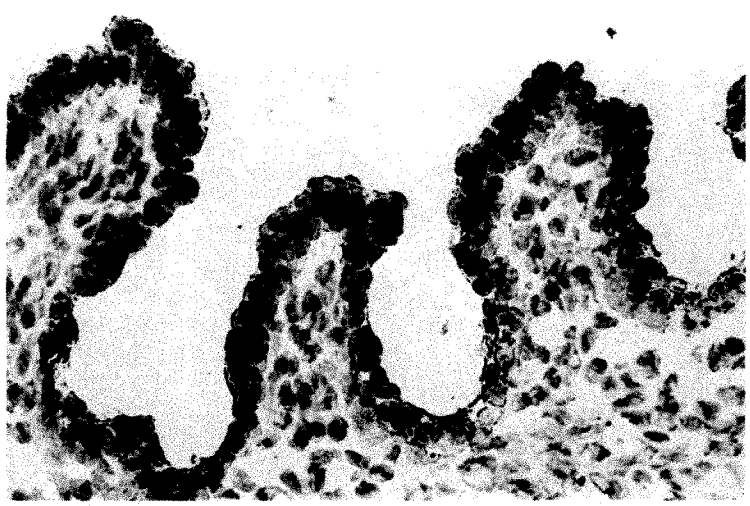


Fig. 4. The fallopian tube at 17 weeks of gestation is reactive with anti-CA 125 along the luminal surface and in the apical portion of the cytoplasm. (Original magnification ×200.)

iderm are positive for anti-CA 125 from 7 weeks of gestation. Thereafter, the periderm is strongly positive for anti-CA 125, and it sloughs from the stratum intermedium by 23 weeks of gestation. The amnion was positive for anti-CA 125 until the end of pregnancy. However, other tissues derived from the ectoderm, mesoderm, and endoderm were negative for anti-CA 125. Moreover, both cytotrophoblasts and syncytiotrophoblasts were negative for anti-CA 125 throughout gestation.

The amnion was reported to be the source of CA 125 in the amniotic fluid.[9] The amniotic cells are continuous peripherally with the embryonic ectoderm, and the latter forms the floor of the amniotic cavity.[11, 12] The periderm is derived from the embryonic ectoderm. Therefore our findings demonstrate that the epithelia covering the amniotic cavity produces CA 125. In addition, the positivity for anti-CA 125 in the amnion and the periderm occurs earlier in embryonic life and is stronger than that of fetal coelomic epithelium and its derivatives. This suggests that CA 125 is not a differentiation antigen that is confined to fetal coelomic epithelium and its derivatives, but rather it is a primitive antigen in the epithelia that is closely related to the embryonic ectoderm such as the periderm and the amnion. However, the primitive streak that originates from the embryonic ectoderm forms the mesoderm of the embryo, and the latter produces fetal coelomic epithelium.[11] Therefore, a connection arises between the embryonic ectoderm and fetal coelomic epithelium.

Although the biologic significance of CA 125 remains unclear, the production of CA 125 from the periderm



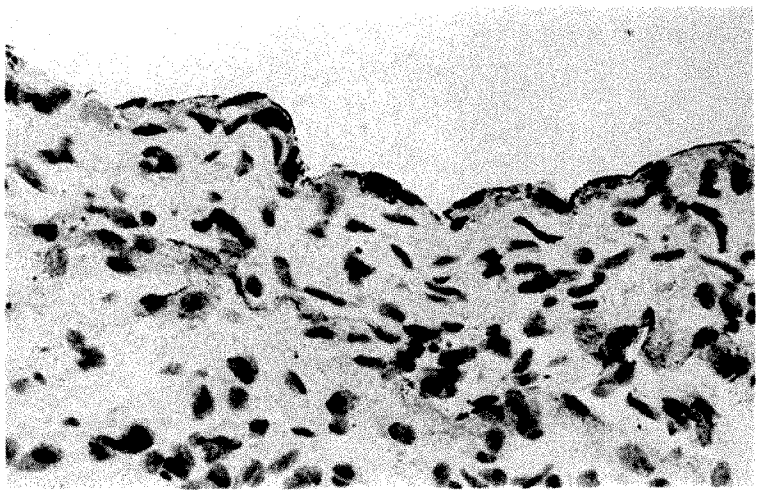


Fig. 5. Pericardium at 15 weeks of gestation is reactive with anti-CA 125 in the apical portion of the cytoplasm. (Original magnification ×400.)

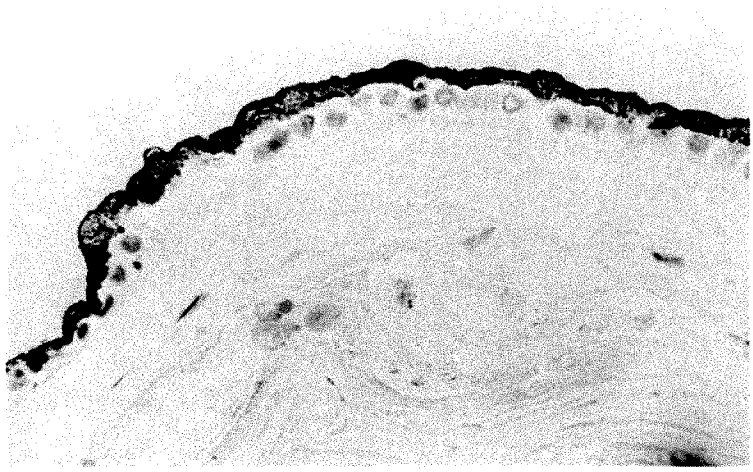


Fig. 6. Amniotic epithelium at 37 weeks of gestation is reactive with anti-CA 125 not only along the luminal surface and in the apical portion of the cytoplasm but also in the cytoplasm. (Original magnification ×400.)

suggests that a high-molecular-weight glycoprotein expressing an antigenic determinant CA 125 may be a primitive substance that covers the skin, at least until midgestation when the periderm is replaced by the vernix caseosa.[11] In contrast, the fetal coelomic epithelium and its derivatives begin to synthesize CA 125 during differentiation into adult müllerian epithelium. Conversely, fetal coelomic epithelium, which is derived from the primitve streak of the ectoderm, may begin to produce CA 125 when it differentiates into cells resembling the stage of cells of the periderm or amnion.

With regard to the production of CA 125 in neoplastic tissues, serous carcinomas of the ovary usually show an increased secretion of CA 125,[2] but most adenocarcinomas of the uterine cervix show a reduced secretion of CA 125.[8, 14] Although it is unknown whether the epitopes of CA 125 glycoprotein molecules produced by neoplastic tissues are equivalent to those of normal tissues, neoplastic tissues may have some acquired genetic abnormality in either the production or secretion of CA 125.

REFERENCES

1. Bast RC Jr, Feeney M, Lazarus H, Nadler LM, Colvin PB, Knapp RC. Reactivity of a monoclonal antibody with ovarian carcinoma. J Clin Invest 1981;68:1331-7.
2. Bast RC Jr, Klug TL, St. Johns E, et al. A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer. N Engl J Med 1983; 309:883-7.
3. Niloff JM, Klug TL, Schaetzl E, Zurawski VR Jr, Knapp RC, Bast RC Jr. Elevation of serum CA 125 in carcinomas of the fallopian tube, endometrium, and endocervix. AM J OBSTET GYNECOL 1984;148:1057-8.

4. Patton PE, Field CS, Harms RW, Coulam CB. CA 125 levels in endometriosis. Fertil Steril 1986;45:770-3.
5. Halila H, Stenman U-H, Seppala M. Ovarian cancer antigen CA 125 levels in pelvic inflammatory disease and pregnancy. Cancer 1986;57:1327-9.
6. Niloff JM, Knapp RC, Schaetzl E, Reynolds C, Bast RC Jr. CA 125 antigen levels in obstetrics and gynecologic patients. Obstet Gynecol 1984;64:703-7.
7. Kabawat SE, Bast RC Jr, Bhan AK, Welch WR, Knapp RC, Colvin RB. Tissue distribution of a coelomic epithelium-related antigen recognized by the monoclonal antibody OC 125. Int J Gynecol Pathol 1983;2:275-85.
8. Nanbu Y, Fujii S, Konishi I, Nonogaki H, Mori T. Immunohistochemical localizations of CA 125, carcinoembryonic antigen, and CA 19-9 in normal and neoplastic glandular cells of the cervix. Cancer 1988;62:2580-8.
9. O'Brien TJ, Hardin JW, Bannon GA, Norris JS, Quick JG. CA 125 antigen in human amniotic fluid and fetal membranes. Am J Obstet Gynecol 1986;155:50-5.
10. Kobayashi F, Sagawa N, Nanbu Y, et al. Immunohistochemical localization and tissue levels of tumor-associated glycoproteins CA 125 and CA 19-9 in the decidua and fetal membranes at various gestational ages. Am J Obstet Gynecol 1989;160:1232-8.
11. Hamilton WJ, Mossman HW. Human embryology. 4th ed. London: Williams & Wilkins, 1972:64-82.
12. Luckett PW. The development of primordial and definitive amniotic cavities in early rhesus monkey and human embryo. Am J Anat 1975;144:149-68.
13. Gardner RL. Origin and differentiation of extra embryonic tissues in mouse. Int Rev Exp Pathol 1983;24:63-131.
14. Fujii S, Konishi I, Nanbu Y, et al. Analysis of the levels of CA 125, carcinoembryonic antigen, and CA 19-9 in the cervical mucus for a detection of cervical adenocarcinoma. Cancer 1988;62:541-7.

Power spectrum analysis of myometrial electromyogram and intrauterine pressure changes in the pregnant rhesus monkey in late gestation

Hsing Wen Hsu, BS, Jorge P. Figueroa, MD, PhD, M. Barbera O. M. Honnebier, MD, Richard Wentworth, PhD, and Peter W. Nathanielsz, MD, PhD

Ithaca, New York

Various methods have been used to quantify myometrial activity in the pregnant rhesus monkey during the last third of gestation. A precise definition of activity must retain the characteristics of epoch duration, amplitude, and the repetitive frequencies of active epochs. We used Fast Fourier Transform and power spectrum analysis to determine whether the patterns of myometrial contractility consist predominantly of contractions or contractures. This analysis allows quantification of activity represented by each of these patterns of activity. We applied these methods to myometrial electromyogram and intrauterine pressure recordings in pregnant rhesus monkeys to characterize the changes in myometrial activity that occur after surgery, after intravenous indomethacin administration, and when food is withdrawn for 48 hours. We conclude that changes in the frequency pattern of myometrial activity provide important information in relation to labor and delivery contractions. (Am J Obstet Gynecol 1989;161:467-73.)

Key words: Power spectrum analysis, myometrial activity, uterine electromyogram

Various methods have been used in experimental animals to characterize and quantify myometrial activity throughout pregnancy.[1-9] The pregnant rhesus monkey has been extensively investigated to study changes in myometrial activity and responses to various physiologic and pharmacologic agents. Myometrial electromyogram electrodes and sensors for measuring intrauterine pressure can be placed in this species at the beginning of the last third of gestation. Several groups of investigators have shown varying degrees of activity at different times of the 24-hour day during various stages of gestation.[5, 10, 11] These cycles of myometrial activity have a clear temporal relationship to the circadian hormone changes in maternal and fetal plasma.[10, 12]

Epochs of myometrial activity differing in duration and intensity have been described in pregnant sheep

From the Laboratory for Pregnancy and Newborn Research, Department of Physiology, New York State College of Veterinary Medicine, Cornell University.
Supported by National Institutes of Health grant HD 18870.
Received for publication July 29, 1988; revised February 28, 1989; accepted March 3, 1989.
Reprint requests: Peter W. Nathanielsz, MD, PhD, Laboratory for Pregnancy and Newborn Research, Department of Physiology, NYS College of Veterinary Medicine, Cornell University, Ithaca, NY 14853.

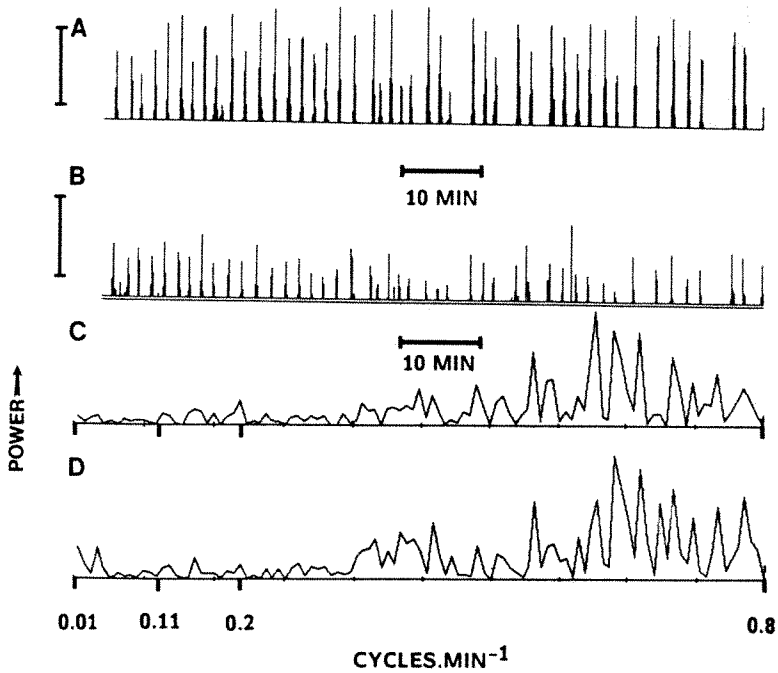


Fig. 1. *A*, Intrauterine pressure recording in a pregnant rhesus monkey 1 day after operation; calibration bar 10 mm Hg. *B*, Myometrial electromyogram at the same time as *A*; calibration bar 50 μV. The running mean threshold baseline has been removed in both *A* and *B*. *C*, Power spectral analysis of intrauterine pressure recording in *A*. *D*, Power spectral analysis of electromyogram recording in *B*.

and monkeys.[2-4] One method of assessing the nature of activity at any moment is to characterize duration and amplitude of each individual event of myometrial electromyogram activity or intrauterine pressure change, either together or separately. In the sheep we have described computer-based methods for the determination of duration of individual epochs of activity.[6] In sheep, throughout the majority of gestation the most common epochs of myometrial activity are contractures, which we have defined as long duration epochs of increased intrauterine pressure or electromyogram activity lasting longer than 3 minutes.[3, 4, 6, 13] During labor and at delivery, myometrial activity shows a contraction pattern during which the epochs of activity are shorter than 2 minutes.[13] To obtain a clear impression of the type of myometrial activity under different physiologic conditions, contractures must be distinguished from labor and delivery contractions. Assessment of myometrial activity as Montevideo units or area of activity under a pressure curve does not permit such a distinction because in the use of these methods important information regarding duration and frequency of individual epochs of activity is lost.

Fast Fourier Transform and power spectrum analysis are alternative methods for studying waveforms that have repetitive frequencies. The Fast Fourier Transform represents the decomposition of the waveform under study into its frequency components. Fast Fourier Transform also permits assessment of amplitude of the peaks that occur with the different frequencies. The retention of amplitude characteristics and division of all observed activity epochs into repetitive frequencies at different frequency ranges allows greater description of patterns of myometrial activity than analyses based solely on counts of individual epochs of activity or Montevideo units or computed area under the curve of the raw electromyogram or intrauterine pressure signal. Depending on the rate of sampling and the frequency of the waveform, Fast Fourier Transform and power spectrum analyses can be performed over variable periods of time appropriate to the experimental question under study. Thus myometrial activity for many hours at different times of the day on successive days or for short periods after administration of various drugs can be compared.

The purpose of this study was to determine whether Fast Fourier Transform and power spectrum analysis of uterine electromyogram and intrauterine pressure make possible rapid distinction of different patterns of myometrial activity. In particular, we wanted to determine whether these methods permit clear distinction of contraction activity that is characteristic of labor and delivery. We performed Fast Fourier Transform and power spectrum analysis of both myometrial electromyogram and intrauterine pressure in the pregnant rhesus monkey obtained during three different situa-

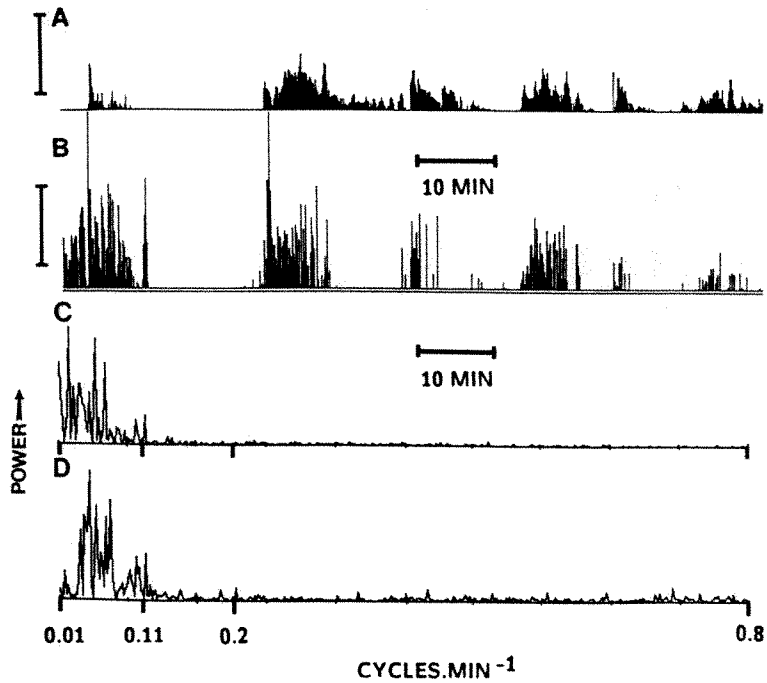


Fig. 2. *A*, Intrauterine pressure recording in a pregnant rhesus monkey 3 days after operation; calibration bar 10 mm Hg. *B*, Myometrial electromyogram at the same time as *A*; calibration bar 50 μV. The running mean threshold baseline has been removed in both *A* and *B*. *C*, Power spectral analysis of intrauterine pressure recording in *A*. *D*, Power spectral analysis of electromyogram recording in *B*.

tions: immediately after operation, after food withdrawal, and during treatment with the prostaglandin synthetase inhibitor indomethacin. The results permit a direct comparison of myometrial electromyogram and intrauterine pressure that is important in the assessment of the many previous studies in which only one of these two variables was measured.

Methods

Care of animals

Surgical procedures. Ten pregnant rhesus monkeys of known gestational age were obtained from the California Regional Primate Center, Davis, Calif., and were acclimated to the laboratory conditions as previously described.[11, 14] The animals were housed in rooms with controlled light cycles (14:10 hours light-dark) with lights on at 8 AM. Purina 5045 Monkey Chow and fresh fruits were provided. Water was provided ad libitum during the experimental period. At the time of operation monkeys ranged from 92 to 107 days' gestational age. Animals were instrumented with techniques described previously.[5, 11, 14] Briefly, with the animals under halothane anesthesia, catheters were placed in the maternal femoral artery and vein in all 10 animals. In each animal, two pairs of myometrial electromyogram leads were placed on the anterior wall of the uterus. In six animals, two catheters made of Tygon microbore tubing (Norton Performance Plastics, Akron, Ohio) (inner diameter 1.27 mm; outer diameter 2.29 mm), with a side hole 1 cm from the tip, were placed with 3 to 6 cm of the catheter within the amniotic cavity. A small retaining collar of Tygon prefabricated around the catheter was located just within the uterus. Heparinized arterial blood samples were drawn at 10 AM, 3 PM, and 9 PM on most days for various hormonal analyses (data not reported). No experimental intervention was initiated for at least 5 days after operation.

Indomethacin protocol. In one animal that was having spontaneous contractions at 132 days' gestation, Indocin (Merck, Sharpe and Dohme) was administered via the maternal inferior vena caval catheter at an infusion rate of 6 mg/hr starting at 3:30 PM and ending at 9 PM.

Food withdrawal protocol. On days that animals were fed, fresh food was provided at 8 AM and the monkey was allowed to feed freely. Three animals were used in the food withdrawal protocols. Food was removed at 3 PM and the next 24-hour period was called the first day of food withdrawal. Food was offered freely after exactly 48 hours of food withdrawal. Only the electromyogram was recorded in the food withdrawal protocol.

Recording of electromyogram and data storage. The uterine electromyogram was recorded from multistranded bipolar stainless steel wires (Cooner AS 632) implanted 3 to 5 mm apart on at least two sites of the

Table I. Percentage of power spectrum activity above and below three different frequency levels in intrauterine pressure and electromyogram records from six instrumented pregnant rhesus monkeys in the first 5 days after surgery

	Activity (%)					
	Cutoff 0.11 cycles/min		*Cutoff 0.2 cycles/min*		*Cutoff 0.25 cycles/min*	
	Above	*Below*	*Above*	*Below*	*Above*	*Below*
Contractions						
Mean	95.4	4.6	83.6	16.4	71.8	28.2
SD	3.7	3.7	5.7	5.7	20.6	20.6
Contractures						
Mean	35.3	64.7	26.0	74.0	23.1	76.9
SD	13.9	13.9	10.9	10.9	10.0	10.0

myometrium.[5, 11, 14] One of the intraamniotic catheters was connected to a pressure transducer (Cobe Lakewood, Colo.) that was placed in a small box on the monkey's back beneath the jacket. Acquisition of the myometrial electromyogram and intrauterine pressure waveforms was performed with a custom-built computer-based data acquisition system previously described in detail.[6] Briefly, the signals (electromyogram and intrauterine pressure) were sampled at 32 Hz, digitized, and the average value over 8 seconds was stored on tape with a time signal. The initial sampling frequency of 32 Hz prevented aliasing problems with changing signals. The lower storage rate of the one reading every 8 seconds represented the averaged data and created a manageable volume of data for subsequent analysis.

Data analysis. Fast Fourier Transform and power spectrum analysis were performed on the digitized intrauterine pressure and electromyogram data by means of the Asyst 2.0 program.[15] Integration of the area under the curve for the power spectrum was performed by addition of all data points for the chosen range of cycle frequencies.

Statistical analysis. In the food withdrawal protocol, the integrated power spectrum data were analyzed by Student's paired t test after one-way analysis of variance. The paired t test was used only for comparison of the increment between the control period (either daylight or darkness) and the same period on the second day of food withdrawal (either daylight or darkness). The effect of the treatment was considered statistically significant when $p < 0.05$. Data obtained during hours of daylight and hours of darkness were analyzed separately and were not compared to each other.

Results

Determination of power spectrum frequency windows. In six animals the power spectrum of either intrauterine pressure and/or electromyogram or both were analyzed for frequencies between 0.01 and 0.8 cycles/min. Because there were no differences between power spectra of intrauterine pressure or electromyogram data in the different frequency windows (Figs. 1 and 2), data for percentage power in different windows of the intrauterine pressure and electromyogram spectra were pooled for analysis of different patterns of contraction and contracture activity (Table I) to determine the percentage of total power at frequencies above and below three different cutoffs (0.11, 0.2, and 0.25 cycles/min). Data were obtained from animals in the first 5 days after operation and were divided into periods when, by visual inspection, the activity consisted primarily of contractions and when activity consisted primarily of contractures (Table I). After evaluation of these data, quantification of contracture activity was performed on frequencies in the window 0.01 to 0.11 cycles/min (i.e., <1 cycle every 9 minutes) and contractions on frequencies in the window 0.2 to 0.8 cycles/min (i.e., >1 cycle every 5 minutes).

Power spectral analysis of contractions. Fig. 1 shows the raw data trace for intrauterine pressure and electromyogram at a time when only contractions occurred. The good correspondence between intrauterine pressure and electromyogram can readily be seen. For the electromyogram recording shown, 94.8% of the activity in repeating frequencies was at a rate >1 every 5 minutes (a frequency >0.2 cycles/min). For the intrauterine pressure recording shown, 97.1% of the activity in repeating frequencies occurred at rates >1 every 5 minutes.

Power spectral analysis of contractures. Fig. 2 shows the raw data trace for intrauterine pressure and electromyogram at a time when only contractures occurred. The good correspondence between intrauterine pressure and electromyogram can readily be seen. For the electromyogram activity in the recording shown, 66.4% of the activity in repeating frequencies was at frequencies lower than 1 every 9 minutes (a frequency <0.11 cycles/min). For the intrauterine pressure in the recording shown, 83.5% of the activity in repeating frequencies occurred at rates <1 every 9 minutes.

Indomethacin effect. Fig. 3 shows the change in the power spectrum of intrauterine pressure and myo-

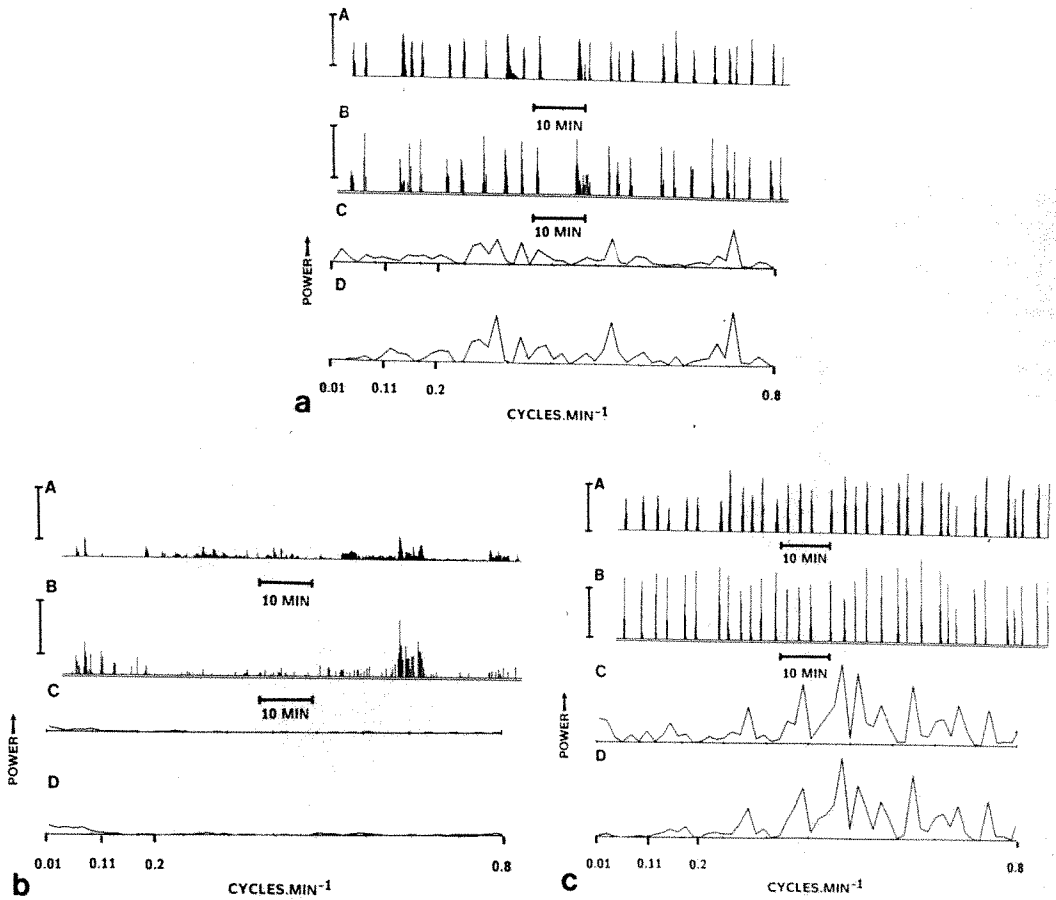


Fig. 3. Myometrial electromyogram and intrauterine pressure in a pregnant rhesus monkey 132 days' gestational age and 20 days after operation taken between 2 PM and 11:30 PM. **a,** Before indomethacin. **b,** During indomethacin infusion at 6 mg/hr via the maternal aorta. **c,** After cessation of indomethacin infusion. *A,* Intrauterine pressure recording: calibration bar 10 mm Hg; *B,* Myometrial electromyogram at the same time as *A*; calibration bar 50 μV. The running mean threshold baseline has been removed in both *A* and *B*. *C,* Power spectral analysis of intrauterine pressure recording in *A*. *D,* Power spectral analysis of electromyogram recording in *B*.

metrial electromyogram after a pregnant monkey that was having spontaneous contractions was treated with indomethacin (Fig. 3, *a*). During the period of indomethacin treatment, the majority of the power in the spectrum for both intrauterine pressure and electromyogram shifted to the low-frequency range (Fig. 3, *b*), but after the contractions returned, the power spectrum shifted back to the high-frequency range (Fig. 3, *c*).

Food withdrawal. The nighttime power spectrum for electromyogram in one animal the night before food withdrawal is shown in Fig. 4, *A*. The majority of the spectral power is at frequencies <1 cycle every 9 minutes. On the second night of food withdrawal, the power spectrum shifts to high frequency (Fig. 4, *B*); after food is returned, the spectrum shifts back to low frequency (Fig. 4, *C*). Fig. 5 shows the total power in the contracture and contractions portions of the power spectrum before, during, and after the food withdrawal for daylight and darkness portions of the 24-hour period. By the second night of food withdrawal the activity in the contractions portion of the power spectrum had risen to 660% of the second control day ($p < 0.05$). There was no change in the contractures spectrum. Power spectrum during daylight hours was analyzed on control day 2, food withdrawal day 2, and recovery day 2 in the three animals that had food withdrawn. On the second food withdrawal day the contractions portion of the power spectrum had risen to 270% of the second control day ($p < 0.05$). This represents a much smaller increase than during the hours of darkness. The contractures portion of the power spectrum also increased significantly above that on control day 2 (Fig. 5).

Comment

Figs. 1 and 2 demonstrate the excellent correspondence between the power spectra of intrauterine pressure and electromyogram. It is sometimes impossible to obtain good intrauterine pressure recordings from

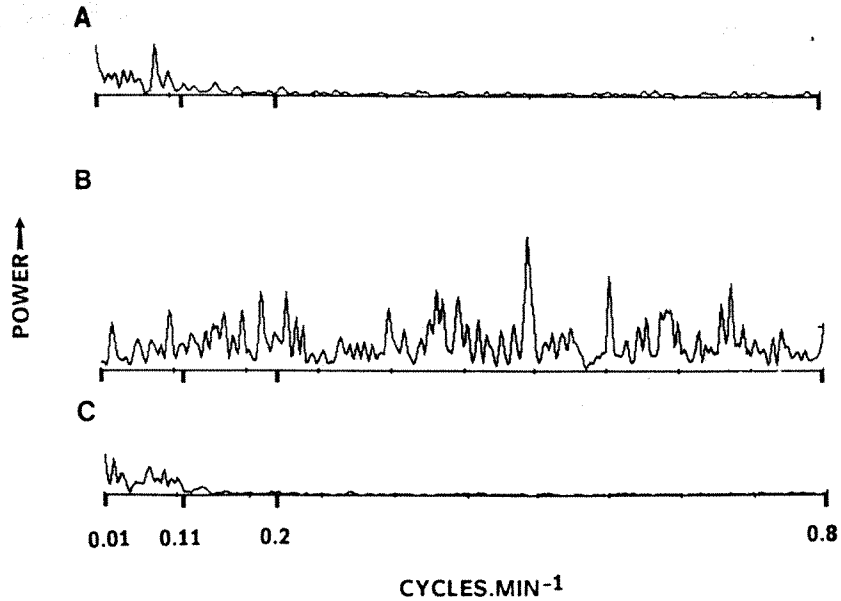


Fig. 4. Electromyogram data from an instrumented pregnant rhesus monkey at 127 to 133 days' gestational age and 28 to 34 days after operation. Power spectrum of myometrial electromyogram between 8 PM (when lights go off) and 5 AM on *A*, the night before food withdrawal; *B*, on the second night of food withdrawal, and *C*, the second night after food was returned.

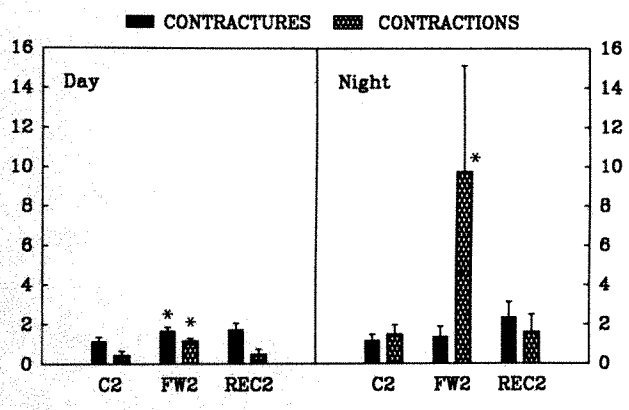


Fig. 5. Daylight *(day)* and darkness *(night)* power spectrum analysis data from three animals in the 24-hour period before (control, *C2*), the second 24-hour period of food withdrawal *(FW2)* and the second 24-hour period after food withdrawal *(REC2)* (mean ± SD, $n = 3$). Ordinate: ▨, activity at frequencies >1 every 5 minutes; ■, activity at frequencies <1 every 9 minutes. (*A significant increase from *C2*; $p < 0.05$). *Day* refers to the daylight period of each 24-hour period. *Night* refers to the dark period of each 24-hour period.

pregnant animals. Movement artifacts (e.g., crouching and straining at stool) produce artificial changes in intrauterine pressure that can only be distinguished from myometrial activity if simultaneous electromyogram is recorded. In addition, in some experimental paradigms it is preferable not to incise the uterus. Thus it is reassuring that recordings of electromyogram over several hours have good correspondence to the information obtained from intrauterine pressure. Our studies with the pregnant sheep show that individual electromyogram complexes may not be accompanied by changes in intrauterine pressure because electromyogram activity before labor may not be propagated over the whole uterus and local contractility of the myometrium may not be reflected by intrauterine pressure changes.[4] At different times of the day and under different physiologic situations, we have noticed that in the pregnant monkey there may also be differences in the amount of intrauterine pressure change generated by individual electromyogram complexes of similar amplitude and duration, but we have not made a detailed study of this relationship. This relationship will be affected by factors that affect activation-contraction coupling such as the density of gap junctions.[16]

The power spectrum showed the majority of activity at low frequency when only contractures were present. The exact frequency peaks depend both on the envelope of duration of the individual contracture and their frequency of occurrence. When the raw data showed mostly contractures, 65% of the power was at frequencies <1 cycle every 9 minutes. When contractions predominated, 84% of the power was at frequencies >1 cycle every 5 minutes. Therefore for future analysis of waveforms from any sensors of myometrial activity we have chosen two windows: <1 cycle every 9 minutes to represent contracture activity and >1 cycle every 5 minutes for contraction patterns.

Quantification of myometrial activity is important if changes throughout gestation and responses to individual pharmacologic agents are to be assessed. The

power spectrum may be precisely quantified by analyzing the power within any spectral frequency range. Thus this method retains both frequency and amplitude characteristics. Analysis of area under either the intrauterine pressure or electromyogram activity envelope provides incomplete information since a given amount of area under curve could reflect periods of either predominantly contraction or contracture activity. Power spectrum analysis also enables information from many hours of recording to be condensed into an easily visible and quantifiable format.

Power spectrum may be used to compare myometrial activity of the same animal between different periods of time, such as the hours of daylight and the hours of darkness. In addition, direct comparison of the same period (e.g., daytime before, during, and after administration of drugs) can be made. Thus when indomethacin was infused into the maternal aorta, contractions were converted to contractures; the main spectral power shifted to the low frequency. This effect was reversed when the indomethacin infusion was stopped.

Power spectrum analysis can also be used to demonstrate whether changes occur at the same time of the day but at different periods of gestation or over successive days of an experimental protocol. For example, in our food withdrawal studies, the power spectral shift from the contractures spectrum to the contractions spectrum was much more marked at nighttime. To study physiologic and pathophysiologic regulation of myometrial activity (e.g., preterm labor), we suggest that computer-based power spectral analysis of features of myometrial activity be applied to the critical times of the day at which a power spectral shift occurs from the contracture to contractions spectrum. In the search for better methods to understand myometrial activity both in experimental animals and in pregnant women, we propose that power spectral analysis will prove to be a powerful tool in the determination of specific patterns of myometrial activity and in the assessment of which patterns are innocuous and which are ominous.

We thank Karen Moore for assistance with the manuscript and Ellen Wolfe and Larry Kantrowitz for their day-to-day attention to the care of the animals. We thank Dr. Robin Gleed for his skilled assistance with the critical anesthesia required for these surgeries.

REFERENCES

1. Harbert GM, Spisso KR. Biorhythms of the primate uterus *(Macaca mulatta)* during labor and delivery. AM J OBSTET GYNECOL 1980;138:686-96.
2. Nathanielsz PW, Jack PMB, Krane EJ, Thomas AL, Ratter S, Rees LH. The role and regulation of corticotropin in the fetal sheep. In: The fetus and birth. Ciba Foundation Symposium no. 47 (new series). Amsterdam: Elsevier/North Holland, 1977:73-91.
3. Jansen CAM, Krane EJ, Thomas AL, et al. Continuous variability of fetal Po_2 in the chronically catheterized fetal sheep. AM J OBSTET GYNECOL 1979;134:766-83.
4. Nathanielsz PW, Bailey A, Poore ER, Thorburn GD, Harding R. The relationship between myometrial activity and sleep state and breathing in fetal sheep throughout the last third of gestation. AM J OBSTET GYNECOL 1980;138:653-9.
5. Taylor NF, Martin MC, Nathanielsz PW, Seron-Ferre M. The fetus determines the circadian oscillation of myometrial electromyographic activity in the pregnant rhesus monkey. AM J OBSTET GYNECOL 1983;146:557-67.
6. Figueroa JP, Mahan S, Poore ER, Nathanielsz PW. Characteristics and analysis of uterine electromyographic activity in the pregnant sheep. AM J OBSTET GYNECOL 1985;151:524-31.
7. Demianczuk N, Towell ME, Garfield RE. Myometrial electrophysiologic activity and gap junctions in the pregnant rabbit. AM J OBSTET GYNECOL 1984;149:485-91.
8. Taverne MA, Naaktegeboren C, Elshesser F, Forsling ML, van der Weyden GC, Ellendorff F, Schmidt D. Myometrial electrical activity and plasma concentrations of progesterone, estrogens, and oxytocin during late pregnancy and parturition in the miniature pig. Biol Reprod 1979b;21:1125-34.
9. Novy MJ. Endocrine and pharmacological factors which influence the onset of labor in rhesus monkeys. In: Knight J, O'Connor M, eds. The fetus and birth. Ciba Foundation Symposium no. 47 (new series). Amsterdam: Elsevier/North Holland, 1979:259-88.
10. Ducsay CA, Cook MJ, Walsh SW, Novy MJ. Circadian patterns and dexamethasone-induced changes in uterine activity in pregnant rhesus monkeys. AM J OBSTET GYNECOL 1983;145:389-96.
11. Nathanielsz PW, Frank D, Gleed R, Dillingham L, Poore ER, Figueroa JP. Methods of investigation of the chronically instrumented pregnant rhesus monkey preparation maintained on a tether and swivel system. In: Nathanielsz PW, ed. Animal models in fetal medicine. Ithaca, New York: Perinatology Press, 1984: vol 3:110-60.
12. Walsh SW, Ducsay CA, Novy MJ. Circadian hormonal interactions among the mother, fetus and amniotic fluid. AM J OBSTET GYNECOL 1984;150:745-53.
13. Harding R, Poore ER, Bailey A, Thorburn GD, Jansen CA, Nathanielsz PW. Electromyographic activity of the nonpregnant and pregnant sheep uterus. AM J OBSTET GYNECOL 1982;142:448-57.
14. Binienda Z, Massmann A, Mitchell MD, Gleed RD, Figueroa JP, Nathanielsz PW. Effect of food withdrawal on arterial blood glucose and plasma 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ concentrations and nocturnal myometrial electromyographic activity in the pregnant rhesus monkey in the last third of gestation: a model for preterm labor? AM J OBSTET GYNECOL 1989;160:746-50.
15. Campbell JA, Mathison M, Pook P, Wyatt A. Up and running with Asyst 2.0. MacMillan Software Company, 1987.
16. Garfield RE, Rabideau S, Challis JR, Daniel EE. Hormonal control of gap junction formation in sheep myometrium during parturition. Biol Reprod 1979;21:999-1007.

Pattern of human chorionic gonadotropin binding in the polycystic ovary

James Brawer, PhD,[a] Martine Richard, MSc,[b,] * and Riaz Farookhi, PhD[c]
Montreal, Quebec, Canada

The histologic evolution of polycystic ovaries in the estradiol valerate–treated rat coincides with the development of a unique plasma pattern of luteinizing hormone. To assess the role of luteinizing hormone in polycystic ovaries, it is necessary to evaluate the luteinizing hormone sensitivity of the specific tissues in the polycystic ovary. Therefore, we examined the pattern of luteinizing hormone binding sites in polycystic ovaries. Rats at 4 or 8 weeks after estradiol valerate treatment each received an intrajugular injection of iodine 125–labeled human chorionic gonadotropin. Some rats also received a 1000-fold excess of unlabeled human chorionic gonadotropin in the same injection. Ovaries were prepared for autoradiography. Dense accumulations of grains occurred over the theca of normal and atretic secondary follicles in all ovaries and over clusters of secondary interstitial cells. The iodine label was variable over the typically hypertrophied theca of precystic follicles. The theca of definitive cysts showed little or no label. These results indicate that cyst formation coincides with the loss of luteinizing hormone/human chorionic gonadotropin binding to the affected follicles. (Am J Obstet Gynecol 1989;161:474-80.)

Key words: Follicular cysts, polycystic ovaries, theca, LH/hCG binding

An injection of estradiol valerate produces a polycystic ovarian condition in the rat.[1-3] The ovaries contain large cystic follicles that exhibit characteristic features such as a highly attenuated granulosa cell layer and marked thecal hypertrophy. Numerous clusters of large lipid-filled secondary interstitial cells are scattered throughout the stroma. In addition, the ovaries contain no new corpora lutea and very few normal large secondary follicles.[1-3]

These distinctive morphologic features emerge between 4 and 8 weeks after estradiol valerate treatment. During the first 4 weeks, the ovaries are characterized by numerous large atretic secondary follicles and, in fact, the transition to the cystic state appears to result from an arrest of the atretic process.[3]

The transformation in ovarian morphologic features to the polycystic state coincides with the appearance of a unique pattern of plasma luteinizing hormone (LH).[4] This specific LH pattern is observed in all animals with established polycystic ovaries regardless of the duration of the condition. This suggests that LH plays a significant role in the development and maintenance of the cystic ovary. The ovary contains a variety of structures that are normally responsive to LH. At present we have no information on the LH receptivity of the tissues in the polycystic ovary; therefore we examined the distribution of LH binding sites in the polycystic ovary by means of autoradiography, with iodine 125–labeled human chorionic gonadotropin (hCG) used as a ligand. hCG binds specifically to the LH receptor with an affinity equal to that of LH.[5] It has been used as a probe of the LH receptor for more than a decade. Our results demonstrate that cyst formation coincides with the loss of LH binding in the cells of the theca interna.

Material and methods

Treatment of animals. Six-week-old female Wistar rats were purchased from Charles River (Canada) Ltd. (St. Constant, Quebec). The animals were maintained under conditions of controlled light (12 hours per day) and temperature (22° C) and had free access to food (Purina Rat Chow, Ralston Purina, St. Louis) and water. Estrous cyclicity was monitored by the daily examination of vaginal smears. Only animals that exhibited two consecutive normal 4-day estrous cycles immediately before treatment were used.

A total of 17 animals (34 ovaries) were studied. All animals exhibited persistent vaginal cornification by 2 weeks after estradiol valerate treatment. Each animal was injected intramuscularly with 2 mg estradiol valerate in 0.2 ml sesame oil. Because we had previously shown that true follicular cysts appear between 4 and 8 weeks after estradiol valerate injection,[3] groups of animals were studied only at each of these time points

From the Departments of Anatomy,[a, b] Physiology,[c] and Obstetrics and Gynecology[a, c] and the McGill Centre for the Study of Reproduction, McGill University.
Supported by the Medical Research Council of Canada.
Received for publication October 31, 1988; accepted March 13, 1989.
Reprint requests: James R. Brawer, PhD, Department of Anatomy, McGill University, 3640 University St., Montreal, Quebec, Canada H3A 2B2.
**M. Richard was the recipient of a studentship of the McGill Centre for the Study of Reproduction (Quebec).*

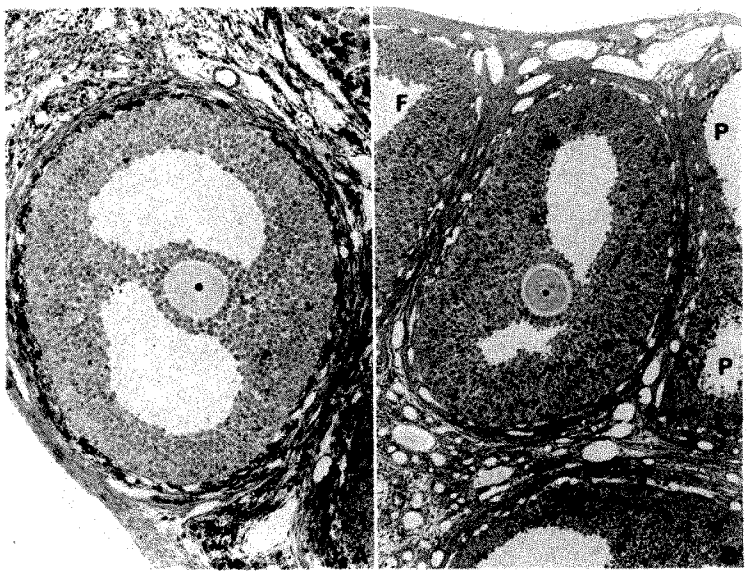


Fig. 1. A, Ovary exposed to ^{125}I-hCG. Theca interna of healthy follicle in center of field is heavily labeled, as are theca and perimural granulosa cell layers of segment of type III large follicular structure in extreme lower right corner of field. (Original magnification ×200.) **B,** Ovary exposed to ^{125}I-hCG and large excess of unlabeled hCG. Healthy follicle appears in center of field with segment of type III large follicular structure *(F)* and portion of precystic follicle *(P)*. Autoradiographic reaction product is absent from all three structures. (Original magnification ×156.)

after estradiol valerate treatment. At 4 weeks after estradiol valerate treatment, two animals each received ^{125}I-hCG (100×10^6 cpm/0.3 ml phosphate-buffered saline solution; specific activity, 50 μCi/μg). Three other animals received the same dose of ^{125}I-hCG and an approximately 1000-fold excess (on a mass basis) of unlabeled hormone. Similarly, at 8 weeks after estradiol valerate treatment, nine animals were injected with labeled hCG alone and three animals received the labeled hormone and a 1000-fold excess of unlabeled hormone. Both ovaries of each of the animals exhibited cystic follicles when studied at 8 weeks. The same was true for the ovaries of the 4-week animals except that there were fewer definitive cysts per ovary.

Hormone injection and perfusion procedure. Animals were anesthetized with 15% urethane injected intraperitoneally (1 ml/100 gm body weight) before injection with hCG. The jugular vein was exposed and the hCG was injected directly therein. Thirty minutes later the animals were perfused. The 30-minute exposure was based on previous reports that maximal ovarian binding of hCG occurs well within this interval.[6, 7]

A ventral incision was then made and the descending aorta and inferior vena cava were exposed. A loose ligature was placed around the two vessels at a level just above the branching of the two renal veins. The aorta was cleared of fat and connective tissue and was clamped at the point of bifurcation of the common iliac arteries. An 18-gauge needle was then introduced into the aorta just above the clamp. The needle was attached to a length of intravenous tubing that branched at the proximal end. Each branch was inserted into a bottle suspended 3 feet above the perfusion table. One bottle contained lactated Ringer's solution and the other a fixative consisting of 1% glutaraldehyde and 1% formaldehyde in 0.2 mol/L phosphate buffer (pH 7.4).

A slow flow of Ringer's solution was started just before insertion of the needle into the aorta; the flow of the lactated Ringer's solution was then increased to its maximum. Simultaneously the aortic ligature was tightened and a small incision was made in the right renal vein. When the right kidney appeared to clear of blood, the perfusate was switched from the lactated Ringer's solution to the fixative. The lactated Ringer's solution and the fixative were both at room temperature. Fixative (100 to 150 ml) was perfused through the system. The ovaries were then removed, cleared of fat and connective tissue, and placed in fixative overnight.

Preparation for autoradiography. Each ovary was cut into four or five pieces. The cuts were made to include grossly invisible follicular structures in the tissue blocks. The tissues were washed in phosphate-buffered dextrose and were postfixed for 2 hours at 4° C in a solution of 2% osmium tetroxide and 2% potassium ferrocyanide.[8] The tissues were then washed twice in a 2.4% sodium chloride solution, dehydrated in a graded methanol series, and embedded in Epon.[9] The Epon blocks were trimmed and 1 μm thick sections

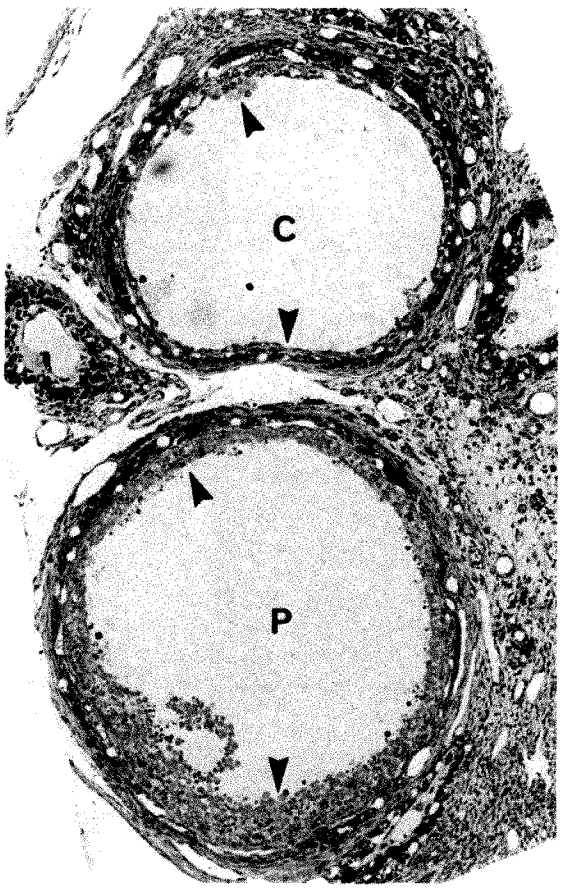


Fig. 2. Cystic and precystic follicles. Precystic follicle in lower half of field *(P)* exhibits several severely atretic layers of granulosa cells *(arrowheads)* in contrast to cystic follicle *(C)* that contains a relatively uniform single layer of granulosa cells *(arrowheads).* (×135.)

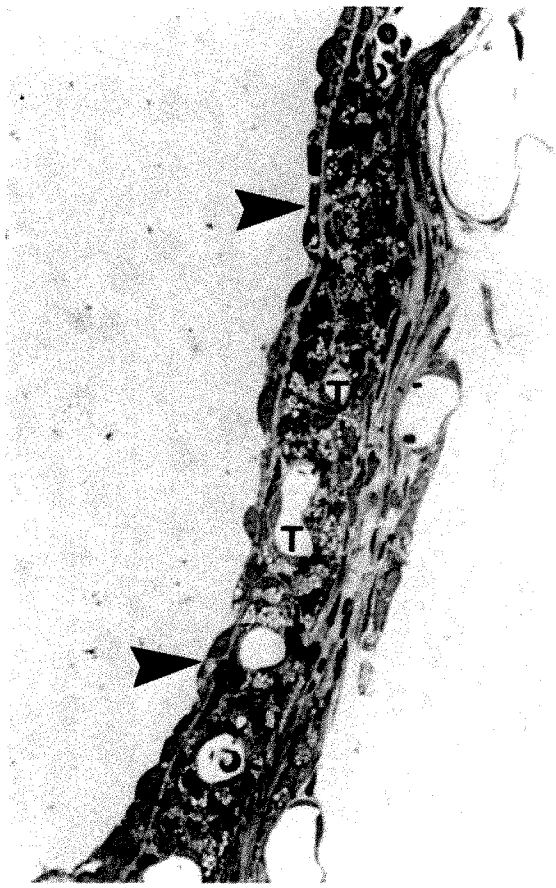


Fig. 3. Cyst wall in ovary exposed to ^{125}I-hCG. Large polygonal cells make up theca interna *(T).* Lipid droplets appear as numerous clear vesicles in thecal cells. Granulosa layer *(arrowheads)* is one cell thick. Note absence of autoradiographic label. (×625.)

were cut with glass knives on a Reichert microtome. All sections were cut within 2 weeks of perfusion.

Slides containing the sections were prewarmed, flooded with 5% ferric ammonium sulfate for 5 minutes (while on the hot plate), and rinsed in distilled water. They were then flooded with Regaud's hematoxylin for 1 minute, washed, and dried.

Autoradiography. Autoradiography of the stained sections was done according to the procedure of Kopriwa and Leblond.[10] The slides were dipped in undiluted Kodak NTB 2 emulsion and were then air-dried for 1 hour in complete darkness. The coated slides were placed in sealed black plastic boxes that contined anhydrous calcium sulfate (desiccant) and stored at 4° C. A test slide removed at 8 days of exposure revealed significant autoradiographic reaction. Therefore most of the slides were removed and developed at this time. A few slides from each ovary were exposed for 21 days. Slides were submerged for 6 minutes in freshly prepared Kodak D-170 developer at 18° C, rinsed in distilled water, fixed in 24% sodium thiosulfate, washed, and air-dried; a coverslip was applied. Photomicrography was done on a Leitz Laborlux D microscope with a Wild photoautomat MPS 45 camera attachment.

Chemicals and reagents. Estradiol valerate (Delestrogen, Squibb Canada, Inc.) was purchased from the Royal Victoria Hospital, Montreal. Highly purified hCG (CR-121; 13,450 IU/mg) was a gift from the Center for Population Research of the National Institute of Child Health and Human Development, Bethesda. Iodination and characterization of iodinated hormone were done in our laboratories in accordance with the method of Ireland and Richards.[11] hCG (1000 IU/mg) used as excess unlabeled hormone in controls was obtained from Ayerst Laboratories (Montreal).

Results

The morphologic features of the precystic and cystic ovaries in the estradiol valerate–treated rat have been described previously.[3, 12] Although the ovaries observed in this study generally conform to those descriptions, the use of perfusion fixation and Epon embedding has

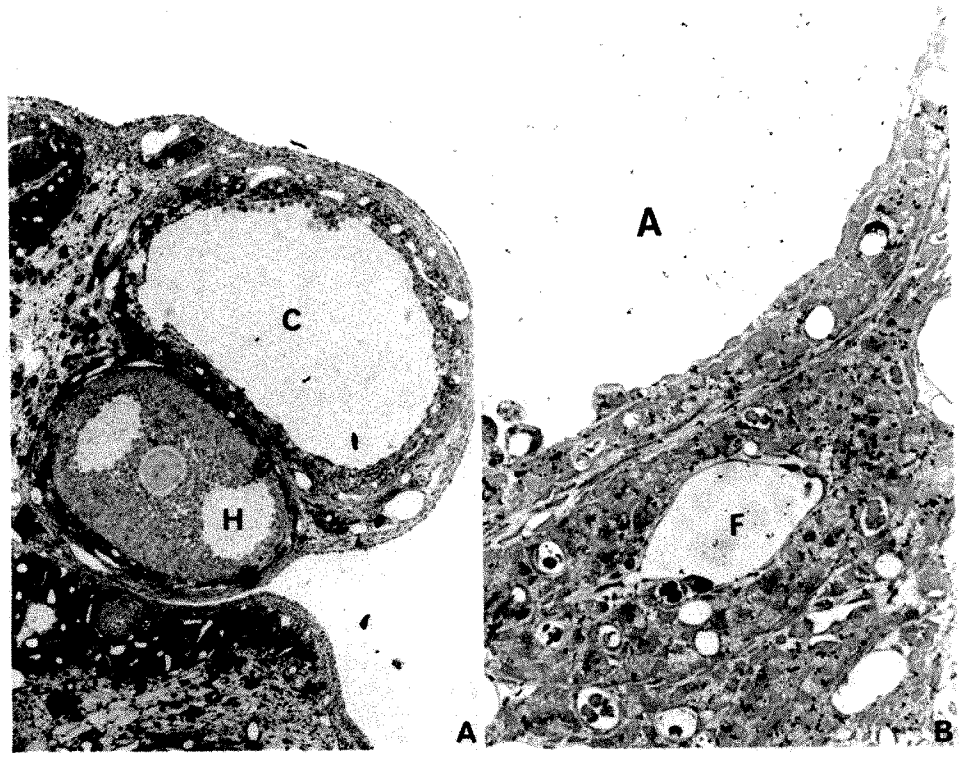


Fig. 4. A, Healthy and cystic follicles in ovary exposed to ^{125}I-hCG. Theca interna of healthy follicle *(H)* is heavily labeled in contrast to that of cyst *(C)*. (Original magnification ×125.) **B,** Wall of cystic follicle in ovary exposed to ^{125}I-hCG. Portion of antrum *(A)* appears in upper left of field. Very old atretic follicle *(F)* is just beneath segment of cyst wall. Large lipid-filled thecal cells in this segment of cyst wall exhibit few grains. (Original magnification ×500.)

permitted a more detailed histologic examination and has revealed several novel features. The specificity of ^{125}I-hCG binding can be appreciated by examining sections from animals injected with labeled hormone to comparable sections from animals that received labeled hormone and a large excess of unlabeled hormone (Fig. 1). The ovaries of all controls that received an excess of unlabeled hormone exhibited negligible autoradiographic labeling.

In this study we observed three classes of large follicular structures, none of which appear in the normal ovary. The first of these structures exhibited thecal hypertrophy and a size that approximated that of the true cyst (Fig. 2). However, the antrum was encompassed by several irregular layers of degenerate granulosa cells. This large follicular structure is identical to the precystic follicle described previously.[3] ^{125}I-hCG binding in these follicles was scant and limited to the theca.

The second type of large follicular structure, the typical follicular cyst, consisted of a large antrum surrounded by one or two layers of granulosa cells and a hypertrophied thecal cell layer (Fig. 2). The thecal cells were often polygonal in shape and very large in contrast to the small fusiform thecal cells observed in healthy secondary follicles. The large thecal cells in cystic follicles frequently contained numerous lipid droplets (Fig. 3). However, in some cysts the theca also contained small fusiform cells. Labeled hCG binding in these follicles was absent in the granulosa and rare to absent in the theca (Figs. 3 and 4).

The third type (type III) of large follicular structure exhibited a unique appearance (Fig. 5). It was the largest of the follicular structures and consisted of four or five layers of healthy granulosa cells surrounding a huge antrum. The granulosa cells, however, differed from those in a normal secondary follicle; they were smaller, more densely stained, more densely packed, and more variable in shape. These differences can best be seen in Fig. 6. Mitotic figures were also occasionally seen randomly scattered throughout the granulosa cell layers. The thecal cell layer was of ordinary thickness and consisted of the small fusiform cells that typify the healthy secondary follicle. Both the theca and granulosa cell layers of these follicles displayed labeled hCG binding. Thecal labeling was intense; dense patches of labeling occurred over the perimural granulosa (Fig. 6).

The precystic follicle was the most commonly observed large follicular structure 4 weeks after estradiol valerate treatment. Definitive cysts were rare and the

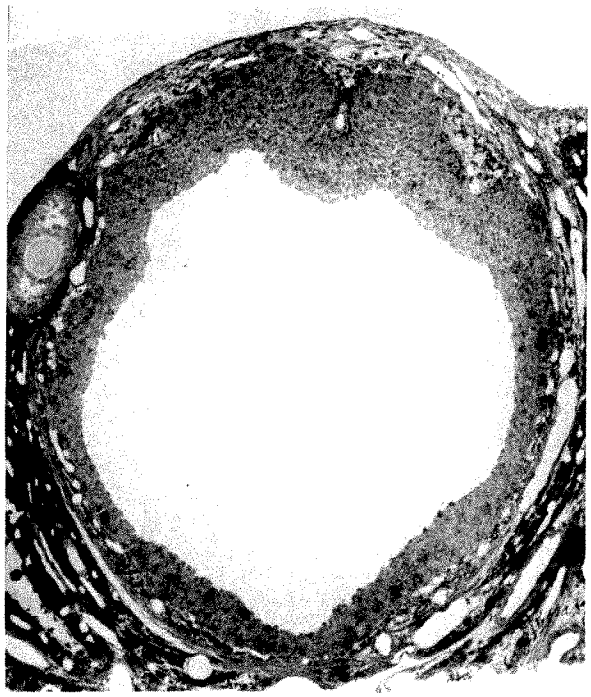


Fig. 5. Type III large follicular structure. This type of follicular structure is the largest seen in polycystic ovary. It is characterized by healthy granulosa cell layers and by modest theca interna consisting of several layers of small fusiform cells. (Original magnification ×140.)

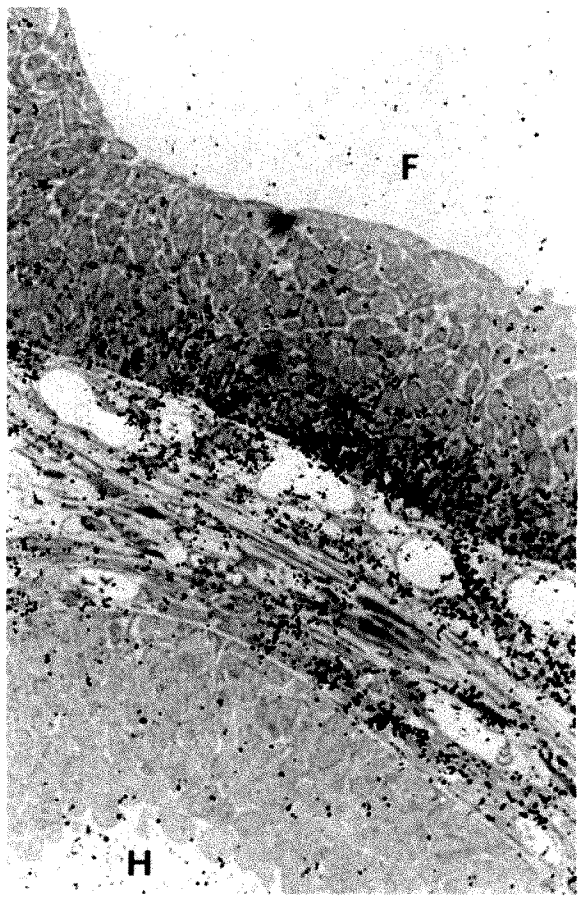


Fig. 6. Segments of type III large follicular structure *(F)* and of healthy follicle *(H)* in ovary exposed to ^{125}I-hCG. Both theca and perimural granulosa cell layers of type III large follicular structure are heavily labeled. Only theca interna is labeled in segment of healthy follicle. (×500.)

type III large follicular structure was never seen. By 8 weeks the precystic follicle was still the most commonly observed large follicular structure, although numerous cysts were also present. The type III large follicular structure also appeared at 8 weeks but occurred approximately half as frequently as the definitive cysts.

There was considerable histologic variation in the multitude of atretic secondary follicles, not only in the granulosa cell layers but also in the theca. For example, the proportion of large vacuolated polygonal thecal cells to the small fusiform variety differed considerably between follicles. Generally, the more severely atretic follicles had the greater proportion of large polygonal cells. Severely atretic follicles in various states of collapse contained exclusively large heavily vacuolated thecal cells that were indistinguishable from secondary interstitial cells. All normal and atretic secondary follicles that were observed exhibited label over the theca. Even severely atretic follicles, in which the granulosa cell layer had been lost and which were in a state of near collapse, exhibited intense thecal labeling (Fig. 7).

Clusters of secondary interstitial cells were numerous throughout the stroma. Apparently healthy primary and small secondary follicles also occurred. Healthy secondary follicles were more numerous at the 4-week posttreatment interval than at 8 weeks. The numerous clusters of secondary interstitial cells were variably labeled. The most recent clusters, in which remnants of the antecedent follicular structure were still visible (e.g., a collapsed antrum), were heavily labeled. However, more irregular clusters of secondary interstitial cells were labeled to a lesser extent and the label was not uniformly distributed over all cells. Some patches of interstitial cells showed little or no label. These were randomly interspersed among intensely labeled clusters.

Comment

Three unusual large follicular structures were observed in the polycystic ovary in this study. Two of these, the cyst and the precystic follicle, had been identified and described previously.[3] To our knowledge, the third variety has not been described. This large follicular structure exhibits a normal theca and several healthy layers of granulosa cells typical of a large preovulatory follicle. Moreover, the presence of dense hCG binding in the perimural granulosa cells also indicates an active preovulatory follicle.[13] However, the total absence of corpora lutea in all ovaries examined, both at 4 and 8 weeks, precludes the possibility that these large

follicles are ovulating. However, they may be capable of ovulation if exposed to an LH surge of sufficient magnitude, an event that under ordinary circumstances would not occur in the rat with polycystic ovaries. We have demonstrated, however, the presence of corpora lutea in the previously polycystic ovary after an LH surge induced by luteinizing hormone–releasing hormone.[12] The most likely antecedent to such corpora lutea would seem to be the type III large follicular structure. However, their remarkable size and the presence of mitotic figures in the mural granulosa cell layers further distinguish these unique structures from ordinary preovulatory follicles. At present, we are unable to explain how and why such structures develop in a milieu in which most follicles become atretic or cystic. The fate of these structures is also unclear at present.

We have previously shown that cystic follicles are not merely preovulatory follicles, but unique follicular structures without counterpart in the normal ovary.[3] The added resolution afforded by perfusion fixation and Epon embedding in this study confirms this finding.

The most significant finding in our study is the paucity of hCG binding to the theca of true cystic follicles. This is in marked contrast to thecal binding in normal and atretic follicles within the same ovary. Indeed, the hCG binding over noncystic theca and secondary interstitial cells was often so intense that individual grains could not be assigned to cells of origin, and cell boundaries could not be accurately determined in the underlying tissue. This rendered grain counting unfeasible and unnecessary because so very few grains occurred over the theca of cystic follicles.

Thus it appears that the arrest of atresia and the transition from the precystic to the cystic condition coincide with a loss in hCG binding and, presumably, a loss in sensitivity to LH. Why this occurs only in selected follicles is unclear. Furthermore, this observation is particularly puzzling in view of the elevated androgen concentrations that have been reported in cyst fluid in human polycystic ovaries.[14-16] We have not measured androgen concentrations in the cysts of estradiol valerate–treated rats (the fluid yield is exceedingly small). However, plasma androgen (testosterone, androstenedione, or dehydroepiandrosterone) concentrations in these animals are similar to those of normally cycling controls.[12] Although it is conceivable that thecal androgen production in cysts may be regulated by a factor other than LH, it appears more likely that much of the androgen production in the polycystic ovary may be explained by the extensive LH-sensitive secondary interstitial tissue,[17] and the LH responsive thecal tissue of noncystic large follicular structures.

In addition to the large follicular structures unique to the polycystic ovary, a variety of ordinary structures commonly encountered in normal ovaries was observed. These include normal and atretic primary and secondary follicles and clusters of secondary interstitial cells in the stroma. Moreover, the pattern of hCG binding to these structures was identical to that reported previously in normal ovaries and in ovaries in which atresia was experimentally induced. It has been shown, for example, that binding of hCG to granulosa cells occurs only in large normal secondary follicles.[18, 19] The hCG binding to granulosa cells of large atretic follicles is very limited.[20-23] Differences in the level of hCG binding to granulosa cells, in these models is inversely proportional to the degree of atresia.

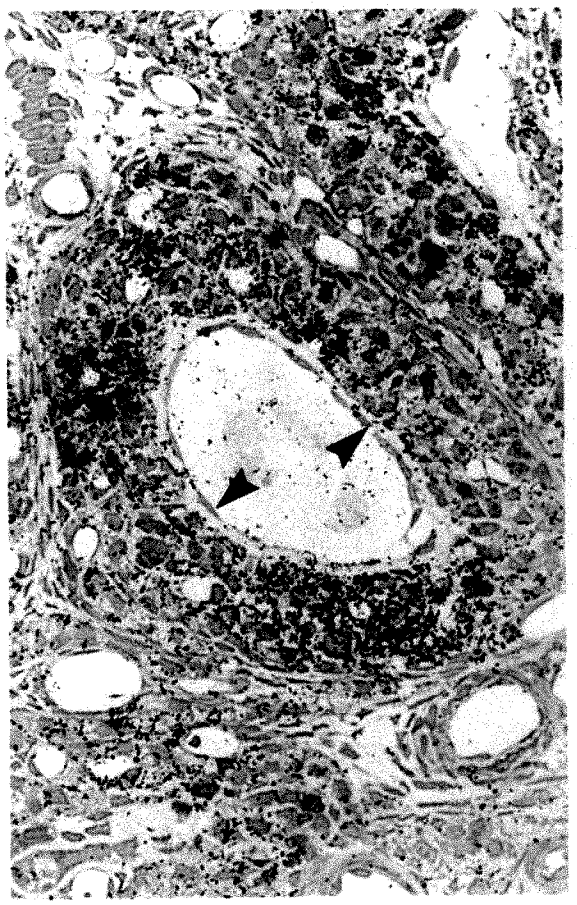


Fig. 7. Follicle in terminal stage of atresia in ovary exposed to ^{125}I-hCG. Thick ellipsoidal theca interna, consisting of hypertrophied lipid-filled polygonal cells, is heavily labeled. Lumen, lined by single layer of granulosa cells *(arrowsheads)*, is near point of collapse. (×500.)

Binding of ^{125}I-hCG to the theca interna of atretic follicles was either greater than[19] or equivalent to[23] that of healthy follicles of similar size. Similarly, the secondary interstitial cells in these models of atresia showed variable binding. As in our study, the most recently formed interstitial clusters exhibited the most intense label.[19]

Thus the histologic components common to both the polycystic and noncystic ovary display similar hCG binding irrespective of the type of ovary in which they

occur. This underscores the fact that not only is the cystic follicle histologically unique but also it exhibits LH binding characteristics unlike that of any other follicular structure.

The results of our study raise a number of important questions. It is not clear whether the loss of thecal LH binding is causal to the transition between the precystic and the cystic state, or whether it is a consequence of cyst formation. Moreover, we are confronted with an unexpected situation in which hypertrophied, lipid-containing thecal cells are apparently insensitive to LH. Paradoxically, thecal cells of identical appearance in atretic follicles and in interstitial cell clusters within the same ovary exhibit intense hCG binding. The functional properties, steroidogenic capabilities, and the roles played by these different thecal populations in polycystic ovaries remain to be determined.

We thank Dr. Beatrix Kopriwa and Mr. Fernando Evaristo for technical assistance and advice in autoradiography.

REFERENCES

1. Brawer JR, Naftolin F, Martin J, Sonnenschein C. Effects of a single injection of estradiol valerate on the hypothalamic arcuate nucleus and on reproductive function in the female rat. Endocrinology 1978;103:501-12.
2. Schulster A, Farookhi R, Brawer JR. Polycystic ovarian condition in estradiol valerate-treated rats: spontaneous changes in characteristic endocrine features. Biol Reprod 1984;31:587-93.
3. Brawer JR, Munoz M, Farookhi R. Development of the polycystic ovarian condition (PCO) in the estradiol valerate-treated rat. Biol Reprod 1986;35:647-55.
4. Grosser P, McCarthy GF, Robaire B, Farookhi R, Brawer JR. Plasma patterns of LH, FSH, and prolactin in the oestradiol valerate-induced polycystic ovarian condition in the rat. J Endocrinol 1987;114:33-9.
5. Richards JS, Midgley AR. Protein hormone action: a key to understanding ovarian follicular and luteal cell development. Biol Reprod 1976;14:82-94.
6. Han SS, Rajaniemi HJ, Cho MI, Hirshfield AN, Midgley AR. Gonadotropin receptors in rat ovarian tissue. II. Subcellular localization of LH binding sites by electron microscopic radioautography. Endocrinology 1974;95:589-98.
7. Rajaniemi HJ, Hirshfield AN, Midgley AR. Gonadotropin receptors in rat ovarian tissue. I. Localization of LH binding sites by fractionation of subcellular organelles. Endocrinology 1974;95:579-88.
8. Karnovsky MJ. Use of ferrocyanide-reduced osmium tetroxide in electron microscopy. Proc Am Soc Cell Biol 1971;11:146a.
9. Brawer JR. The role of the arcuate nucleus in the brain-pituitary-gonad axis. J Comp Neurol 1971;143:411-36.
10. Kopriwa B, Leblond CP. Improvements in the coating technique of radioautography. J Histochem Cytochem 1962;10:269-84.
11. Ireland JJ, Richards JS. A previously undescribed role for luteinizing hormone (LH:hCG) on follicular cell differentiation. Endocrinology 1978;102:1458-65.
12. Hemmings R, Farookhi R, Brawer JR. Pituitary and ovarian responses to luteinizing hormone releasing hormone in a rat with polycystic ovaries. Biol Reprod 1983;29:239-48.
13. Uilenbroek J, Richards J. Ovarian follicular development during the rat estrous cycle: gonadotropin receptors and follicular responsiveness. Biol Reprod 1979;20:1159-65.
14. Short R. Further observations on the defective synthesis of ovarian steroids in the Stein-Leventhal syndrome. J Endocrinol 1962;24:359-65.
15. Short R, London DR. Defective biosynthesis of ovarian steroids in the Stein-Leventhal syndrome. Br Med J 1961;1:1724-7.
16. Tanabe K, Gagliano P, Channing CP, Nakamura Y, Yoshimura Y, Iizuka R, Fortuny A, Sulewsky J, Rezai N. Levels of inhibin F activity and steroids in human follicular fluid from normal women and women with polycystic ovarian disease. J Clin Endocrinol Metab 1983;57:24-31.
17. Erickson GF, Magoffin DA, Dyer CA, Hofeditz C. The ovarian androgen producing cells: a review of structure/function relationships. Endocr Rev 1985;6:371-99.
18. Amsterdam A, Koch Y, Lieberman ME, Lindner HR. Distribution of binding sites for human chorionic gonadotropin in the preovulatory follicle of the rat. J Cell Biol 1975;67:894-900.
19. Bortolussi M, Marini G, Reolon ML. A histochemical study of the binding of ^{125}I-HCG to the rat ovary throughout the estrous cycle. Cell Tiss Res 1979;197:213-26.
20. Uilenbroek J, Woutersen P, van der Schoot P. Atresia of preovulatory follicles: gonadotropin binding and steroidogenic activity. Biol Reprod 1980;23:219-29.
21. Carson RS, Findlay JK, Burger HG, Trouson AO. Gonadotropin receptors of the ovine ovarian follicle during follicular growth and atresia. Biol Reprod 1979;21:75-87.
22. Peluso JJ, Steger RW, Hafez ESE. Sequential changes associated with the degeneration of preovulatory follicles. J Reprod Fertil 1977;49:215-8.
23. Shaha C, Greenwald GS. Autoradiographic analysis of changes in ovarian binding of FSH and hCG during induced follicular atresia in the hamster. J Reprod Fertil 1982;66:197-201.

Effect of a 48-hour intravenous Δ^4-androstenedione infusion on the pregnant rhesus monkey in the last third of gestation: Changes in maternal plasma estradiol concentrations and myometrial contractility

Jorge P. Figueroa, MD, PhD, M. Barbera O. M. Honnebier, MD, Zbigniew Binienda, DVM, Jeffrey Wimsatt, DVM, and Peter W. Nathanielsz, MD, PhD

Ithaca, New York

Increased myometrial activity in the pregnant rhesus monkey occurs in situations in which plasma estrogen concentrations are elevated. Examples of such situations are after laparotomy, with or without hysterotomy, and before delivery. The increased activity occurs primarily in the hours of darkness. To investigate the possibility of a causal relationship between the rise in estrogens and myometrial activity, we infused androstenedione intravenously for 48 hours to the rhesus monkey in the last third of gestation. Myometrial activity was quantified either as an increase in the number of individual contraction events or as a change in the power spectrum at high frequencies characteristic of contractions. Androstenedione infusion was followed by increased myometrial activity. Maternal plasma 17β-estradiol concentration was significantly elevated at 10 AM on the second day of androstenedione infusion. We conclude that, in the rhesus monkey late in gestation, estradiol may play a role in the regulation of the contraction activity observed during the hours of darkness in several different situations. (AM J OBSTET GYNECOL 1989;161:481-6.)

Key words: Estrogens, estrogen precursors, myometrial contractility in pregnancy

Maternal plasma estrogen concentrations in the pregnant rhesus monkey are high throughout gestation and rise at delivery.[1] This increase is not as dramatic as that described in the pregnant sheep.[2] In the nonpregnant sheep, estradiol administration results in both stimulatory and inhibitory effects on the myometrium.[3] However, the role of estrogens in stimulating myometrial activity during pregnancy in the primate has not been studied in detail.

In the pregnant rhesus monkey, 24-hour patterns of maternal plasma steroid hormone concentrations have been demonstrated.[4] Because of possible delays in the passage of estradiol formed in the placenta and membranes in reaching maternal blood plasma,[5] it is difficult to assess the potential for a causal relationship of changes in plasma estradiol concentration and the well-established patterns of myometrial activity that have been described by several investigators.[6, 7] Myometrial activity in the relatively unstressed instrumented pregnant rhesus monkey is generally of the contracture type when recorded either with electromyogram leads or intrauterine pressure measurement.[7] This is true unless the mother has recently undergone surgery, is stressed in some other fashion, or is about to deliver. In each of these situations contractures change to predominantly contractions around the hours of darkness.[7, 8] We have demonstrated that the administration of the aromatase inhibitor 4-hydroxyandrostenedione will inhibit the change to contractions that is normally observed after laparotomy and hysterotomy.[9] The possible causal relationship linking changes in hormone concentrations to the increase in nocturnal contraction activity that has been demonstrated after surgery,[7] during certain forms of stress, such as food withdrawal,[8] and before labor and delivery[7] remains to be determined.

Short-term administration of androstenedione to either the fetus or mother[10] results in increased plasma concentration of estradiol in pregnant monkeys. Estrogens will cross the primate placenta.[11] In this study we administered Δ^4-androstenedione for 48 hours to increase placental estrogen production in the pregnant rhesus monkey. We wished to determine whether maintained elevation of maternal plasma estrogen concentrations would be accompanied by a change from myometrial contractures to contractions around the hours of darkness.

From the Laboratory for Pregnancy and Newborn Research, Department of Physiology, New York State College of Veterinary Medicine, Cornell University.

Supported by National Institute of Child Health and Development grant HD 18870.

Received for publication July 29, 1988; revised March 8, 1989; accepted March 24, 1989.

Reprint requests: Peter W. Nathanielsz, MD, PhD, Laboratory for Pregnancy and Newborn Research, Department of Physiology, New York State College of Veterinary Medicine, Cornell University, Ithaca, NY 14853.

Table I. Arterial plasma estradiol concentration in the pregnant rhesus monkey

Experiment No.	Second-day baseline period			Second-day experimental period			Second-day recovery period		
	9 PM	10 AM	3 PM	9 PM	10 AM	3 PM	9 PM	10 AM	3 PM
Controls infused with intralipid 10% (0.25 ml/min for 48 hr)									
1	532.8	308.0	399.2	217.8	9341.4	262.2	248.6	343.8	332.0
2	326.2	317.4	295.1	417.1	414.8	259.6	388.3	331.3	337.8
3	312.1	231.2	292.4	357.4	357.4	357.4	*	*	*
Mean	390.4	285.5	328.9	330.8	371.2	293.1	318.5	377.6	334.9
SEM	71	27	35	59	22	32	69	6.2	2.9
Animals infused with Δ^4-androstenedione (2 mg/hr in intralipid)									
4	313.8	270.9	650.0	1931.2	1735.6	1053.6	381.1	393.3	361.4
5	224.7	384.3	287.5	281.7	1198.7	1749.6	1367.3	343.3	643.7
6	321.5	385.1	237.8	1672.8	435.3	379.0	306.1	306.1	306.1
7	248.6	343.8	332.0	2288.8	1585.6	1519.9	447.7	*	354.5
Mean	277.1	346.0	376.8	1544.0	1239.0	1176.0	626	347.6	416.4
SEM	23	27	93	439	291	302	249	25	77

*Data not available.

Material and methods

Care of animals

Surgical procedures. Five pregnant rhesus monkeys of known gestational ages were obtained from the California Regional Primate Center in Davis, Calif., and acclimated to the laboratory conditions as previously described.[7, 8] The animals were housed in rooms with controlled light cycles (14 hours light and 10 hours dark) (Fig. 1), with lights on at 6 AM. Food provided was Purina 5045 Monkey Chow (Purina, St. Louis) and fresh fruits. Water was provided as desired during the experimental period. Animals were instrumented using techniques described previously.[7, 8] Briefly, catheters were placed in the maternal femoral artery and vein with the animal under halothane anesthesia. In each animal, two pairs of myometrial electromyogram leads were placed on the anterior wall of the uterus. Two catheters made of Tygon microbore tubing (Norton Performance Plastics, Akron, Ohio; inner diameter 1.27 mm, outer diameter 2.29 mm) with a side hole 1 cm from the tip were placed with 3 to 6 cm of the catheter within the amniotic cavity. A small retaining collar of Tygon prefabricated around the catheter was located just within the uterus. At surgery monkeys ranged from 92 to 107 days' gestational age.

Recording of intrauterine pressure and myometrial electromyogram and data storage. The uterine electromyogram was recorded from multistranded bipolar stainless steel wires (Cooner AS 632, Chatsworth, Calif.) implanted 3 to 5 mm apart on at least two sites of the myometrium.[7, 8] One of the intraamniotic catheters was connected to a pressure transducer (Cobe, Lakewood, Colo.) that was placed in a small box on the monkey's back, beneath the jacket. Acquisition of the myometrial electromyogram and intrauterine pressure waveforms was performed with a custom-built computer-based data acquisition system. Details of sampling frequency and filters have been previously published.[12] Briefly, the signals (electromyogram and intrauterine pressure) were sampled at 32 Hz, and the average value over 8 seconds was digitized and stored on tape with a time signal. The initial sampling frequency of 32 Hz prevented aliasing problems with changing signals, whereas the lower storage rate of one reading every 8 seconds creates a manageable volume of data for subsequent analysis.

Data analysis. Fast Fourier Transform and power spectrum analysis were performed on the digitized intrauterine pressure and electromyogram data with the Asyst 2.0 system. Since the Fast Fourier Transform[13] software program works on multiples of 4096 data points, we analyzed only the hours 10:54 AM to 8 PM for periods of light and 8 PM to 5:06 AM for the periods of darkness. Integration of the area under the curve of the power spectrum was performed by addition of all the data points: for low frequencies 0.01 to 0.11 cycle/min or less than one cycle every 9 minutes, and for high frequencies 0.2 to 0.8 cycle/min or greater than one cycle every 5 minutes.[14]

Individual epochs of activity were calculated with a minimum duration of 16 seconds as a requirement, a 2-hour running mean as threshold, and a 40-second delimiter to establish the termination of an event. Contractions were classified in a 16- to 96-second window, and contractures were classified in a 96- to 896-second window, with techniques similar to those we have reported in sheep.[12]

Administration of Δ^4 androstenedione in intralipid. Forty-eight milligrams of Δ^4-androstenedione (Sigma Chemical, St. Louis) was prepared daily and mixed with 6 ml of a 10% fat emulsion (intralipid 10%, Kabi Vitrum, Inc., Alameda, Calif.). In the four animals in the Δ^4-androstenedione group this emulsion was infused for 48 hours at a rate of 0.25 ml/hr with a C-99 battery-operated pump (Razel Scientific, Stamford, Conn.). Intralipid alone was infused as a control in three animals.

In two animals the intralipid study was performed 1 week before the androstenedione infusion.

Blood sampling. Maternal arterial blood samples were taken into sterile, cold, heparinized syringes, placed in ice-cold heparinized tubes, and immediately cooled in ice. Blood was centrifuged at 4° C for 3 minutes at 6000 rpm. Plasma was removed at once with sterile pipette and was flash-frozen in liquid nitrogen. All samples were stored at −20° C. The red cells were resuspended in a volume of saline solution equal to the volume of plasma and reinjected into the maternal arterial catheter. Samples were taken at 9 PM, 10 AM, and 3 PM.

Statistical analysis. Plasma estradiol data were analyzed by paired *t* test comparing data obtained on the second control day versus the second experimental day at each individual sampling time in both the androstenedione- and intralipid-infused groups. Uterine electromyogram activity was analyzed by use of the previously described output of the computerized power spectrum analysis and the epoch counting analysis. Total area under the curve data for the power spectrum windows was normalized for amplitude with the value for each animal on control day 2 considered 100%. The paired *t* test was used to compare myometrial activity on the second control day and the second experimental day in both the androstenedione- and intralipid-infused groups.

Radioimmunoassay of estradiol. Maternal plasma estradiol concentration was measured in duplicate aliquots of 200 μl of plasma after ether extraction. The Coat-a-Count antibody-coated tube with the iodine 125-labeled estradiol kit (TKE 25, Diagnostic Products Corp., Los Angeles) was used. Specificity was further evaluated by comparing values obtained after ether extraction of nine individual pregnant monkey plasma samples followed by column chromatography on Sephadex LH 20 (Pharmacia Fine Chemicals, Piscataway, N.J.) with values for the same sample extracted but not chromatographed. Chromatographed samples had values that were 98.0% ± 15.9% (mean ± SD, $n = 9$) of the estradiol concentration obtained on the same sample without chromatography. Parallelism was confirmed by assaying serial dilutions of normal rhesus monkey pregnancy plasma and nonpregnant rhesus monkey plasma to which estradiol had been added.

Results

Table I shows the changes in maternal plasma 17β-estradiol concentration in the three daily samples on the second day of the control period, on the second day of the androstenedione infusion period, and the second day of the recovery period in four monkeys infused with androstenedione in intralipid, and in three control animals infused with intralipid alone. On the second day of the androstenedione infusion period, maternal plasma estrogen concentration was elevated at all three sampling times. This increase was statistically significant compared with the same times of the second control day, respectively ($p < 0.05$). Although only the 9 PM samples were available from all three control animals, estradiol concentrations at this time showed no rise from the values on the control day 2.

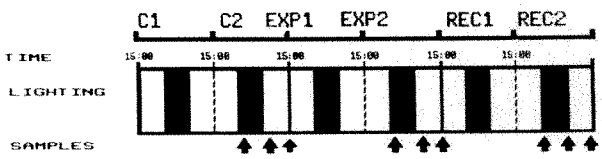


Fig. 1. Time line for the 6 days of the experiment. All animals were infused with saline solution on the two control days, *C1* and *C2*. On the experimental days, *EXP1* and *EXP2*, animals were infused with either androstenedione (2 mg/hr) in intralipid or intralipid alone. Blood samples were taken on the second control day *(C2)*, experimental day two *(EXP2)*, and the second day of recovery *(REC2)*.

Myometrial electromyogram recordings

Effect of androstenedione on myometrial contractility. Fig. 2A shows the effect of two days of intravenous androstenedione infusion on the power spectrum of myometrial electromyogram during the hours of darkness in one pregnant rhesus monkey. By the second night of infusion, maternal plasma estradiol was elevated and the concentration portion of the power spectrum was increased. In contrast, there was no change in the power spectrum at any time when the intralipid vehicle was infused alone (Fig. 2B).

Fig. 3, *A* and *B*, shows the changes that occur in the power spectrum areas in the contractures and contractions windows in the hours of light and darkness during the androstenedione and intralipid infusions, respectively. During the hours of darkness, there was an increase in contraction type activity. Similarly, when the individual epochs of activity were separated into contractures (96 to 896 seconds' duration) and contractions (16 to 96 seconds' duration) throughout the 3 days when analysis was conducted, there was a significant increase in contraction frequency on the second night of androstenedione infusion ($p < 0.05$) (Fig. 4, *A*).

Comment

The continuous intravenous administration of androstenedione for 48 hours to the pregnant rhesus monkey resulted in a significant, maintained rise in maternal plasma estradiol concentration. This observation confirms the findings of others using 4-hour infusion that, in the nonhuman primate, intravenously administered C19 androgen precursors can be converted to estrogens late in pregnancy.[10] In this study, estrogen concentrations rose to levels slightly higher than those previously described as being present at

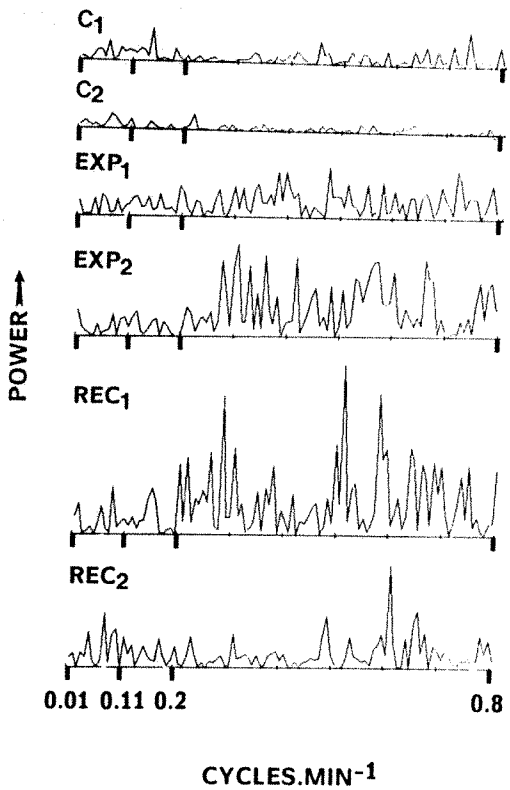


Fig. 2A. Effect of androstenedione infusion (2 mg/hr in intralipid 10% at 0.25 ml/hr) intravenously to a pregnant rhesus monkey on myometrial electromyogram power spectrum during the hours of darkness; control days 1 and 2 (C_1 and C_2) followed by 2 days of androstenedione (EXP_1, EXP_2); followed by 2 days of recovery (REC_1, REC_2).

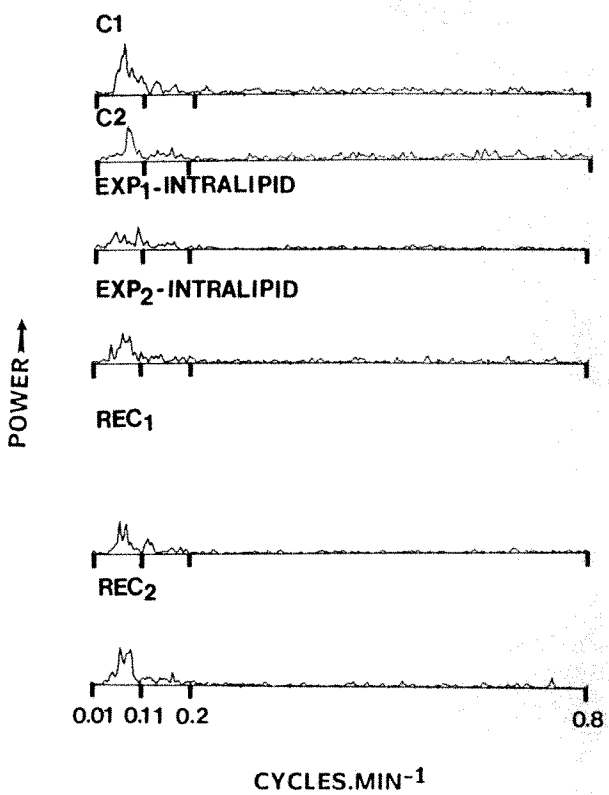


Fig. 2B. Effect of intralipid 10% infusion (0.25 ml/hr) on myometrial electromyogram power spectrum during the hours of darkness; days *C1*, *C2* correspond to the 2 control days of the androstenedione infusion; EXP_1 and EXP_2 were the days of intralipid infusion; REC_1 and REC_2 were equivalent to the recovery days of androstenedione infusion.

term in the pregnant rhesus monkey.[1] This elevation in plasma estrogen concentrations was accompanied by a statistically significant increase in the incidence of nocturnal contractions. Although intrauterine pressure was also recorded in our study, we have not examined intrauterine pressure data statistically since movement artifacts in the free-ranging, tethered animal may compromise analysis. These concerns are probably of lesser importance when studies are conducted with the primate in a restraining chair.

In the pregnant sheep the stress of food withdrawal late in pregnancy also increases myometrial activity and may lead to preterm labor.[15] Maternal plasma estrogen concentrations are increased in this situation.[16] We have previously demonstrated that 48 hours of food withdrawal in the pregnant rhesus monkey in the last third of gestation also leads to an increase in contraction-type myometrial activity.[8] During the same study an increase in maternal estradiol was noted.[17] Because of the presence of a fetoplacental unit the locations of the biochemical pathways leading to estrogen production in the nonhuman primate are not directly comparable to those in the sheep.[10] Estrogen has been shown to play a role in progesterone production in the baboon[18]; thus the possible interplay of these steroids complicates the picture.

Suppression of maternal and fetal adrenal function by adminstration of dexamethasone to the pregnant monkey not only depresses myometrial activity, but also abolishes the 24-hour rhythm observed in myometrial activity.[19] This depressant effect may well be due to inhibition of estrogen production. However, other mechanisms are likely to play a role because mean uterine activity returned to baseline levels in the 48 hours after dexamethasone treatment, but the peak in nocturnal activity remained absent.[19]

Dexamethasone has been shown to prevent the normal increase in maternal plasma estrogens that occurs in the pregnant rhesus monkey at term. Administration of dexamethasone was accompanied by a prolongation of gestation.[19] In contrast, estradiol administration failed to produce preterm delivery in the presence of a live fetus.[20] However, amniotic implants of estradiol will result in preterm delivery in the rhesus monkey, suggesting that the site of generation or administration of estrogen may be critical.[20] Partly because of these

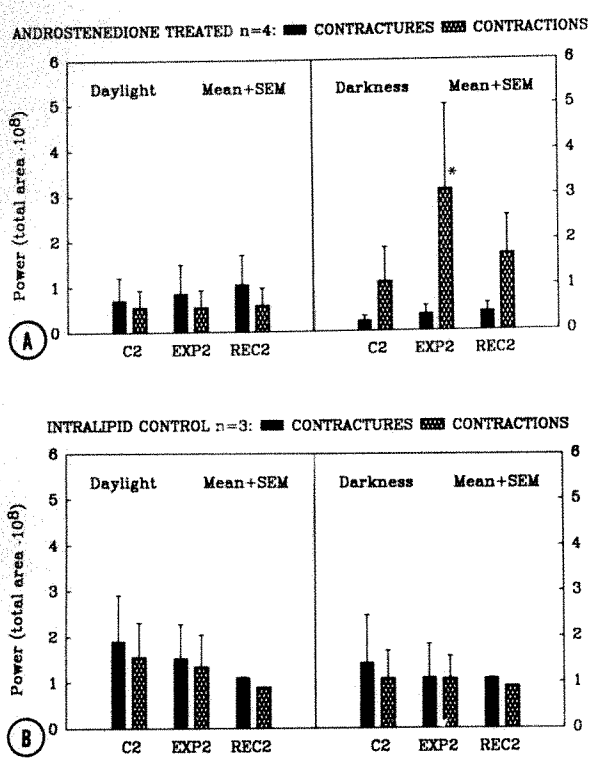


Fig. 3. Effect of 48-hour infusions of intravascular androstenedione **(A)** and intralipid **(B)** to the pregnant rhesus monkey. Two power spectrum windows were analyzed. Contractures (■) were represented in the 0.01 to 0.11 cycles/min window; contractions (▩) were represented in the 0.2 to 0.8 cycles/min window. No changes occurred in either window during the hours of light (10:54 AM to 8 PM). During the hours of darkness (8 PM to 5:06 AM) the power in both the contractions and contractures window was significantly increased on the second day of androstenedione infusion ($p < 0.05$). Mean ± SEM; $n = 4$ for androstenedione infusion and $n = 3$ for intralipid controls. *C2* is the second control day; *EXP2* is the second day of either intralipid or androstenedione infusion. *REC2* is the second recovery day of the 6-day protocol. *Asterisk*, $p < 0.05$ when *EXP2* was compared with *C2*. Intralipid 10% or androstenedione 2 mg/hr in 10% intralipid infused on the 2 experimental days.

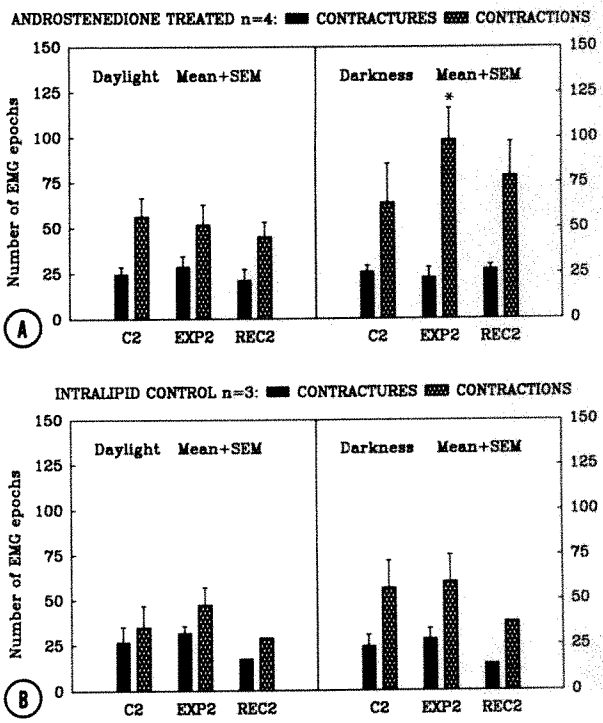


Fig. 4. Effect of intravascular androstenedione **(A)** and intralipid **(B)** infusions to the pregnant rhesus monkey for 48 hours on the number of myometrial contraction and contracture type epochs. Contractions (▩) represent epochs lasting 16 to 96 seconds and contractures (■) epochs lasting 96 to 896 seconds. The only significant change was the increase in contraction frequency on the second night of androstenedione infusion ($p < 0.05$). Hours of daylight analyzed from 10:54 AM to 8 PM; hours of darkness were 8 PM to 5:06 AM.

observations we chose to infuse androstenedione as an estrogen precursor to stimulate estrogen production at its normal site rather than infuse estradiol directly.

The change from contractures to labor contractions that occurs at term in many species is accompanied by changes in absolute maternal plasma estrogen levels or the estrogen/progesterone ratio.[1,2] The most likely explanation of our data is that estrogen production at the normal site from precursors administered intravenously can modify myometrial activity. A consistent feature of postsurgical stress, food withdrawal,[8] and the current study is the observation that in the presence of continuous stress the change in myometrial activity is periodic and apparently entrained to the light-dark cycle. In this study the major changes in myometrial activity occurred in the early hours of darkness, although maternal plasma estradiol was elevated throughout the entire day. The mechanism by which estrogens stimulate myometrial contractions remains unclear. Estrogens may alter the population of adrenergic receptors on the myometrial cell, thereby directly influencing myometrial activity.[21] It is possible that other agonists are secreted during the hours of darkness and the estrogens serve merely to lower the myometrial threshold to such putative agonists. Further studies are needed to explore this relationship.

Our study was designed in part to evaluate a possible role for androgen precursors in the increased myometrial activity that accompanies several forms of stress. We postulate that in situations of acute or chronic stress, androgen precursor production rises and may lead to increased estrogen production. In turn, the estrogens stimulate increased myometrial contractility. Further studies are required to prove this hypothesis. An alternative mechanism that would be compatible with our observations is that androstenedione itself induced an increase in myometrial activity. This explanation would

have implications for the role of androgens released from maternal and fetal adrenals during different stress states. This pregnant nonhuman primate model may prove to be useful not only in the study of factors that determine myometrial activity in general, but also with respect to putative mechanisms that can precipiate premature labor.

We thank Ms. Tami Myers and Dr. James Owiny for assistance with the estradiol assay.

REFERENCES

1. Challis JRG, Davies IJ, Benirschke K, Hendrickx AG, Ryan KJ. The concentrations of progesterone, estrone and estradiol-17β in the peripheral plasma of the rhesus monkey during the final third of gestation, and after the induction of abortion with $PGF_{2\alpha}$. Endocrinology 1974; 95:547.
2. Challis JRG. Sharp increase in free circulating oestrogens immediately before parturition in sheep. Nature 1971; 229:208.
3. Lye SJ, Wathes DC, Porter DG. Oestradiol-17β both inhibits and stimulates myometrial activity in ewes in vivo. J Reprod Fertil 1983;67:335.
4. Walsh SW, Ducsay CA, Novy MJ. Circadian hormonal interactions among the mother, fetus and amniotic fluid. Am J Obstet Gynecol 1984;150:745.
5. Challis JRF, Mitchell BF. Steroid production by the fetal membranes in relation to the onset of parturition. In: McNellis D, Challis JRG, MacDonald PC, Nathanielsz PW, Roberts JM, eds. The onset of labor: cellular and integrative mechanisms. Ithaca, New York: Perinatology Press, 1988:233.
6. Harbert GM, Spisso KR. Biorhythms of the primate uterus *(Macaca mulatta)* during labor and delivery. Am J Obstet Gynecol 1980;138:686.
7. Taylor NF, Martin MC, Nathanielsz PW, Seron-Ferre M. The fetus determines the circadian oscillation of myometrial electromyographic activity in the pregnant rhesus monkey. Am J Obstet Gynecol 1983;146:557.
8. Binienda Z, Massmann A, Mitchell MD, Gleed RD, Figueroa JP, Nathanielsz PW. The effect of food withdrawal on arterial blood gluocse and plasma 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ concentrations and nocturnal myometrial electromyographic activity in the pregnant rhesus monkey in the last third of gestation: a model for preterm labor? Am J Obstet Gynecol 1989;160:746-50.
9. Butler WR, Figueroa JP, Frank DA, Gleed RD, Nathanielsz PW, Poore ER. Myometrial electromyographic (e.m.g.) activity in the pregnant rhesus monkey following fetal surgery with or without maternal administration of the aromatase inhibitor 4-hydroxyandrostenedione (4-OHA). J Physiol (Lond) 1985;369:197P.
10. Walsh SW, Resko JA, Grumbach MM, Novy MJ. In utero evidence for a functional fetoplacental unit in rhesus monkeys. Biol Reprod 1980;23:264.
11. Hill DE, Slikker W Jr, Helton ED, et al. Transplacental pharmacokinetics and metabolism of diethylstilbestrol and 17β-estradiol in the pregnant rhesus monkey. J Clin Endocrinol Metabol 1980;5:811.
12. Figueroa JP, Mahan S, Poore ER, Nathanielsz PW. Characteristics and analysis of uterine electromyographic activity in the pregnant sheep. Am J Obstet Gynecol 1985;151:524.
13. Campbell JA, Mathison M, Pook P, Wyatt A. Up and running with Asyst 2.0. MacMillan Software Company, 1987.
14. Hsu HW, Figueroa JP, Honnebier MB, Wentworth R, Nathanielsz PW. Power spectrum analysis of myometrial electromyogram and intrauterine pressure changes in the pregnant rhesus monkey in late gestation. Am J Obstet Gynecol 1989;161:467.
15. Fowden AL, Silver M. The effect of the nutritional state on uterine prostaglandin F metabolite concentrations in the pregnant ewe during late gestation. Q J Exp Physiol 1983;69:337.
16. Milvae R, Mitchell MD, Nathanielsz PW, Pimentel G, Rosen ED. The effect of food withdrawal on myometrial electromyographic (EMG) activity and maternal plasma concentrations of 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ (PGFM) and oestrone sulphate in the pregnant ewe at 122-127 days gestation (d G.A.). J Physiol (Lond) 1986; 381:410P.
17. Binienda Z, Massmann A. Wimsatt J, Honnebier MBOM, Figueroa JP, Reimers TJ, Nathanielsz PW. Endocrine changes during 48 hours of food withdrawal in the pregnant rhesus monkey in the last third of gestation. J Clin Endocrinol Metab 1989;68:1184.
18. Albrecht ED, Pepe GJ. Regulation of progesterone and estrogen formation during baboon pregnancy. In: Albrecht E, Pepe GJ, eds. Research in perinatal medicine (IV). Ithaca, New York: Perinatology Press, 1985:175.
19. Ducsay CA, Cook MJ, Walsh SW, Novy MJ. Circadian patterns and dexamethasone-induced changes in uterine activity in pregnant rhesus monkeys. Am J Obstet Gynecol 1983;145:389.
20. Mead G, Walsh SW, Dolney AM, Novy MJ. Amniotic fluid (AF) steroid hormone implants: effect on gestational length and prostaglandins (PGs) in rhesus monkeys. In: Proceedings of the twenty-eighth annual meeting of the Society for Gynecologic Investigation, St. Louis, Missouri, March 18-21, 1981. St. Louis, Missouri: Society for Gynecologic Investigation, 1981:77.
21. Weiland NG, Wise PM. Estrogen alters the diurnal rhythm of α_1-adrenergic receptor densities in selected brain regions. Endocrinology 1987;121:1751.

Flow cytometry of normal, hyperplastic, and malignant human endometrium

A study of ploidy and proliferative indices including comparison with in vitro S-phase labeling

James G. Thornton, MD,[a] Philip Quirke, PhD,[b] and Michael Wells, MD[b]
Leeds, England

The validity of 48 endometrial proliferative indices derived from flow cytometric deoxyribonucleic acid histograms was tested by comparison with S-phase labeling indices. There was a positive but weak correlation. Despite this correlation, there was a good relationship between the flow cytometric proliferative indices and both the menstrual phase and degree of tissue differentiation in a larger series of 162 normal, hyperplastic, and malignant endometrial specimens examined by flow cytometry alone. Flow cytometric proliferative indices may be useful for prediction of tumor behavior in non-DNA aneuploid endometrial carcinomas that are assessed as being a good prognostic type by other tumor parameters. (AM J OBSTET GYNECOL 1989;161:487-92.)

Key words: Flow cytometry, labeling indices, endometrium, hyperplasia, carcinoma

Endometrial carcinoma is conventionally classified by tumor stage and histologic grade. Other hormone receptor and cell kinetic parameters have been claimed to have prognostic importance, but their detailed relationships to tumor behavior are still uncertain.[1, 2] Tumor kinetic measurements may be performed by labeling of cells in culture with various deoxyribonucleic acid (DNA) precursors or by measurement of the quantity of DNA in individual tumor cells. From the DNA histogram, it is possible to identify diploid cells in the G_0 or G_1 phase of the cell cycle, tetraploid cells in the G_2 phase or mitosis, and to calculate the proportion of cells intermediate between diploid and tetraploid that are presumably in S phase. If there is a second G_0/G_1 population peak of nondiploid cells, the tumor is said to be DNA aneuploid; this second peak interferes with the counting of S-phase cells. Until recently cellular DNA was measured by microspectrophotometric methods,[3] but these are laborious and flow cytometry has superseded them for rapid and precise DNA measurements on large numbers of cells.[4]

In a number of tumor types, flow cytometric measurement of DNA content has identified a DNA aneuploid group with a poor prognosis and a diploid group with a more favorable prognosis.[4, 5] Such studies can be performed on paraffin-embedded tissue,[6] which allows examination of archival material and greatly expedites the development of detailed prognostic indicators. There have been a number of studies of endometrium.[1-3, 7-10] DNA aneuploid tumors appear to have a worse prognosis. Although S-phase fractions were greater in poorly differentiated tumors, the S-phase fraction itself did not correlate with prognosis.[2] However, others have claimed that larger S-phase fractions are associated with more deeply invasive tumors.[10]

Other kinetic parameters such as S-phase labeling indices[11] should provide similar results but are more laborious and require fresh tissue. Experiments to compare flow cytometric proliferative indices with thymidine or bromodeoxyuridine labeling indices have primarily been confined to cell culture systems or animal tumor models.[12, 13]

The purpose of our study was to use such proliferative indices as a prognostic indicator in endometrial cancer. As a preliminary step to a large retrospective study, we report the proliferative indices and ploidy status of normal, hyperplastic, and malignant endometrium. To validate the results, a number of fresh endometrial specimens were labeled in vitro with the thymidine analog bromodeoxyuridine, and S-phase indices were derived and compared with the flow cytometric proliferative indices.

Methods

The study was performed in two parts. First, 48 non–DNA aneuploid endometrial specimens were labeled in vitro with bromodeoxyuridine to derive S-phase in-

From the Departments of Obstetrics and Gynecology[a] and Pathology,[b] University of Leeds.
Supported by grants from the Yorkshire Cancer Research Campaign.
Received for publication June 28, 1988; accepted March 22, 1989.
Reprint requests: James G. Thornton, MD, Department of Obstetrics and Gynecology, University of Wales College of Medicine, Heath Park, Cardiff, CF4 4XN, South Glamorgan, Wales.

Table I. Number of specimens examined by each technique

Histologic features	*I_S and I_P*	*Flow only*		*Total*
		Diploid	*Aneuploid*	
Proliferative	15	61	0	76
Secretory	9	33	0	42
Hyperplasia				
Simple cystic	4	9	1	14
Architectural atypia	5	18	2	25
Cytologic atypia	5	3	0	8
Carcinoma				
Well-differentiated	1	18	7	26
Moderately differentiated	2	12	2	16
Poorly differentiated	2	8	1	11
Other				
Atrophic or menstrual	5	0	0	5
TOTAL	48	162	13	223

dices (I_S). Adjacent portions of the same specimens were examined by flow cytometry. From the DNA histograms, proliferative indices (I_P) were calculated and compared with the I_S values. Second, flow cytometric measurement of ploidy and I_P was performed on another 175 normal and abnormal endometrial specimens obtained from the archives. The histopathologic diagnosis of the fresh and archival cases combined with those of the whole series are listed in Table I. The flow cytometric results are only reported in detail for the 223 normal, hyperplastic, and malignant specimens. Five atrophic and menstrual specimens were included in the first part of the study to test the correlation between I_S and I_P.

Comparison of in vitro labeling and flow cytometry. Forty-eight endometrial specimens were obtained fresh from the operating room. Curettage specimens were immediately placed in phosphate-buffered saline solution (pH 7.4), and adjacent specimens were placed in formalin and routinely processed for histologic examination. The method of bromodeoxyuridine labeling correlates well with thymidine labeling.[11] Briefly, 1 mm^3 pieces of endometrium were incubated for 1 hour at 37° C on grids in phosphate-buffered saline solution containing 2×10^{-4} mol/L bromodeoxyuridine and fixed in Carnoy's solution. Three-micron paraffin sections were denatured in hydrochloric acid and labeled with a monoclonal anti-bromodeoxyuridine antibody by means of an indirect peroxidase technique. A moderately differentiated adenocarcinoma labeled with bromodeoxyuridine is shown in Fig. 1. Labeling indices (I_S) were derived for glands and stroma separately by the following formula:

$$I_S = \frac{\text{Labeled cells}}{\text{Labeled and unlabeled cells}} \times 100$$

Since flow cytometric specimens were not separated into glandular and stromal components, a total specimen I_S was also derived as follows. A 7 μm section was taken from the paraffin-embedded tissue and stained with hematoxylin and eosin. This specimen was adjacent to the 60 μm section used for flow cytometry (see following section). This was examined on a Leitz microscope at ×200 magnification. The relative proportions of glands and stroma were calculated by counting the number of times they were overlaid by the intersections on an eyepiece graticule. An adjusted value for I_S that accounted for the proportions of glands and stroma in the flow cytometric specimen was calculated as follows:

P_{gl} = Proportion of glands
P_{st} = Proportion of stroma
Adjusted specimen I_S = Gland $I_S \times P_{gl}$ + Stroma $I_S \times P_{st}$

Flow cytometry. Adjacent specimens of endometrium were routinely processed in paraffin and, after removal of sections for histology and morphometry, examined by flow cytometry according to a modification of the method of Hedley et al.[6] Sixty-micron sections from paraffin blocks were cut, transferred to glass slides, dewaxed in xylene, rehydrated through graded alcohols (100%, 95%, 90%, 70%, and 50%) and washed twice in water. The tissue was removed from the slide with a scalpel and incubated in 0.9% sodium chloride adjusted to pH 1.5 with 2N hydrochloric acid plus 0.5% pepsin at 37° C in a water bath for 30 minutes. For archival material in which hysterectomy specimens that included myometrium were examined, the nonendometrial portion of the specimen was discarded when the tissue was removed from the glass slide. The cells were centrifuged at 2000 *g* and washed twice in phosphate-buffered saline solution. Cells were stained by incubation at 20° C for 30 minutes in RPMI 1640 tissue culture medium plus 4′-6′-diamidino-2-

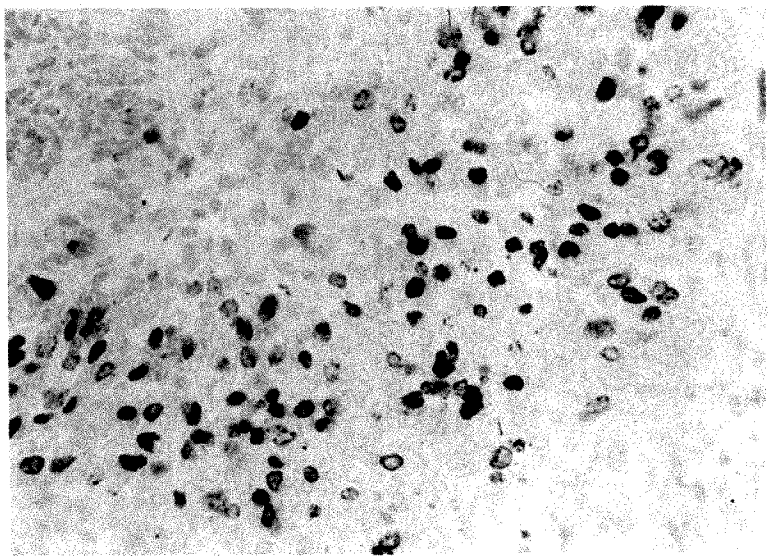


Fig. 1. Moderately differentiated endometrial cancer with S-phase nuclei labeled with bromodeoxyuridine.

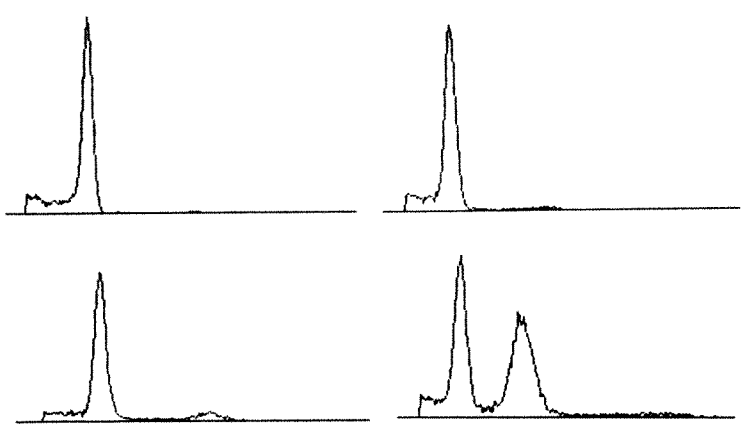


Fig. 2. Typical DNA histograms of secretory endometrium with low proliferative fraction *(top left)*, proliferative endometrium with medium proliferative fraction *(top right)*, moderately differentiated carcinoma with high proliferative fraction *(bottom left)*, and aneuploid carcinoma *(bottom right)*.

phenylindole dihydrochloride 1 μg/ml. The cells were syringed through a 23-gauge needle and filtered through 24 layers of gauze to remove debris.

Samples were analyzed on an EPICS V flow cytometer. Excitation was by a Coherent Inova-90 5W ultraviolet enhanced argon ion laser used at 100 mW and a wavelength of 488 nm. Specimens were rejected if the median half-peak coefficient of variation of the diploid peak was >10%. A minimum of 10,000 nuclei were counted. Typical histograms showing the normal diploid cell population of specimens with low, medium, and high proliferative fractions and the DNA aneuploid histogram of a poorly differentiated carcinoma are shown in Fig. 2. Tumors were recorded as aneuploid only if two G_0/G_1 peaks could be clearly identified.[12] The DNA index of each DNA aneuploid cell population was calculated by measuring its modal channel number and dividing this by the modal channel number of the first diploid G_0/G_1 peak.

Cell cycle analysis. Histogram analysis was performed with a standard program, Para 1. The percent S phase and percent G_2 phase were summed to derive the proliferative index since this has been found to yield more accurate results when samples demonstrating varying percentage coefficients of variation are analyzed. Proliferative indices were only calculated for diploid specimens.

Statistical analysis. Labeling indices and flow cytometric proliferative indices are not usually normally distributed. Therefore correlations between tests were

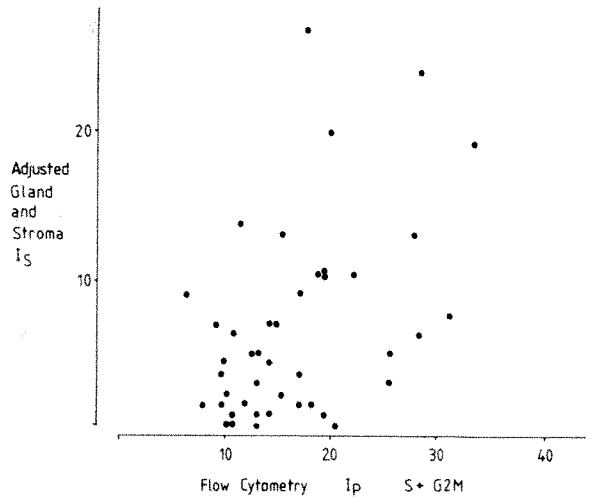


Fig. 3. Plot of bromodeoxyuridine-labeling indices against flow cytometric proliferative indices.

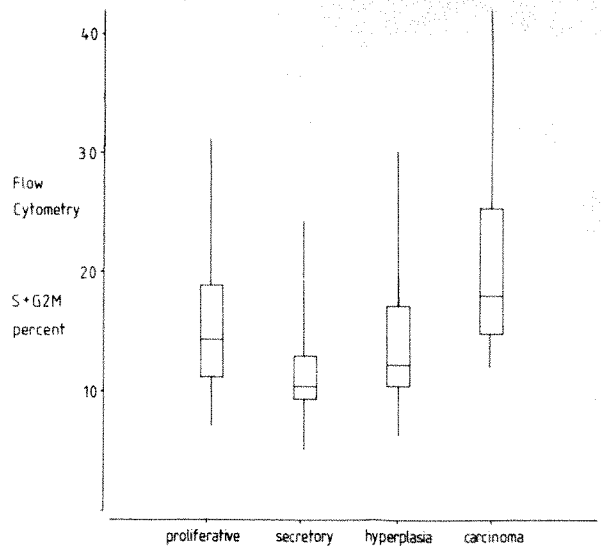


Fig. 4. Flow cytometric proliferative indices for various endometrial types. (Medians, interquartile ranges, and ranges.)

measured with Spearman's rank coefficient. Tests of the significance of differences between paired samples were tested with a Wilcoxon rank-sum test and between unpaired samples using a Mann-Whitney U test. Data analysis was performed using the Statistical Program for the Social Sciences (SPSS^X) version 2.2.

Results

The adjusted specimen labeling indices plotted against the flow cytometric proliferative indices for the 44 diploid specimens examined by both techniques are shown in Fig. 3. Four specimens with adjusted I_S values of 0 were excluded. There is a significant but weak correlation between them ($R = 0.36$, $p < 0.01$). I_P values tend to be higher than I_S. This trend is particularly marked at low levels of I_P when the values are rarely <10%. The median, interquartile ranges, and ranges of I_P for proliferative, secretory, and grouped hyperplastic and carcinomatous specimens are shown in Fig. 4. The median value for proliferative endometrium was 14.3% and for secretory endometrium was 10.3% ($p = 0.0001$), whereas that for hyperplasia was 12.0%, which is only slightly lower than proliferative endometrium ($p = 0.02$). Carcinomas had a median value of 17.8%, which is significantly higher than normal proliferative ($p = 0.002$) and hyperplastic endometrium ($p = 0.0001$). The results for the various subtypes of hyperplasias and of carcinomas are shown in Fig. 5. Although there is considerable overlap, there is a consistent trend toward higher values of I_P being associated with more atypical hyperplasias and less differentiated carcinomas. The histologic features and DNA indices of the 13 DNA aneuploid specimens are listed in Table II. Most aneuploid tumors were well-differentiated carcinomas, but the highest DNA indices occurred in the less-differentiated carcinomas.

Comment

Our study has shown by flow cytometry that proliferative indices in normal endometrium are related to the phase of the menstrual cycle and, in malignant endometrium, to the degree of differentiation. Although the correlation with S-phase labeling indices provides some confirmation of the validity of the flow cytometric measurements, it also demonstrates that flow cytometry appears to be a relatively crude method of measuring proliferative indices when this cell cycle program is used.

There are several possible causes for the poor correlation between flow cytometry and S-phase labeling. First, although care was taken to correct the I_S values for the proportion of gland and stromal tissue in the flow cytometric specimens, it was not always possible to exclude myometrial contamination. In normal endometrium, myometrium was removed from the thick tissue sections before analysis by flow cytometry, but removal was not always possible in tumor or curettage specimens in which myometrium and endometrium were intimately mixed. Second, flow cytometric measurements of proliferative indices rely on the assumption that cells with a DNA content above an arbitrary value are in S phase. Unless the coefficient of variation of DNA content is 0, it is inevitable that some G_0/G_1 cells at the upper end of the normal distribution will be misclassified. Third, there may be some cells with a genuine S-phase DNA content that are not actually synthesizing DNA, the so-called S_0 cells. These have been clearly demonstrated in cell culture[13] and in mouse tumors.[14] Such cells are recorded as S phase on flow cytometry, but do not take up the bromodeoxyuridine label on culture. We are now investigating whether such

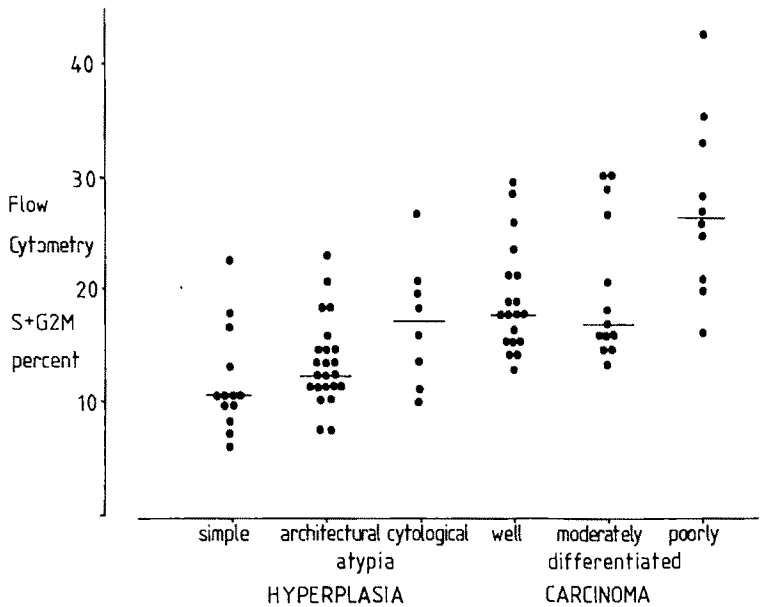


Fig. 5. Flow cytometric proliferative indices for the various subtypes of endometrial hyperplasia and carcinoma. (*Horizontal lines* represent median values.)

a cell population exists in endometrium by dual-channel flow cytometry of bromodeoxyuridine-labeled tissue. A fourth source of error may be that the bromodeoxyuridine-labeling method underestimated the number of S-phase cells in some specimens because of necrosis, poor oxygenation, or poor penetration of the nucleotide in culture. Necrosis did occur in some carcinomas, but we do not believe that it was a major factor in our study because all cases with a labeling index of 0 were excluded from analysis. Finally, the I_F value is somewhat higher than I_S because all G_2 cells and approximately half of cells in mitosis are included as well.

Similar work has been performed on breast tumors by McDivitt et al.[15] They compared thymidine-labeling indices with flow cytometric S-phase calculations by means of the same histogram analysis program (Para 1) and obtained an excellent correlation of $R = 0.8$ when collagenase digestion was used but only $R = 0.6$ when mechanical dissociation was used. These correlation coefficients are better than those obtained in our study. One explanation may be that McDivitt et al.[15] performed the flow cytometry on fresh tissue and obtained lower coefficients of variation.

There have been a number of studies of flow cytometry of endometrium, but most have concentrated on measurements of ploidy and none have compared proliferative indices with labeling indices. Two studies have shown that normal endometrium is always diploid and that mean S-phase fractions are greater in normal proliferative (9.7%) than secretory endometrium (6.2%)[8] but less than in carcinomas.[7] There is only one previous report of flow cytometric S-phase fractions in hyperplasia.[16] In nine cases of cystic hyperplasia, mean S-phase fraction was 3.4%, in six cases of "adenomatous" hyperplasia it was 4.8%, and in five carcinomas, 4.8%. In endometrial carcinomas, S-phase fractions were greater in poorly differentiated than well-differentiated tumors.[1, 8]

Table II. DNA indices of 13 aneuploid endometrial specimens

Histologic features	*No.*	*DNA indices*
Cystic hyperplasia	1	1.30
Architectural atypia	2	1.20, 1.42
Well-differentiated carcinoma	7	1.15, 1.16, 1.26, 1.27, 1.31, 1.42, 1.52
Moderately differentiated carcinoma	2	1.17, 1.75
Poorly differentiated carcinoma	1	1.71

The rate of DNA aneuploidy among endometrial carcinomas in our study (9 of 43) is slightly lower than most previous reports that estimate a rate of approximately 30%.[1, 3, 7, 8, 10] According to two groups, DNA aneuploidy was more common among low-grade tumors,[7, 8] but this was not shown in the smaller study by Geisinger et al.[1] DNA aneuploid tumors appear to have a worse prognosis than diploid tumors,[7, 10] and it has been claimed that DNA ploidy is a better prognostic indicator than tumor grade, depth of invasion, or even estrogen receptor concentration.[2] The finding of one aneuploid case of cystic hyperplasia and two of aneuploid hyperplasia with architectural atypia has not been

previously described but may represent cases with an increased malignant potential. One preliminary report suggested that the proliferative indices in carcinomas may also be predictive of a poor prognosis,[10] but this has not been confirmed.[2]

Therefore it is clear that the rate of DNA aneuploidy in endometrial carcinomas is lower than in most other nonhematopoietic malignancies in which 50% to 80% are DNA aneuploid.[5] Like other parameters of endometrial malignancy, ploidy may be a poor discriminator of the majority of well-differentiated good prognosis carcinomas, and proliferative indices may be better. It remains to be determined whether flow cytometric proliferative indices or S-phase labeling indices are better predictors of tumor behavior, but a major advantage of flow cytometric measurements is that they can be performed retrospectively on stored tissue and are much less time consuming than culture techniques.

We thank Mrs. C. A. North and Mr. R. Stretton for technical assistance.

REFERENCES

1. Geisinger KR, Kute TE, Marshall RB, Homesley HD, Morgan TM. Analysis of the relationships of the ploidy and cell cycle kinetics to differentiation and the female sex steroid hormone receptors in adenocarcinoma of the endometrium. Am J Clin Pathol 1986;85:536-41.
2. Lindahl B, Alm P, Ferno M, et al. Prognostic value of flow cytometrical DNA measurements in stage I-II endometrial carcinoma: correlations with steroid receptor concentration, tumor myometrial invasion, and degree of differentiation. Anticancer Res 1987;7:791-8.
3. Iverson OE, Laerum OD. Ploidy disturbances in endometrial and ovarian carcinomas. A review. Anal Quant Cytol Histol 1985;7:327-36.
4. Quirke P, Dyson JED. Flow cytometry: methodology and applications in pathology. J Pathol 1986;149:79-87.
5. Friedlander ML, Hedley DW, Taylor IW. Clinical and biological significance of aneuploidy in human tumours. J Clin Pathol 1984;37:961-74.
6. Hedley DW, Friedlander ML, Taylor IW, Rugg CA, Musgrove EA. Method of analysis of cellular DNA content of paraffin embedded pathological material using flow cytometry. J Histochem Cytochem 1983;31:1333-5.
7. Iverson OE. Flow cytometric deoxyribonucleic acid index: a prognostic factor in endometrial carcinoma. AM J OBSTET GYNECOL 1986;155:770-6.
8. Lindahl B, Alm P, Killander D, Langstrom E, Trope C. Flow cytometric DNA analysis of normal and cancerous human endometrium and cytological-histopathological correlations. Anticancer Res 1987;7:781-90.
9. Oud PS, Henderik JBJ, Beck HLM. Flow cytometric analysis and sorting of human endometrial cells after immunocytochemical labeling for cytokeratin using a monoclonal antibody. Cytometry 1985;6:159-64.
10. Naus GJ, White RD, Richart RM, Deitch AD. Predictive value of flow cytometric DNA content analysis of paraffin-embedded tissue in endometrial carcinoma. Lab Invest 1985;52:48A.
11. Thornton JG, Wells M, Hume WJ. Flash labelling of S-phase cells in short-term organ culture of normal and pathological human endometrium using bromodeoxyuridine and tritiated thymidine. J Pathol 1988;154:321-8.
12. Hiddeman W, Schumann J, Andreeff M. Convention on nomenclature for DNA cytometry. Cytometry 1984;5:445-6.
13. Dolbeare F, Gratzner H, Pallavicini MG, Gray JW. Flow cytometric measurement of total DNA content and incorporated bromodeoxyuridine. Proc Natl Acad Sci USA 1983;80:5573-7.
14. Allison DC, Ridolpho PF, Anderson S, Bose K. Variations in the [3H]thymidine labeling of S-phase cells in solid mouse tumors. Cancer Res 1985;45:6010-6.
15. McDivitt RW, Stone KR, Meyer JS. A method for dissociation of viable human breast cancer cells that produces flow cytometric kinetic information similar to that obtained by thymidine labeling. Cancer Res 1984;44:2628-33.
16. Feichter GE, Hoffken H, Heep J. DNA-flow-cytometric measurements on the normal, atrophic, hyperplastic and neoplastic human endometrium. Virchows Arch [A] 1982;398:53-65.

Bound volumes available to subscribers

Bound volumes of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY are available to subscribers (only) for the 1989 issues from the Publisher, at a cost of $56.00 ($79.00 international) for Vol. 160 (January-June) and Vol. 161 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue in the volume. The binding is durable buckram with the JOURNAL name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact The C.V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318, USA; phone (800) 325-4177, ext. 351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular JOURNAL subscription.

Atrial natriuretic peptide receptors in guinea pig placentas

Christos G. Hatjis, MD, and David M. Grogan, BA
Winston-Salem, North Carolina

The multiple physiologic effects of atrial natriuretic peptide are mediated through specific atrial natriuretic peptide plasma membrane receptors. We have attempted to determine whether atrial natriuretic peptide receptors are present in guinea pig placentas and subplacentas at approximately 45 and 65 days' gestation. A microsomal plasma fraction was prepared from each component. Atrial natriuretic peptide receptors were detected by in vitro radioligand techniques with α-human atrial natriuretic peptide labeled with iodine 125. In placentas we identified one set of specific binding sites for atrial natriuretic peptide at both gestational ages. Although the maximal concentration of the receptors did not change with advancing gestation, the dissociation constant was higher at 65 than at 45 days' gestation. In subplacentas two sets of binding sites were identified, one of low capacity–high affinity and the other of low affinity–high capacity. The maximal concentration of the atrial natriuretic peptide receptors did not change as a function of gestation. However, the dissociation constant, for both the high- and low-affinity sites, significantly decreased with advancing gestation. (Am J Obstet Gynecol 1989;161:493-6.)

Key words: Atrial natriuretic peptide receptors, guinea pig placentas, guinea pig subplacentas

Atrial natriuretic peptide (ANP) is a hormone or a group of hormones that is secreted by specialized cells in atrial myocytes in many species.[1] The presumed active peptide in humans is designated α-hANP.[2] Although the exact physiologic or pathologic role of ANP has not been elucidated, this peptide has been shown to be a potent natriuretic, diuretic, and vasodilatory agent.[3] The multiple effects of ANP appear to be mediated through specific membrane receptors.[4] We and others have identified specific receptors for ANP in various mammalian organs, including human placentas.[5-7] There is no information about ANP receptor properties in placentas obtained from pregnant guinea pigs. Moreover, guinea pig placentation is unique with regard to the presence of subplacentas.[8] In addition to their endocrine, respiratory, and nutritional functions, guinea pig placentas and subplacentas may be involved in fluid and electrolyte homeostasis. If placental ANP receptors are present in several species showing different types of placentation, then one could infer that they may be involved in important aspects of placental function across species. Thus we have attempted to determine whether specific ANP receptors can be detected in plasma membranes from placentas and subplacentas of pregnant guinea pigs at 45 and 65 days' gestation.

From the Section on Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Bowman Gray School of Medicine of Wake Forest University.
This work was partially supported by the Bowman Gray School of Medicine United Way Venture Grant and by United Cerebral Palsy Grant R-356-87.
Received for publication September 22, 1988; revised February 15, 1989; accepted March 13, 1989.
Reprint requests: Christos G. Hatjis, MD, Director of Perinatology, Riverside Methodist Hospitals, 3535 Olentangy River Road, Columbus, OH 43214.

Material and methods

Material. Nonradioactive α-hANP (124-151) was obtained from Peninsula Laboratories. α-hANP labeled with iodine 125 was obtained from Amersham and was used fresh on a monthly basis. The other reagents were obtained commercially and were of the highest purity available.

Tissue. Primigravid Hartley guinea pigs with time-dated gestations were put to death under xylazine and ketamine anesthesia at 40 to 45 days' gestation or at 60 to 65 days' gestation (term, 65 days). At hysterectomy, placentas and subplacentas were obtained from four animals at each gestational age. The tissue was snap-frozen in liquid nitrogen and stored at −70° C. Under these conditions no significant loss in ANP receptor activity was noted with freezing and storage for up to 6 months.

Membrane preparations. Membrane preparation of a plasma membrane fraction was done as previously described.[5-7] On the day of the assay approximately 2 to 5 gm of either placenta or subplacenta was allowed to thaw in 10 to 20 ml of ice-cold 0.9% sodium chloride, 20 mmol/L Tris hydrochloride, 250 mmol/L sucrose, 5 mmol/L ethylenediaminetetraacetic acid (EDTA), and 0.6 mmol/L phenylmethylsulfonyl fluoride (PMSF), pH 7.5. All subsequent procedures were carried out at 4° C. The tissue was minced with scissors and subsequently homogenized with a Brinkman Polytron blender on setting No. 7, twice, with each cycle

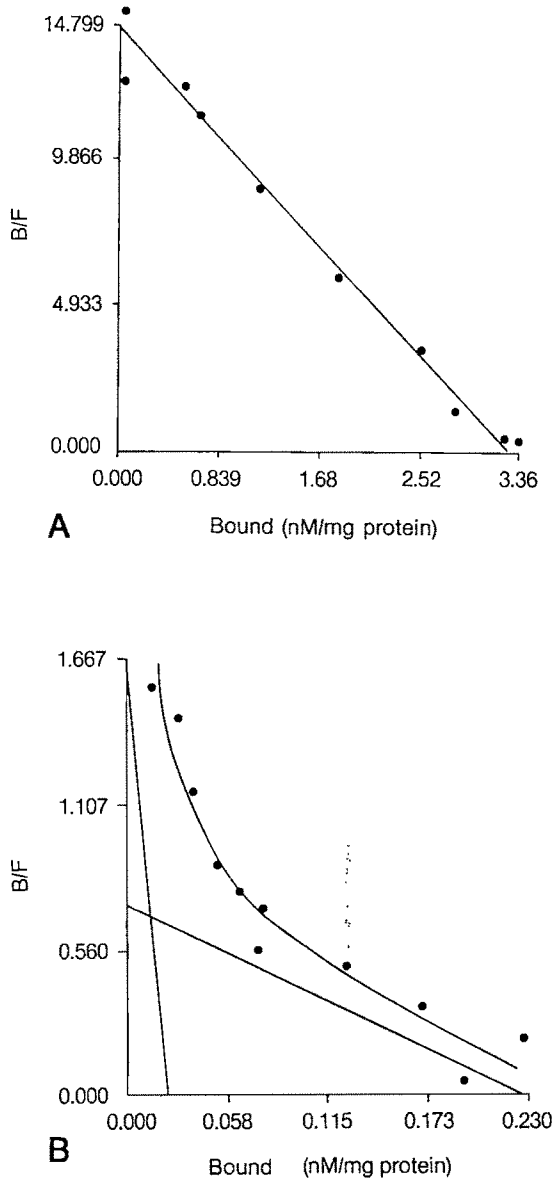


Fig. 1. Representative binding curves of ^{125}I-α-hANP to guinea pig placenta **(A)** and subplacenta **(B)** at 65 days' gestation. Each point was determined in triplicate.

lasting approximately 45 seconds and a 20-second cooling period between homogenizations. This crude homogenate was then centrifuged at 600 *g* for 15 minutes. The resultant pellet was discarded and the supernatant was centrifuged at 12,000 *g* for 30 minutes. The supernatant of the above was centrifuged at 40,000 *g* for 40 minutes and the pellet was resuspended in 50 mmol/L potassium phosphate (pH 7.5, 2 mmol/L EDTA and 0.6 mmol/L PMSF) on the Brinkman Polytron blender at setting No. 7 for 15 seconds. This suspension was then centrifuged at 40,000 *g* for 40 minutes twice and the final pellet was resuspended in the incubation buffer of 50 mmol/L potassium phosphate, 2 mmol/L EDTA, 0.6 mmol/L PMSF, 0.1% bacitracin, and 1 μmol/L aprotinin (pH 7.5) in a volume that gave a final concentration of 11 to 23 μg per tube.

Table I. Binding characteristics of ANP receptor in guinea pig placentas

Gestational age	K_d *(nmol/L)*	B_{max} *(nmol/L/mg protein)*
45 days (n = 4)	0.133 ± 0.012*	1.770 ± 0.249
65 days (n = 4)	0.288 ± 0.056*	2.470 ± 0.394

*p = 0.035.
n, Number of animals studied.

ANP receptor assay. Placental and subplacental plasma membranes were incubated at 25° C for 45 minutes in the presence of ^{125}I-ANP with or without unlabeled α-hANP. Preliminary experiments had shown that ANP binding to placental membranes achieved equilibrium by 20 minutes of incubation and was stable for at least 90 minutes. ^{125}I-ANP was present at concentration of 10 to 15 pmol/L. Unlabeled α-hANP was present in concentrations ranging from 10^{-13} to 10^{-7} mol/L. The latter concentration (10^{-7} mol/L) was designated as nonspecific binding. α-hANP was diluted in 0.1% acetic acid and 0.2% bovine serum albumin. Polypropylene tubes were used throughout. The total volume of incubation was 250 μl.

At the end of incubation, 4 to 5 ml of incubation buffer without the additives was used to stop the reaction. The tubes were decanted into pressure manifolds containing GF/C (Whatman) filters presoaked in 1% polyethylenimine and 1% bovine serum albumin. These filters were then washed in 15 ml of buffer, dried, and counted in a γ-counter (Packard Auto Gamma, efficiency 80%).

We used a competitive binding assay of displacement of ^{125}I-α-hANP by α-hANP to arrive at Scatchard plots.[9] These were then analyzed by LIGAND.[10] The dissociation constants, K_d, and maximum binding capacities, B_{max}, were calculated for each one of the curves. The results are expressed as mean ± SEM. The amount of protein in the assay was determined by the method of Bradford,[11] with bovine serum albumin used as the standard.

The parameters of interest, (K_d and B_{max}), were compared by two-tailed t test. A $p < 0.05$ was considered as significant.

Results

Fig. 1 shows representative binding curves for placental *(A)* and subplacental *(B)* ANP receptors of an animal at 65 days' gestation. Similar results were obtained in the other animals studied. We detected ANP receptors in the microsomal fraction in all cases. The binding was specific, saturable, and was inhibited by

Table II. Binding characteristics* of ANP receptors in guinea pig subplacentas

Gestational age	K_H *(pmol/L)*	K_L *(nmol/L)*	R_H *(pmol/L/mg protein)*	R_L *(nmol/L/mg protein)*
45 days (n = 3)	86.97 ± 8.86†	6.14 ± 3.78‡	15.90 ± 5.10	0.094 ± 0.032
65 days (n = 3)	21.23 ± 4.10	0.56 ± 0.28	27.95 ± 24.48	0.228 ± 0.200

Statistical analysis for R_H and R_L was performed after log transformation. n, Number of animals studied.
*K_H and R_H, K_d and B_{max} for high-affinity site. K_L and R_L, K_d and B_{max} for low-affinity site.
†p = 0.004.
‡p = 0.0408.

increasing the concentration of unlabeled α-hANP. Note that in the placental component one set of binding sites is seen as opposed to the subplacental fraction, which demonstrates two sets of receptors for α-hANP. Table I shows the calculated K_d and B_{max} from replicate experiments in guinea pig placental tissues at 45 and 65 days' gestation. In all cases there appears to be one set of receptors for α-hANP that shows a uniform high affinity and low capacity. Statistically, the best curve fit is obtained when one set of ANP binding sites is postulated to be present. Of interest is the observation that the K_d increases with advancing gestation, suggesting a decrease in the affinity of the placental ANP receptor for α-hANP.

Table II shows the calculated K_d and B_{max} from replicate experiments in guinea pig subplacentas. In all cases there appear to be two sets of receptors for α-hANP that show high-affinity/low-capacity and low-affinity/high-capacity binding characteristics, respectively. Statistically, the best curve fit is obtained when two sets of binding sites are postulated to be present. Although the B_{max} for either the high- or low-affinity site does not change with advancing gestation, in contrast to the observations with placental tissue, K_d for the high- and low-affinity binding sites decreased from 45 to 65 days' gestation, suggesting an increase in the affinity of the subplacental ANP receptor to α-hANP.

Comment

This is the first instance where ANP receptors have been reported in guinea pig placentas and subplacentas. These observations extend our previous results concerning human placentas[7] and sheep cotyledons.[12] These results strongly suggest that placental ANP receptors are found in species with different types of placentation (from both the functional and the morphologic standpoint).

Similar to the situation in human placentas,[5, 7] one set of binding sites is found in guinea pig placentas. Although we do not have any information regarding the ontogenesis of ANP binding sites in human placentas with advancing gestation, in pregnant guinea pigs there appears to be a decrease in the affinity of the placental ANP receptor from 45 to 65 days' gestation. However, it should be noted that these studies were conducted at only two time points—midpregnancy (45 days) and term (65 days). Finally, whether this change represents a primary effect or is secondary to an increase in circulating ANP concentrations is not known.

Pregnant guinea pigs are the only mammals that show a subplacental structure.[8] The latter has been thought to be involved in fluid and electrolyte homeostasis. Interestingly, ANP receptors are present in the microsomal fraction from subplacentas. In contrast to the situation in the placental ANP receptors, two sets of binding sites are observed. Although the maximal concentration of these binding sites is unchanged as a function of gestation, there is an increase in the affinity of these binding sites for α-hANP from 45 to 65 days' gestation. We doubt that the presence of these two sets of binding sites reflects the use of a probe that was developed from human material, since only one set of binding sites was seen in guinea pig placentas. ANP receptor heterogeneity has been previously reported in other tissues and species.[2] Thus a plausible explanation might be that the presence of two sets of binding sites reflects actual functional receptor subpopulations in guinea pig subplacentas.

The change in placental and subplacental ANP receptors from 45 to 65 days' gestation in pregnant guinea pigs could be a primary effect or could be secondary to changes in circulating ANP concentrations in the maternal and fetal compartments. However, very little is known about circulating levels of ANP in pregnant or nonpregnant guinea pigs. Further purification of the placental and subplacental ANP receptors will be necessary to rule out the contribution of an intrinsic change in ANP receptors. This work represents the necessary first step toward the latter goal.

The presence of ANP receptors in placental and subplacental plasma membrane fractions is interesting in that it suggests that ANP may have significant effects on placental hemodynamics, fluid shifts, and electrolyte

transport, as well as endocrine function. There is a paucity of in vitro or in vivo experiments to support this hypothesis. However, in a preliminary report in human placentas, ANP appears to modulate sex steroid production.[13] Further experiments need to be performed to define the functional implications of ANP receptors in guinea pig placentas and subplacentas.

REFERENCES

1. Atlas SA. Atrial natriuretic factor: a new hormone of cardiac origin. Recent Prog Horm Res 1986;42:207.
2. Adams SP. Structure and biologic properties of the atrial natriuretic peptides. Endocrinol Metab Clin North Am 1987;16:1.
3. Ballermann BJ, Brenner BM. Biologically active atrial peptides. J Clin Invest 1985;76:2041.
4. Jacobs JW, Vlasuk GP, Rosenblatt M. Atrial natriuretic factor receptors. Endocrinol Metab Clin North Am 1987; 16:63.
5. Sen I. Identification and solubilization of atrial natriuretic factor receptors in human placenta. Biochem Biophys Res Commun 1986;134:480.
6. Leitman DC, Murad F. Atrial natriuretic factor receptor heterogeneity and stimulation of particulate guanylate cyclase and cyclic GMP accumulation. Endocrinol Metab Clin North Am 1987;16:79.
7. Hatjis CG, Grogan D. Atrial natriuretic peptide receptors (ANP) in normal human placentas. Am J Obstet Gynecol 1988;159:587.
8. Ramsey EM. The placenta of laboratory animals and man. New York: Holt, Rinehart & Winston, 1975.
9. De Lean A, Munson PJ, Rodbard D. Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay, and physiological dose-response curve. Am J Physiol 1978;235(2):E97.
10. Munson PJ, Rodbard D. LIGAND: a versatile computerized approach for characterization of ligand-binding systems. Anal Biochem 1980;107:220.
11. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976; 72:248.
12. Hatjis CG, Grogan D. Atrial natriuretic peptide receptors in sheep cotyledons. Am J Obstet Gynecol 1988; 159:1337.
13. Craig HR, Yasue I, Benveniste R, Schumacher GFB. Effect of alpha-trial natriuretic peptide on estrogen and progesterone secretion by human placental cell cultures [Abstract]. In: Proceedings of the thirty-fifth annual meeting of the Society for Gynecologic Investigation, Baltimore, Maryland, March 17-20, 1988:342.

CORRESPONDENCE

Attempted suicide and pregnancy

To the Editors:

It was interesting to read the article written by D. Lester and A. T. Beck (Attempted suicide and pregnancy. AM J OBSTET GYNECOL 1988;158:1084-5) with regard to 15 consecutive pregnant women because we have had experience with >500 cases in Hungary. There is only one hospital for the provision of health care to chemically poisoned adult inhabitants of Budapest and the surrounding area, which involves 3 million people. The number of hospitalized cases of poisoning is about 10,000 annually. More than 90% of the cases involve self-poisoning (i.e., suicide attempts). There is an obvious preponderance of women and among them are pregnant women. Our purpose was to find a human experimental epidemiologic model for the study of teratogenicity of high doses of drugs and other chemicals.[1]

The first project consisted of 209 poisoned pregnant women who were hospitalized between 1960 and 1979.[2,3] Of these 37% were unmarried. The main reasons of suicide attempts were as follows: familial troubles that mainly involved the potential loss of a lover (78%), economic crisis (9%), unwanted pregnancy or a desire for abortion (13%). Higher rates of stillbirths, mental retardation, and behavioral deviation and a lower mean birth weight as a result of fetal growth retardation were found in the offspring. However, an increased occurrence of spontaneous abortions, congenital abnormalities, and infant deaths was not detected.

In the second project the behavioral development of offspring and their sibs in 176 self-poisoned pregnant women hospitalized in 1980 to 1984 was studied. The aim of this approach was to separate the familial-maternal sociocultural effect in postnatal life and the biologic-teratologic effect of self-poisoning in prenatal life.[4]

Since 1985 a very sensitive pregnancy test has been performed in the third project in all poisoned women from ages 14 to 50 years. The annual total numbers of self-poisoned pregnant women ranged from 76 to 102 in 1985 to 1987. These pregnancies and offspring development are being followed-up. Most of the suicide attempts occurred at the earliest inapparent phase of pregnancy. The incidence of so-called *chemical pregnancies* is significantly higher[4] and the rates of chromosome breakages and sister chromatid exchanges in self-poisoned pregnant women were significantly lower than in nonpregnant self-poisoned women.[5] It indicates some protective effect of pregnancy concerning mutagenic factors.

The purpose of this letter is to give some information about these studies and to call for collaboration in the evaluation of the data base.

Andrew Czeizel, MD
Agnes Lendvay, PhD
Department of Human Genetics and Teratology
National Institute of Hygiene
World Health Organization Collaborating Centre for the Community Control of Hereditary Diseases
Budapest, Hungary 1966 Gyáli ut 2-6

REFERENCES

1. Czeizel A. Self-poisoning as a model for the study of mutagenicity and teratogenicity of chemicals in human beings. In: Ramel C, Lambert B, Magnusson J, eds. Genetic toxicology of environmental chemicals. Part B: Genetic effects and applied mutagenesis. New York: Alan R. Liss, 1986: 237-44.
2. Czeizel A, Szentesi I, Glauber A, Bucski P, Molnár G. Pregnancy outcomes and health condition of offspring on self-poisoned pregnant women. Acta Pediatr Hung 1984;25: 209-36.
3. Czeizel A, Szentesi I, Szekeres I, Molnár G, Glauber A, Bucski P. A study of adverse effects on the progency after intoxication during pregnancy. Arch Toxicol 1988;62:1-7.
4. Czeizel A. Endpoints of reproductive dysfunction in an experimental epidemiological model: self-poisoned pregnant women. In: Chambers PL, Gehring P, Sakai F, eds. New concepts and developments in toxicology. New York: Elsevier Science, 1986:49-61.
5. Huong TTT, Szentesi I, Czeizel A. Lower prevalence of chromosome aberrations and SCEs in self-poisoned pregnant women. Mutat Res 1988;198:255-9.

Sperm antibodies and infertility

To the Editors:

The excellent report by Witkin and David (Effects of sperm antibodies on pregnancy outcome in a subfertile population. AM J OBSTET GYNECOL 1988;158:59-62) deserves high praise for giving emphasis to the significance of sperm antibody in relation to infertility. They have also, quite correctly, urged gynecologists to give more consideration to this possible etiologic factor. I would add to their arguments the concept that the occasional occurrence of pregnancy despite the presence of sperm antibody is no more an argument against the antibody's significance than is the occasional pregnancy that occurs despite mild endometriosis or moderate oligospermia, or other such conditions. These are still the exceptions, not the rule. As the authors say, furthermore, these pregnancies usually are quite delayed, hence, do *not* represent normal fertility.

I am gratified that they found a significant difference in the incidence of sperm antibody in women who are infertile (38%) and those who are fertile (12%). If we subtract one value from the other, we obtain 26%,

which could be taken as their infertile value if the "background" fertile value were zero. They made use of an enzyme-linked immunosorbent assay (ELISA) to detect this antibody. In our work with sperm agglutination, we reported in 1975 an incidence of 18% in infertile women, and we found 1% or 2% in the fertile group.[1] The moderate degree of difference may be because of the difference in methods, but also, the rather small number of samples that they studied (as they point out).

However, we do have a disagreement concerning the study of men. Witkin and David found no distinction between infertile and fertile groups, whereas we found a 9% incidence among the infertile men and a ≤1% incidence in the fertile group.[1] Others have found the same value, namely, 8% to 10%, as was recently reviewed.[2] There may need to be more studies on the ELISA technique itself. These authors have used the individual sperm from the patient or the patient's partner as the target antigen; other workers have used pools of sperm—not only pooled but unrelated to the patients.[3,4]

The authors put great emphasis on the desirability of the use of "purified, living, and motile spermatozoa." However, in the ELISA procedure, they are certainly dead sperm, in contrast (we may point out) to those in sperm agglutination assays in which the sperm cells are living and motile (even if not purified) during the procedure. I suppose that they mean that their test sperm *had* been "purified, living, and motile." However, this does raise a question. Did they also use sperm preparations that had *not* been purified (as by their swim-up procedure), to compare the results of at least some of their sera? In fact, did they also try to use completely immotile sperm? It would be interesting to know how much change in ELISA results this would cause.

As a different point, their finding that sperm antibodies have an association with miscarriage is very interesting and provocative. Here they would have some support from the similar conclusions reported by Haas et al.[5]

Finally, their comments with regard to prednisone treatment would seem to suggest some degree of success for this treatment. Our study with methylprednisolone did show a very good success rate,[6] as did also the work of Hargreave.[7] I would welcome more details eventually on the outcome of their steroid medication efforts.

Sidney Shulman, PhD

Sperm Antibody Laboratory
Fertility Antibody Diagnostics, Inc.
945 West End Avenue
New York, NY 10025

REFERENCES

1. Shulman S, Jackson H, Stone ML. Antibodies to spermatozoa. Am J Obstet Gynecol 1975;123:139.
2. Shulman S. Infertility as caused by sperm antibodies. Gynecol Obstet Invest 1986;22:113.
3. Lynch DM, Leali BA, Howe SE. A comparison of sperm agglutination and immobilization assays with a quantitative ELISA for antisperm antibody in serum. Fertil Steril 1986;46:285.
4. Howe SE, Lynch DM. Antibody binding specificity to donor sperm in sera from infertile patients. Am J Reprod Immunol Microbiol 1987;13:104.
5. Haas GG, Kubota K, Quebbeman JF, Jijon A, Menge AC, Beer AE. Circulating antisperm antibodies in recurrently aborting women. Fertil Steril 1986;45:209.
6. Shulman JF, Shulman S. Methylprednisolone treatment of immunologic infertility in the male. Fertil Steril 1982; 38:591.
7. Hargreave TB, Elton RA. Treatment with intermittent high dose methylprednisolone or intermittent betamethasone for antisperm antibodies: preliminary communication. Fertil Steril 1982;38:586.

Reply

To the Editors:

Sperm antibody testing of infertile couples will gain acceptance among clinicians only when physicians can be assured that the analysis measures antibodies that are relevant to infertility. It is because of this that assays that directly measure immunoglobulin reactivity with living, motile sperm (Immunobead binding,[1] mixed antiglobulin reaction,[2] radioimmunoassay,[3] and enzyme-linked immunosorbent assay or ELISA[4]) are rapidly replacing assays that measure a secondary consequence of antibodies (sperm agglutination and immobilization). Inasmuch as antibodies that neither agglutinate nor immobilize sperm can nevertheless interfere with fertility,[5] and agglutination of sperm can occur in the absence of antisperm antibodies,[5] reliance on these latter two assays will yield a significant number of false-positive and false-negative results.

Our article presented data suggesting that only antibodies on the surface of ejaculated sperm, and not antisperm antibodies present in a man's serum, correlated with infertility.[4] Other studies have demonstrated a lack of correlation between antisperm antibodies in serum and in semen.[6-8] Naturally, if circulating antibodies never come into contact with sperm they can have no effect on fertility. Therefore the direct analysis for antibodies on the sperm surface appears to be the preferred procedure to detect relevant antibodies in men.

A purified population of living motile sperm were reacted with patients' sera in our assay. Fixation of sperm to wells of a microtiter plate was performed *after* their incubation with serum. As shown previously, the ELISA assay correlates with the Immunobead assay only when the ELISA employs purified and motile sperm free in solution.[9]

The issues of treatment for immunologic infertility can be addressed only after vaid antisperm antibody detection assays are performed. If the antisperm antibodies that are detected are not relevant to infertility, then any treatment will be ineffective.

Steven S. Witkin, PhD

Immunology Division
Department of Obstetrics and Gynecology
Cornell University Medical College
New York, NY 10021

REFERENCES

1. Bronson R, Cooper G, Rosenfeld D. Sperm antibodies; their role in infertility. Fertil Steril 1984;42:171-83.
2. Jager S, Kremer J, Van Slochteren-Draaisma T. A simple method of screening for antisperm antibodies in the human male. Int J Fertil 1978;23:12-21.
3. Haas GG Jr, Cines DB, Schreiber AD. Immunologic infertility: identification of patients with antisperm antibodies. N Engl J Med 1980;303:722-7.
4. Witkin SS, David S. Effects of sperm antibodies on pregnancy outcome in a subfertile population. AM J OBSTET GYNECOL 1988;158:59-62.
5. Haas GG Jr. How should sperm antibody tests be used clinically? Am J Reprod Immunol Microbiol 1987;15:106-11.
6. Ayvaliotis B, Bronson R, Rosenfeld D, Cooper G. Conception rates in couples where autoimmunity to sperm is detected. Fertil Steril 1985;43:739-42.
7. Clark GN, Elliott PJ, Smaila C. Detection of sperm antibodies in semen using the Immunobead test: a survey of 813 consecutive patients. Am J Reprod Immunol Microbiol 1985;7:118-23.
8. Meinertz H. Indirect mixed antiglobulin reaction (MAR) as a screening procedure for antisperm antibodies. Am J Reprod Immunol Microbiol 1987;15:101-5.
9. Bronson R, Cooper G, Rosenfeld D, Witkin SS. Detection of spontaneously occurring sperm-directed antibodies in infertile couples by Immunobead binding and enzyme-linked immunosorbant assay. NY Acad Sci 1984;438:504-7.

Vaginosonographic examination—Routine procedure?

To the Editors:

The article by Bernaschek et al. (Bernaschek G, Rudelstorfer R, Csaicsich P. Vaginal sonography versus serum human chorionic gonadotropin in early detection of pregnancy. AM J OBSTET GYNECOL 1988; 158:608-12) refers to the use of a vaginal transducer to diagnose pregnancy as early as 4 weeks and 2 days and for early diagnosis of ectopic pregnancy. The authors suggest repeating the scan within 2 days if the initial study is equivocal. Unfortunately, they make no unequivocal reference to a need for patient selection. In view of their emphasis on the suitability of vaginal scanning as an office procedure, it could be interpreted as suggesting that this is a reasonable means of routine diagnosis of pregnancy. It is totally inappropriate to publish such an article without any cautionary comment with regard to the safety of ultrasonography.

Margaret E. Furness, FRACR, DDU
Director of Radiology
Queen Victoria Hospital
Rose Park, South Australia 5067

Reply

To the Editors:

The allegations center on three issues: (1) safety of ultrasound; (2) need for patient selection for repeat scans; and (3) reasonable means of routine diagnosis of pregnancy.

1. The energy output of this modern vaginal ultrasonographic transducer is well beyond the safety margins of the American Institute of Ultrasound in Medicine (i.e., considerably <100 mW/cm²). The recommendations of the World Federation of Ultrasound in Medicine and Biology are not based on scientific evidence that ultrasound delivered clinically is deleterious, but rather on the realization that scientific evidence is inconclusive. To date, studies with negative results predominate. Nevertheless, exposures should be as short as possible and administered by qualified personnel.[1]

2. An indication for a repeat scan after 2 days is only suggested when no intrauterine gestational sac has been seen in connection with a positive pregnancy test (<750 mIU/ml) and the absence of any clinical evidence of spontaneous abortion.

3. In consideration of the very low energy output and the great value of a definite identification of an intrauterine pregnancy, we believe that vaginal ultrasonography is a new means for routine diagnosis of early pregnancy. There are patients for whom the very early diagnosis of an intrauterine pregnancy is of great value (e.g., patients with first-trimester bleeding or with a history of ectopic pregnancy, pelvic inflammatory disease, or tubal surgery or after in vitro fertilization).[2]

Finally, years ago the monitoring of follicles was introduced into fertility praxis with the same equipment without any reported hazard to the oocytes.

Gerhard Bernaschek, MD
Rudolf Rudelstorfer, MD
Second Universitäts-Frauenklinik
Spitalgasse 23
A-1090 Vienna, Austria

REFERENCES

1. Martin CA. Biologic effects. In: Sabbagha RE, ed. Diagnostic ultrasound applied to obstetrics and gynecology. Philadelphia: Lippincott, 1987:41.
2. Goldstein STR, Snyder JR, Watson C, Danon M. Very early pregnancy detection with endovaginal ultrasound. Obstet Gynecol 1988;72:200-6.

Nerve entrapment after Pfannenstiel incision

To the Editors:

I was surprised to read the conclusion made by Sippo et al. (Nerve entrapment after Pfannenstiel incision. AM J OBSTET GYNECOL 1987;157:420-1) that entrapment of the iliohypogastric nerve is a common complication of the Pfannenstiel incision. Because the authors have not reported the total number of patients who had undergone a Pfannenstiel incision, the prevalence rate of nerve entrapment after Pfannenstiel incision in their patients cannot be calculated. Thus, their statement that "entrapment of the iliohypogastric neve is a common complication of the Pfannenstiel incision" might be a result of their beliefs rather than a conclusion based on statistical calculations.

We perform each year at the Soroka Medical Center in Beer-Sheva, Israel, some 700 laparotomies by the Pfannenstiel incision and have never encountered such a complication. However, I must admit that we have

not actively sought this complication in our patients. Nevertheless, I agree with the authors' recommendations that the Pfannenstiel incision should be extended through the external oblique aponeurosis only and should not involve incision of the internal oblique muscle, and that widely placed corner sutures should be avoided in the closure of the anterior fascia.

Benjamin Piura, MD, MRCOG
Division of Obstetrics and Gynecology
Soroka Medical Center
P.O. Box 151
Beer-Sheva 84101, Israel

Reply

To the Editors:

As you have pointed out, the true incidence of nerve entrapment after Pfannenstiel incision is not known, nor can it be estimated by our preliminary work. However, the fact that nine cases were identified within a 16-month period suggests that this entity is prevalent and could more commonly be identified if physicians who perform large numbers of Pfannenstiel incisions actively sought a history of its symptoms in their postoperative patients.

Why we were first to describe this entity, only fate can explain. But since the publication of this article we have received correspondence from physicians and patients who suggest that this syndrome is real and universal.

Our investigation arose from the serendipitous recognition that the hysterectomized women who have what our gynecology colleagues had termed a "psychosomatic post hysterectomy syndrome," in fact had complaints that closely resembled the symptoms of the nerve entrapment seen after inguinal herniorrhaphy. Until we looked closer at these patients who had been within our midst all along, the true organic source of their symptoms had remained unrecognized and misdiagnosed as psychosomatic. This phenomenon may be compared with mitral valve prolapse, which, although unheard of even 20 years ago, is now recognized to be present in 10% to 15% of the population because it has been described and is actively sought on examination.

It is large centers like yours that do a significant number of procedures with Pfannenstiel incision that will show us how prevalent this nerve entrapment syndrome really is, but only if the residents and staff earnestly consider nerve entrapment and look for it.

Another factor that may be deceptive as to the actual extent of this problem is that the patients who have symptoms remote from the time of surgery may consult their primary care physician first and not their gynecologist or surgeon. In our article (Sippo WC, Burghardt A, Gomez AC. Nerve entrapment syndromes from lower abdominal surgery. Fam Pract 1987;25: 585-7) we emphasized this point with the hope that primary care physicians might consider nerve entrapment as a possible source of lower abdominal pain in patients with a history of surgery.

Perhaps the most important lesson we can learn from this matter is what my Mentor urged us to do as residents: "Believe what the patient tells you and assume it to be so, for the patient has no reason to lie. You may be surprised at what you find if you look hard enough."

William C. Sippo, MD
Major, Medical Corps
Keller Army Hospital
West Point, NY 10996

Unexplained elevated maternal serum α-fetoprotein level associated with third-trimester problems

To the Editors:

I would like to raise a question with regard to a personally observed association between unexplained elevated maternal serum α-fetoprotein level and third-trimester high-risk pregnancy problems.

In following up delivery outcomes of patients in whom I performed genetic amniocentesis in my Maternal Serum α-Fetoprotein Program Follow-up Center, which is approved by the California State Health Department, I have observed problems in the third trimester: (1) premature separation of the placenta, (2) toxemia, (3) premature labor, and (4) unexplained stillbirth.

If this is more than just a personal experience, should not maternal serum α-fetoprotein pregnancy screening be indicated not only as a screening test for neural tube and other defects such as chromosomal disorder (Down syndrome) but also as a predictor for potential third-trimester high-risk problems?

The issue is not only one of concern for professional liability but also potentially one of compliance. It may be easier to obtain compliance for maternal serum α-fetoprotein screening if the presentation to the patient for justification is not just screening for birth defects, which has a connotation of being associated with pro choice and the subsequent implications for termination of pregnancy. The possibility of premature labor might permit obstetricians to recommend the test in patients who have had previous premature labor, toxemia, unexplained stillbirth, premature separation of the placenta, and intrauterine growth retardation, and focus on that as the primary justification for the test.

The next problem that would surface is what to do and what to recommend if the patient has an unexplained elevated maternal serum α-fetoprotein value. Would genetic amniocentesis automatically be necessary if the fetal ultrasonogram did not reveal a neural tube or other related defect?[1]

John D. Stephens, MD
Medical Director
California Prenatal Diagnosis Institute
1390 S. Winchester Blvd.
San Jose, CA 95128

REFERENCE

1. Richards DS, Seeds JW, Katz VL, Lingley LH, Albright SG, Cefalo RC. Elevated maternal serum alpha-fetoprotein with normal ultrasound: is amniocentesis always appropriate? A review of 26,069 screened patients. Obstet Gynecol 1988;71:203.

Neurofibromatosis and pregnancy

To the Editors:

We congratulate Dr. Blickstein et al. on their detailed review (The obstetric perspective of neurofibromatosis. AM J OBSTET GYNECOL 1988;158:385-9) in which they consider the obstetric problems involved with neurofibromatosis. However, we believe that two problems should receive more attention.

The authors pointed out that the incidence of nontoxemic hypertension is in conflict. To differentiate between primary arterial hypertension, renal hypertension (renal arterial stenosis), pheochromocytoma (paroxysmal hypertonic crises), hypertension because of hyperthyroidism, and hypertension as a symptom of preeclamsia, the following laboratory tests should be performed routinely: plasma renin, 24-hr urine catecholamines, vanillylmandelic acid, homovanillic acid, aldosteron, angiotensin, triiodothyronine and thyroxine. Additionally, we recommend ultrasonography of the heart and the kidneys for these high-risk pregnant patients.

We agree with the authors that even in these women vaginal delivery should be attempted. Because of hypertension, continuous peridural analgesia should be performed analogous to preeclamptic patients.[1, 2] However, if there is any intraspinal lesion (e.g., meningioma), indication for continuous peridural analgesia may not be given. If cesarean section is required, anesthesia should be primarily focused on the patient's hypertension. Therefore specific interactions of perioperatively administered antihypertensive drugs, anesthetics, and narcotics must be emphasized. Hypersensitivity of these patients to muscle relaxation agents must be considered to schedule postoperative care.[3]

G. Mitterschiffthaler, MD
Department of Anesthesiology and General Intensive Medicine
L. C. Fuith, MD
Department of Gynecology and Obstetrics
University of Innsbruck
Anichstraße 35
A-6020 Innsbruck, Austria

REFERENCES

1. Jouppila P, Jouppila R, Hollmen A, Kouvila A. Lumbar epidural analgesia to improve intervillous blood flow during labor in severe preeclampsia. Obstet Gynecol 1982;59:158-61.
2. Newsome LR, Bramwell RE, Curling PE. Severe preeclampsia: hemodynamic effects of lumbar epidural anesthesia. Anesth Analg 1986;65:31-6.
3. Mitterschiffthaler G, Maurhard U, Huter O, Brezinka C. Verlängerte Wirkung von Vecuronium bei Neurofibromatose (M. von Recklinghausen). Anaesthesiol Reanim [In press].

Reply

To the Editors:

We thank Drs. Mitterschiffthaler and Fuith of Austria for their informative comment. We agree that their recommendations for laboratory and ultrasonographic tests may help in the differential diagnosis of the conflicting problem of nontoxemic hypertension in pregnant patients with neurofibromatosis.

Because the literature is scant that considers labor of patients with neurofibromatosis, one must rely on one's own clinical judgment. We therefore welcome the suggestion of continuous peridural analgesia in hypertensive neurofibromatosis parturients. Continuous peridural analgesia, however, may be found to be technically difficult to perform in cases complicated by scoliosis.

After the publication of our review, we propose a worldwide study of the reproductive performance of as many patients with neurofibromatosis as possible. Perhaps we could take this opportunity to invite our colleagues to participate in this project.

Isaac Blickstein, MD
Department of Obstetrics and Gynecology
Kaplan Hospital
76100 Rehovot, Israel

Isolation of human immunodeficiency virus from the placenta or from maternal blood contaminating the placenta?

To the Editors:

The title of the case report by Drs. Hill, Bolton, and Carlson (Isolation of acquired immunodeficiency syndrome virus from the placenta. AM J OBSTET GYNECOL 1987;157:10-1) seems misleading.

Although the authors pointed out that "The positive viral culture of the placenta was probably a result of the patient's positive blood culture," they nevertheless claim that they isolated the virus from placental tissue. The figure that shows a human T-lymphocyte with extra cellular human immunodeficiency virus simply suggests that the human immunodeficiency virus is in proximity to a lymphocyte. This does not support the contention that the virus had infected placental tissue. Both the lymphocyte and the alleged virions were quite likely from maternal blood.

The authors also might have emphasized that maternal antibodies pass to the fetus as immunoglobulin; thus the fetus has positive serologic test results whether they screen with tests that are simple, cheap, and sensitive (enzyme-linked immunosorbent assay) or complex, expensive, and specific (Western blot). Thus we expect seropositivity in neonates with waning titers over time. A waxing titer would suggest neonatal infection, now anticipated in 50% of children born to infected women. In future reports it would be important to state whether the neonatal serologic tests are performed at birth or at intervals of 6 months or later to assist the reader in knowing whether the positive test results may be because of passive maternal antibody transfer.

The authors do not indicate whether they made any attempt to remove maternal blood from the placenta. (We are not aware of any technique in which this could be accomplished completely.) Therefore their conclusion that the positive test results were probably caused by maternal blood seems to be correct.

In this case the report would have been better titled "Maternal blood contaminates placenta and makes this organ infectious for whatever agents maternal blood may contain."

Jeffrey S. Greenspoon, MD
Robert Hurd Settlage, MD, MPH
Department of Obstetrics and Gynecology
Los Angeles County/University of Southern California Medical Center
Women's Hospital
Room 5-K-40,
1240 N. Mission Road
Los Angeles, CA 90033

REFERENCE

1. Hill WC, Bolton V, Carlson JR. Isolation of acquired immunodeficiency syndrome virus from the placenta. Am J Obstet Gynecol 1987;157:10-1.

Reply

To the Editors:

We appreciate the comments of Dr. Greenspoon with regard to our case report of the "Isolation of acquired immunodeficiency syndrome virus from the placenta." Dr. Greenspoon makes several valid points. However, the human immunodeficiency virus (HIV) was isolated from the placenta of this patient. Therefore, the case report was titled "Isolation of acquired immunodeficiency syndrome virus from the placenta." As we pointed out in our report, this was probably a result of the patient's positive HIV status. Because the maternal HIV antibodies are immunoglobulin G, they do always pass to the fetus. Therefore our neonate will initially have positive serologic test results. Over time, which appears to be 9 to 15 months, the titers may wane.

As pointed out by Dr. Greenspoon, in approximately 50% of children born to HIV-positive women, the neonate will have an HIV infection. The test performed in our neonate was at birth and was therefore positive. The infant showed no HIV antibodies at 8 months of age. Unfortunately, both parents have died from acquired immunodeficiency syndrome. The infant is now asymptomatic and 3 years old. The serologic status is HIV negative.

There was an attempt to remove maternal blood from the placenta; however, as Dr. Greenspoon indicated, this cannot be accomplished completely. Finally, it is important to remember that maternal blood does contaminate the placenta and makes this organ infectious for whatever agents maternal blood may contain. This includes HIV, hepatitis, or other infectious agents. As health care providers we should remember this fact and properly dispose of the placenta.

Washington C. Hill, MD
James R. Carlson, PhD
Veronica Bolton
Department of Obstetrics and Gynecology
Creighton University School of Medicine
601 N. 30th St., Suite 4810
Omaha, NE 68131

Race and gender factors omitted from results

To the Editors:

I read with interest the article by Goldstein et al. (Goldstein I, Lockwood C, Belanger K, Hobbins J. Ultrasonographic assessment of gestational age with the distal femoral and proximal tibial ossification centers in the third trimester. Am J Obstet Gynecol 1988; 158:127-30).

After the development of x-ray film, Pozier (1912)[1] and Adair and Scammon (1921)[2] studied the osseous development of the knee joint in newborn infants. From these studies it was determined that the distal femoral epiphysis usually did not appear until after the seventh lunar month, was present in 50% of the fetuses at 32 weeks' gestation, 84% at 36 weeks' gestation, and 95% at 40 weeks' gestation. The proximal tibial epiphysis appeared in the ninth lunar month and was present in 41% of the infants at 40 weeks' gestation.

In 1949 Christie,[3] who studied 1112 single born neonates, noted that the presence of the distal femoral epiphysis was correlated with birth weight, sex, and race. If the fetus was <2000 gm, distal femoral epiphyses were present in 9% of white males, 18% of black males, and 50% of white or black females. Between the weights of 2000 gm and 2499 gm, 75% of white males, 89% of black males, 92% of white females, and 94% of black females had distal femoral epiphyses present. If >3000 gm, 85% of white males, 91% of black males, 98% of white females, and 99% of black females had distal femoral epiphyses present.

Classical teaching stresses that there is greater maturity of female infants as compared with male infants, and of black infants as compared with white infants of equal weights.

It would appear that the results from the study by Goldstein and his co-workers would be more meaningful if the sex and race of the fetuses they studied were given. If there were no differences, this should be stated.

Rudi Ansbacher, MD, MS
Professor of Obstetrics and Gynecology
Department of Obstetrics and Gynecology
University of Michigan Medical Center
Ann Arbor, MI 48109-0718

REFERENCES

1. Pozier J. Etude radiographique de l'ossification du genou chez le nouveau-ne'. Paris: These, 1912.
2. Adair FL, Scammon RE. A study of the ossification centers of the wrist, knee, and ankle at birth, with particular reference to the physical development and maturity of the newborn. Am J Obstet Gynecol 1921;2:35-60.
3. Christie A. Prevalence and distribution of ossification centers in the newborn infant. Am J Dis Child 1949;77:355-61.

Reply

To the Editors:

My colleagues and I are grateful to Professor Ansbacher for his insightful comments on our study of the

appearance of the ossification centers during the third trimester. He is, of course, correct when he points out that traditionally there appear to be gender and race differences in fetal development. However, such findings were based on examinations by use of x-ray film. As the correspondent's references indicate, these examinations were of newborns.

Our methodology, on the other hand, was based on the use of high-resolution ultrasonographic technology and antepartum measurements. We attempted to determine fetal maturity on the basis of nonbiometric measurements. Previous investigators found no differentiation by race in the appearance of the proximal tibial and distal femoral ossification centers.[1] The purpose of our study was to measure the distal femoral and the proximal tibial ossification centers in the axial plane (medial lateral to the epiphysis) to maximize precision and minimize observer variation.

In addition to the ossification centers, there are other parameters that are indicative of fetal maturity. For example, echogenicity of the colon and peristalsis of the small intestine are useful in the prediction of gestational age.[2] Characterization of the nonbiometric measurement can improve the estimation of gestational age during the third trimester and it is advisable to use such assessments for clinical purposes.

Israel Goldstein, MD
Yale University School of Medicine
333 Cedar St.
P. O. Box 3333
New Haven, CT 06510

REFERENCES

1. Mahony BS, Callen PW, Filly RA. The distal femoral epiphyseal ossification center in the assessment of third-trimester menstrual age: sonographic indentification and measurement. Radiology 1985;155:201-4.
2. Goldstein I, Lockwood C, Hobbins JC. Ultrasound assessment of fetal intestinal development in the evaluation of gestational age. Obstet Gynecol 1987;70:682-6.

Candida warrants concern

To the Editors:

I would like to make a couple of comments concerning an article by Smith et al. (Intraamniotic infection with *Candida albicans* associated with a retained intrauterine contraceptive device: A case report. Am J Obstet Gynecol 1988;159:123-4). Their case report represents the usual reaction by clinicians who perform amniocentesis and find supposedly low virulence organisms that were unexpected. Our natural bias is that these organisms cannot be pathogenic and we tend to minimize their significance. This case, in which *Candida* was discovered at genetic amniocentesis, is an extension of data published by Bruner et al.[1] in which six cases of *Candida* chorioamnionitis diagnosed by amniocentesis were published.

Candida infection in those patients (three of six) was associated with an intrauterine contraceptive device as was the patient in the Smith et al. case report. *Candida* certainly is capable of crossing intact fetal membranes and causing intrauterine infection with potentially catastrophic consequences for the neonate. This case extends the data that we reported and confirms that *Candida albicans* can cause significant intrauterine infection and that perhaps a foreign body such as a retained intrauterine contraceptive device or cerclage may be key to the pathogenesis of this infection.

It is important for clinicians to realize that if *Candida* is discovered at the time of amniocentesis this represents a significant isolate and should be treated with the same concern that would be given to a bacterial isolate from amniotic fluid, either in early gestation or at the time of fetal viability.

John P. Elliott, MD
Associate Director, Maternal-Fetal Medicine
Good Samaritan Medical Center
1111 E. McDowell Road
Phoenix, AZ 85006

REFERENCE

1. Bruner JP, Elliott JP, Kilbride HW, et al. *Candida* chorioamnionitis diagnosed at amniocentesis with subsequent fetal infection. Am J Perinatol 1986;3:213.

Reply

To the Editors:

We greatly appreciate Dr. Elliott's interest in our case report and respond to his comments. At the time of our case report we were unaware of his and Dr. Bruner's extensive experience with *Candida* chorioamnionitis. After review of our case report and the expanding experience with this particular diagnosis we would agree that the appearance of *Candida albicans* on an amniocentesis specimen should be viewed with the same concern as any other bacterial isolate. It is our hope that this case report will inform clinicians of the rare but potentially serious complications associated with intrauterine contraceptive device and pregnancy outcome.

Carl V. Smith, MD
Department of Obstetrics and Gynecology
Division of Maternal-Fetal Medicine
University of Nebraska Medical Center
42nd and Dewey Ave.
Omaha, NE 68105-1065

ITEMS

Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60.00 U.S. per insertion and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, The C.V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318.

The First International Symposium for the Treatment of Post Surgical Adhesions. September 15-17, 1989, the Ritz-Carlton, Phoenix, Arizona. For further information/formal brochure, contact: Symposia Medicus, 2815 Mitchell Dr., Suite 128, Walnut Creek, CA 94598-1622. Tel.: (415) 935-7889.

American Uro-Gynecologic Society, Tenth Annual Meeting, October 4-7, 1989, Marriott's Camelback Inn, Scottsdale, Arizona. **Post Graduate course in Uro-Gynecology,** October 4, 1989. For further information contact: Jeanne Boyea, Office of the Secretary-Treasurer, c/o The University of Michigan Medical Center, Department of Ob/Gyn, 1500 E. Medical Center Dr., MPB D2239 0718, Ann Arbor, MI 48109. Tel.: (313) 764-8429.

Two Postgraduate Gynecology Courses: Comprehensive Review of Gyn for a Clinician, Sept. 12-15, 1989, and **Reproductive Endocrinology,** Sept 15-18, 1989, Massachusetts General Hospital, Boston, Massachusetts. For information contact: Harvard Medical School, Department of Continuing Education, 25 Shattuck St., Boston, MA 02115. Tel.: (617) 732-1525.

International Perinatal Doppler Society (I.P.D.S.), Paris, France, September 13-15, 1989. For more information contact: Dr. Ph. Arbeille, Secretary of the I.P.D.S., Unite INSERM 316, C.H.U. Bretonneau, 37044 Tours, France.

Update in Gynecologic Oncology: An International Perspective, October 16 and 17, 1989, Worcester, Massachusetts. Sponsored by the University of Massachusetts Medical School, Department of Obstetrics and Gynecology. For further information contact: Office of Continuing Education, UMMS, 55 Lake Avenue North, Worcester, MA 01655. Tel.: (508) 856-3041.

Hysteroscopy Workshop: A Didactic and Practical Hands-On Experience, November 10-11, 1989, Ambassador West Hotel, Chicago, Illinois. For registration fees and information, contact the Office of Continuing Medical Education, Northwestern University Medical School, 301 East Chicago Ave., Ward Building 1-004, Chicago, IL 60611. Tel.: (312) 908-8533.

The Fetus & Newborn: State of the Art Care, San Diego, California, October 11-13, 1989. For information contact: Contemporary Forums, 530 La Gonda Way, Suite E, Danville, CA 94526. Tel.: (415) 820-2800.

Developmental Interventions in Neonatal Care, New Orleans, Louisiana, October 4-6, 1989. For information contact: Contemporary Forums, 530 La Gonda Way, Suite E, Danville, CA 94526. Tel.: (415) 820-2800.

FACULTY POSITIONS IMMEDIATELY AVAILABLE

Maternal Fetal Medicine - Reproductive Endocrinology - Obstetrics/Gynecology - Urogynecology

The Department of Obstetrics and Gynecology at the University of South Dakota School of Medicine in Sioux Falls, South Dakota, a delightful city located directly on the Sioux River and serving a tri-state area and one of the best kept secrets in the Midwest, is building a full-time academic department. Qualified individuals to contribute, develop and provide leadership in all aspects of the specialty are actively being sought.

Candidates should have interests in clinical practice activities and medical education. Clinical and/or basic research opportunities are available and encouraged. An opportunity for a creative, motivated individual with a pioneer spirit.

Financial remuneration and incentives are competitive and commensurate with experience, credentials and rank. BC or BE. Position open until filled.

Please send Curriculum Vitae to: R. H. Wiebe, M.D., Professor and Chairman, Department of Obstetrics and Gynecology, University of South Dakota School of Medicine, 1100 South Euclid Avenue, Sioux Falls, SD 57117-5039, (605) 333-7327.

An Equal Opportunity/Affirmative Action Employer

FACULTY POSITION OB/GYN GENERAL SPECIALIST

The Knoxville Unit of The University of Tennessee College of Medicine is currently accepting applications for a General Specialist in the Department of Obstetrics and Gynecology at The University of Tennessee Medical Center at Knoxville.

This position requires board certification or eligibility in Obstetrics and Gynecology. Responsibilities include: resident and medical student education; participation in Regionalization Program; and clinical or basic research.

Specialized areas of interest such as benign GYN surgery and/or reproductive immunology are encouraged.

Academic rank is commensurate with both qualifications and experience.

Interested persons should sent curriculum vitae:

Michael R. Caudle, M.D.
Associate Professor and Chairman
Department of Obstetrics and Gynecology

THE UNIVERSITY OF TENNESSEE
Medical Center at Knoxville
1924 ALCOA HIGHWAY
KNOXVILLE, TENNESSEE 37920
EEO/An Affirmative Action/Title IX/Section 504 Employer

CHIEF OF MATERNAL FETAL MEDICINE UNIVERSITY OF TEXAS MEDICAL BRANCH AT GALVESTON

Faculty position in the Division of Maternal-Fetal Medicine in the Department of Obstetrics and Gynecology at the University of Texas Medical Branch at Galveston. A highly qualified individual is sought to become the Chief of the Maternal Fetal Medicine Division. Successful candidates should be board certified or eligible in maternal fetal medicine. They should have a proven record of research and commitment to an academic career. Academic rank is commensurate with qualification and experience. Interested candidates please send curriculum vitae to Garland D. Anderson, M.D., Professor and Chairman, Department of Obstetrics and Gynecology, University of Texas Medical Branch at Galveston, Galveston, Texas 77550.

UTMB is an equal opportunity M/F/H/V Affirmative Action employer. UTMB hires only individuals authorized to work in the United States.

Request for Participation in Maternal Autoimmune Thrombocytopenia Study

We have plans for a treatment study of Maternal Autoimmune Thrombocytopenia. Intravenous Gamma Globulin will be given at 32 + 37 weeks of pregnancy. This will be compared to a similar group receiving no gamma globulin. This study will be done on a randomized basis. History of well documented autoimmune thrombocytopenia prior to present pregnancy will be required for patient entry. Other therapy (steroids, C-section, treatment of infant) will be at local option. We are looking for 10+ centers to participate in such a study. Each center should be able to enroll 5 patients or more for 1 year. If interested in receiving protocol and specifics, please write Dr. William Krivit, Professor of Pediatrics, University of Minnesota Department of Pediatrics, 1252 Ingerson Road, St. Paul, MN 55112.

Immediate Opening
ASSISTANT OR ASSOCIATE PROFESSOR
MATERNAL-FETAL MEDICINE

The Department of Obstetrics & Gynecology, MacDonald Hospital for Women, University Hospitals of Cleveland, and Department of Reproductive Biology, Case Western Reserve University, is actively recruiting two full-time Perinatologists for its Division of Maternal-Fetal Medicine. The department of OB-GYN delivers approximately 3,000 infants per year, with a large number of high risk referrals.

Excellent opportunity to be involved in teaching, ultrasound and prenatal diagnosis including CVS, PUBS, and Fetal Echocardiography. Clinical and basic research are encouraged. There is a Board approved fellowship program. Board eligibility or certification in Maternal-Fetal Medicine is required. Interested candidates should send curriculum vitae to:

J. C. Veille, M.D.
Director, Maternal-Fetal Medicine
Department of Obstetrics & Gynecology
MacDonald Hospital for Women
University Hospitals of Cleveland
2074 Abington Road
Cleveland, OH 44106
Phone: 216-844-3338

The University is an equal opportunity/affirmative action employer.

MATERNAL-FETAL MEDICINE FACULTY POSITION

The Department of Obstetrics and Gynecology, University of Florida College of Medicine, under the direction of Byron J. Masterson, M.D., Professor and Chairman, is seeking applicants for one full-time, tenure accruing faculty position with the rank of Assistant Professor, Associate Professor or Professor for the Division of Maternal-Fetal Medicine.

Board eligibility or certification in maternal-fetal medicine is required. Appointment is contingent upon having a current Florida medical license.

Salary and rank commensurate with training and experience. Application deadline: 9/15/89. Anticipated starting date: 7/1/90.

Send curriculum vitae to:
Amelia C. Cruz, M.D.
Department of Obstetrics and Gynecology
J-294 JHMHC
University of Florida College of Medicine
Gainesville, Florida 32610

"An equal employment opportunity/affirmative action employer"

Obstetrician-gynecologist wanted to join a progressive and prosperous group. Outstanding opportunity for a well trained, board-eligible OB-GYN physician. This practice is strong in high-risk obstetrics, infertility, etc. Working out of a level III hospital. Send CV to:

Drs. Trabin, Manko, and Litt
1401 Forum Way #200
West Palm Beach, FL 33401
(407) 689-8780



OBSTETRICIAN-GYNECOLOGIST

Opening for qualified Obstetrician/Gynecologist in the Department of Ob/Gyn in a newly established affiliate clinic of a large multispecialty group in western Kentucky.

Competitive salary and attractive benefit package including malpractice insurance. Partnership eligibility in two years and no buy-in is required.

Opportunity to practice state-of-the-art medicine in a semi-rural setting. Large lakes nearby which offer recreational opportunities—metropolitan areas easily accessible. Community is family oriented with relaxed lifestyle not available in large cities.

Interested parties send curriculum vitae to:

Medical Director
TROVER CLINIC
Madisonville, Kentucky 42431
Phone: 502-825-7470

OBSTETRICS - GYNECOLOGY
Immediate Openings

Board certified/Board eligible faculty physicians for teaching hospital with residency program. Emphasis on general OB-GYN. 5,000 deliveries per year. Mid-sized urban community in Central California; moderate cost of living. Academic rank and salary commensurate with experience. Supplemental faculty practice plan. Send inquiries and C.V. to:

MEDICAL STAFF OFFICE
KERN MEDICAL CENTER
1830 Flower St.
Bakersfield, CA 93305

OB/GYN

46-physician multi-specialty group located 40 miles north of Sacramento seeks a physician to join our OB/GYN Department. Excellent compensation package the first year. Partnership opportunity thereafter. Contact:

Warren J. Boyer, Jr., M.D.
Marysville Medical Group
800 Third Street
Marysville, CA 95901



MARYSVILLE MEDICAL GROUP

Obstetrics/ Gynecology

WISCONSIN: Marshfield Clinic, a multi-specialty group practice with 300 physicians, is seeking BE/BC obstetrician/gynecologists to join expanding regional centers. Positions available in three locations: Two in northwestern Wisconsin within 90 miles of Minneapolis and one, a group practice of 25 physicians, in north central Wisconsin, about 1 1/2 hours from Lake Superior. Communities offer exceptional recreational, cultural, and educational opportunities. An emphasis on quality practice and life-style is combined with a total compensation package on reaching membership in excess of $200,000. Starting salary negotiable. No start-up expense. Send C.V. and references to:

David L. Draves
Director Regional Development

1000 North Oak Avenue
Marshfield, WI 54449

or call collect at (715) 387-5376



Marshfield Clinic

OBSTETRICS AND GYNECOLOGY WISCONSIN

We are looking for a Board Certified/ Board Eligible obstetrician/gynecologist to join a 7 physician OB/GYN Department in a large multi-specialty group practice located near Milwaukee, Wisconsin, to share coverage at two clinic locations and two hospitals. Competitive salary and fringe benefits.

Forward inquiries and CV to:

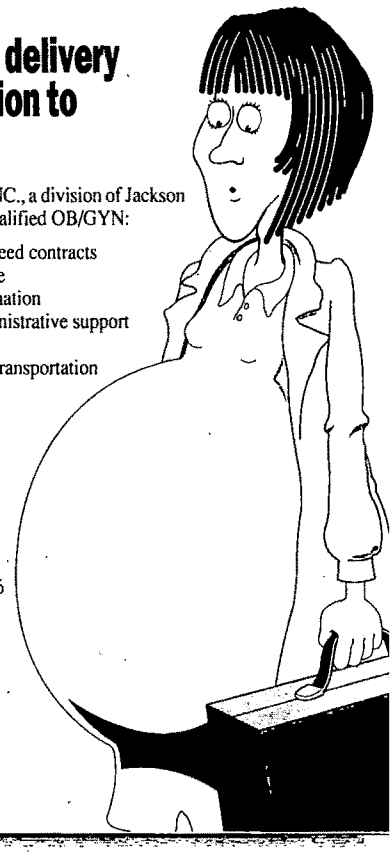
Administrator
P.O. Box 427
Menomonee Falls, WI 53051

Kentucky – Louisville — We have an immedite opening for a B.C./B.E. OB/GYN to provide in-house coverage. We are currently doing approximately 3500 deliveries per year. We are supported with N.I.C. high risk unit. Our unit is complemented by perinatology coverage. Also available are several private practice opportunities. Excellent salary and benefit package. Send C.V. and references to:

Humana Hospital Audubon

Bob Bowman
P.O. Box 17555
Louisville, KY 40217

Be in on the delivery of the solution to OB staffing.



LOCUM TENENS, INC., a division of Jackson & Coker, offers the qualified OB/GYN:

- 3 to 9 month guaranteed contracts
- competitive daily rate
- licensing and coordination
- knowledgeable administrative support
- round trip airfare
- on-site housing and transportation
- permanent practice opportunities

Call 1-800-544-1987
or write to:
Locum, Tenens, Inc.
400 Perimenter
Center Terrace
Suite 760 OGLT9
Atlanta, Georgia 30346

LOCUM TENENS, INC.
A Division of Jackson and Coker

CHIEF OF OB/GYN
BALTIMORE, MARYLAND

The Johns Hopkins Health Plan is recruiting a Chief for its OB/GYN Department consisting of six physicians and two midwives. Position involves recruiting, utilization review and quality assurance in addition to an active clinical practice in OB/GYN.

The Health Plan is affiliated with The Johns Hopkins School of Medicine and the succesful candidate will receive a clinical faculty appointment and have the opportunity for some teaching. We offer a competitive salary and comprehensive benefits, including paid malpractice.

Interested candidates should reply to: Medical Affairs, The Johns Hopkins Health Plan, 1000 E. Eager Street, Baltimore, MD 21202.

SPECIAL DELIVERIES. SPECIAL CHALLENGES.

The Southern California Permanente Medical Group invites you to join a unique prepaid health care program. Our multispecialty group practice is a partnership composed of and managed by SCPMG physicians.

Board eligible/certified **PERINATOLOGISTS** are needed for our high-volume obstetric services which handles 3,000-6,000 deliveries per year. Opportunities exist both to join and create high risk services.

There are positions available to practice Perinatology exclusively. Perinatologists who are interested in performing other aspects of obstetrics and gynecology may do so. We also have positions available for Endocrinologists and General Obstetrician-Gynecologists. University affiliation and clinical research are encouraged.

Our compensation is competitive and benefits are outstanding. They include: professional liability, medical and dental coverage, vacation and sick leave, continuing education, life insurance and retirement plans. **After two years full-time employment, physicians are eligible to be considered for partnership.**

For a physician application, please call or write to: William G. McCormick, M.D., Physician Recruitment, Dept. 006, Walnut Center, Pasadena, CA 91188-8013. (714) 829-5365.

Equal Opportunity Employer M/F/H

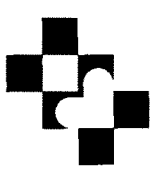
KAISER PERMANENTE
Good People. Good Medicine.

REPRODUCTIVE ENDOCRINOLOGIST

Fargo Clinic—MeritCare is seeking a BE/BC Reproductive Endocrinologist to join our staff of seven Obstetrician-Gynecologists in the Department of Ob-Gyn. Fargo Clinic is a 240 physician multi-specialty group practice located next to St. Luke's Hospital. St. Luke's is a 400 + bed level III hospital with a designated Children's Hospital. The department of Ob/Gyn is active in donor and husband insemination. Excellent opportunity exists to develop Invitro and GIFT Program.

Fargo-Moorhead is a tri-college community of 130,000. The sister cities and surrounding area offer a wide range of cultural and recreational activities.

Guaranteed first year salary with attractive long term compensatory arrangements. Reply to Robert C. Montgomery, M.D., 737 Broadway, Fargo, ND 58102 or call 1-800-437-4010 ext. 2151.



Fargo Clinic
MeritCare

Life.

It's our life's work.

If your mission in life is to practice the ideals of medicine without administrative demands, we invite your inquiry.

Group Health Plan in St. Louis is a staff model HMO that promotes the ideals of private practice while freeing doctors from the financial baggage of the private practitioner.

Group Health Plan isn't for every physician. Just for those few who want to practice medicine without compromise.

For an interview please talk with Tim Walton at (314) 993-5955. Or write Group Health Plan, 11475 Olde Cabin Road, St. Louis, Mo. 63141. An equal opportunity employer.

GROUP HEALTH PLAN

HARBOR-UCLA MEDICAL CENTER
DEPARTMENT OF OBSTETRICS AND GYNECOLOGY
TORRANCE, CALIFORNIA

FULL-TIME FACULTY POSITIONS

The Department of Obstetrics and Gynecology at the Harbor-UCLA Medical Center is seeking candidates for the following positions:

1. Chief of Gynecology
2. Maternal-Fetal Medicine
3. Perinatal Genetics
4. General Obstetrics and Gynecology

Applicants should be Board Certified or Active candidates. The positions offer a mix of resident and student teaching and supervision, direct patient care, opportunities and encouragement to carry out clinical/basic research, and clinical practice. Academic rank commensurate with qualifications and experience. To apply send a copy of resume or for further information, please contact:

Charles R. Brinkman, III, M.D.
Professor, Chairman and Program Director
Harbor-UCLA Medical Center-Box 3
1000 West Carson Street
Torrance, California 90509
(213) 533-3561

MATERNAL-FETAL MEDICINE ASHEVILLE, NORTH CAROLINA

The University of North Carolina Department of OB/GYN and the Mountain Area Health Education Center has a full time faculty position open in Asheville, NC.

Assistant/Associate Professor: Individual should be experienced in prenatal diagnosis to include ultrasonography, fetal therapy and counseling with experience in management of high risk pregnancy. Candidates should have demonstrated abilities in clinical practice, teaching and research and have completed fellowship training in Maternal-Fetal Medicine.

The salary and rank are commensurate with experience and training. To apply send CV to:

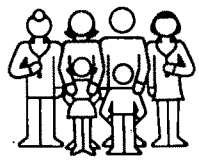
Tom Bacon, DrPH
Chairman, Search Committee
Mountain Area Health Education Center
501 Biltmore Avenue
Asheville, NC 28801

MAHEC and the University of North Carolina is an Affirmative Action Equal Opportunity Employer.

OBSTETRICS & GYNECOLOGY

Obstetrician/Gynecologist needed to join a five physician department at a major regional multispecialty clinic in Billings, Montana. Clinic setting includes all specialties and is adjacent to an outstanding tertiary care referral hospital. Excellent opportunity for a top notch board eligible or board certified Obstetrican/Gynecologist looking for a challenging practice in a geographic area offering unlimited recreational potential. Those interested send CV to:

John W. Heizer, M.D.,
Medical Director
The Billings Clinic
P.O. Box 35100
Billings, Montana 59107-5100



Central Minnesota
Group Health Plan

OB/GYN

Rapidly growing, innovative primary care clinic has openings for two board eligible/certified OB/GYN physicians. New clinic facility in the fastest growing urban area in Minnesota. St. Cloud is a community of 45,000 located on the upper Mississippi River 70 miles from Mpls./St. Paul. Be close to lakes, ski areas and excellent schools, including three universities. Join a supportive primary care staff and receive competitive salary, outstanding benefits, liberal vacation and educational leave, malpractice coverage, etc. Contact Physician Services, Central Minnesota Group Health Plan, 1245 15th Street North, St. Cloud, MN 56303. 612/253-5220. EOE.



Center for *Women's* Health
Opportunity Available
GYNECOLOGIST
Kalamazoo, Michigan

Bronson Methodist Hospital Center for Women's Health is actively seeking a second board eligible/board certified gynecologist.

- Practice quality gynecologic care in a relaxed atmosphere
- On-site mammography
- PMS, post-mastectomy and menopause programs
- Dedicated staff of nurse practitioners, nutritionists and counselors
- Affiliation with Michigan State University College of Human Medicine

Kalamazoo, located midway between Detroit and Chicago, has a metropolitan population of 220,000. Residents enjoy a high quality life style:

- Excellent schools, four colleges and a major university
- Theatre, art, symphony and ballet
- Lake Michigan is 30 miles away; many inland lakes
- Full spectrum of sporting activities
- Outdoor recreation accentuated by Michigan's four seasons

For more information about this practice opportunity contact:
Debra C. Hartman
Bronson Management Services Corporation
210 East Vine Street
Kalamazoo, Michigan 49001
(616) 344-6444 (collect)

DIRECTOR OF PERINATOLOGY SERVICES

The Irvine Medical Center, located in Irvine, California, is seeking a full-time, Board Certified Perinatologist to oversee the perinatology services of a 177-bed hospital with a current Medical Staff of 600 physicians. The Irvine Medical Center is currently under construction and is the only hospital in Irvine, a rapidly growing, well established community located in Orange County. The Perinatology Director will have the opportunity to develop the perinatology services for the Medical Center complex.

Please send CV to: Perinatology Search Committee, Irvine Medical Center, 4605 Barranca Parkway, Suite 200, Irvine, CA 92714.

INDEX TO ADVERTISERS

Bay Shores Medical Group
Opportunity Available 43A
Berlex Laboratories, Inc.
Tri-Levlen 30A,31A,32A
Billings Clinic, The
Opportunity Available 54A
Bronson Management Services Corporation
Opportunity Available 54A
Central Minnesota Group Health Plan
Opportunity Available 54A
Current Science
Journal 29A
Fargo Clinic
Opportunity Available 53A
Group Health Plan
Opportunity Available 53A
Harbor-UCLA Medical Center
Opportunity Available 53A
Humana Hospital Audubon
Opportunity Available 52A
Irvine Medical Center
Opportunity Available 54A
Johns Hopkins Health Plan
Opportunity Available 52A
Kaiser Permanente
Opportunity Available 52A
Kern Medical Center
Opportunity Available 51A
Locum Tenens, Inc.
Opportunity Available 52A
Marshfield Clinic
Opportunity Available 51A
Marysville Medical Group
Opportunity Available 51A
Medical College of Georgia
Opportunity Available 42A
Medical West
Opportunity Available 22A
Michael Reese Health Plan
Opportunity Available 22A
Mountain Area Health Education Center
Opportunity Available 53A
Ohio Permanente Medical Group, Inc.
Opportunity Available 20A
Ortho Pharmaceutical Corporation
All-Flex 4A
Ortho-Novum 7/7/7 ... Third Cover,Fourth Cover
Osler Institute, The
Review Course 56A
Pennsylvania Hospital
Opportunity Available 20A
Permanente Medical Group, Inc.
Opportunity Available 41A
Roerig
Unasyn 19A,20A,21A,22A,23A
Rose Medical Center
Opportunity Available 41A
Searle & Company
Demulen 1/35 ..... 33A,34A,35A,36A
Serono Laboratories, Inc.
Serophene 6A
St. John Hospital
Opportunity Available 41A
Stackhouse
PT 1000 ..... 42A,43A
Syntex Laboratories, Inc.
Tri-Norinyl ..... Second Cover,1A,2A
2V Medtech International Corp.
2V STAT 45A
Trabin, Manko, and Litt
Opportunity Available 50A
Trover Clinic
Opportunity Available 50A
University Hospitals of Cleveland
Opportunity Available 41A,50A
University of Florida College of Medicine
Opportunity Available 50A
University of Minnesota
Opportunity Available 49A
University of South Dakota School of Medicine
Opportunity Available 49A
University of Tennessee Medical Center at Knoxville
Opportunity Available 45A,49A
University of Texas Medical Branch at Galveston
Opportunity Available 49A
University of Wisconsin Medical School
Opportunity Available 45A
West Penn Hospital
Opportunity Available 45A
Wyeth-Ayerst Laboratories
Lo/Ovral 46A,47A,48A
Triphasil 37A,38A,39A,40A
Carl Zeiss, Inc.
Colposcope 1-FC 3A

While every precaution is taken to insure accuracy, we cannot guarantee against the possibility of an occasional change or omission in the preparation of this index.

The Obstetrics and Gynecology Review Course

*November 27-December 6, 1989 — Chicago
Spring 1990 — Site to be Chosen

Now, your comprehensive review of basic and clinical science

OBJECTIVES:
- Increase basic knowledge and clinical skills in ob-gyn.
- Assist residents and fellows to organize study
- Prepare candidates to enter their specialty
- Provide practicing gynecologists with an update

METHODS:
- HOME STUDY MATERIALS with questions and answers
- SEMINAR with projection slides and syllabus
- LABORATORY with microscopic slides
- PRACTICE EXAMS with oral and written parts

*Any six days will complete the program. 8:00 AM to 9:30 PM Nov. 27-Dec. 2 (live); Dec. 3-6 (Video replay of Nov. 27-30).

*"The faculty was outstanding. The most pleasant thing was learning a tremendous amount, not only from world-famous authorities but from people who are relatively unknown as well."**

TOPICS

Clinical Science
- Surgical Anatomy
- Embryology
- Radiology
- Genetics
- Teratology
- Anesthesiology
- Antibiotics

Pathology
- Vulva
- Vagina
- Cervix
- Endometrium
- Myometrium
- Ovary
- Placenta

Oncology
- Chemotherapy
- Vulva and Vagina
- Cervix
- Uterus
- Ovary
- Trophoblast
- Breast

Benign Gynecology
- Infectious Diseases
- Urinary Retention
- Urinary Incontinence
- Pediatric Gynecology
- Sexual Assault
- Ectopic Pregnancy
- Endometriosis

Reproductive Endo.
- Contraception
- Infertility
- Amenorrhea
- Abnormal Bleeding
- Androgen
- Prolactin
- Menopause

Maternal and Fetal Med.
- Pharmacology
- Diabetes
- Hypertension
- Hematology
- Antepartum Testing
- Premature Labor
- Birth Trauma

Faculty

Guy Benrubi, M.D.
University of Florida

Phillip Galle, M.D.
Southern Illinois University

Raymond Glassenberg, M.D.
Northwestern University

Edward Hill, M.D.
Univ. of California, San Francisco

Allan Jacobs, M.D.
St. Louis University

Victoria Maclin, M.D
Rush Medical College

Edward Marut, M.D.
University of Chicago

Roy Petrie, M.D.
Washington University

Gerald Quirk, M.D.
University of Arkansas

William Roddick, M.D.
Southern Illinois University

Joseph Sanfilippo, M.D.
University of Louisville

Robert Scully, M.D.
Harvard University

Gary Shangold, M.D.
University of Chicago

Marion Verp, M.D.
University of Chicago

Richard Voet, M.D.
University of Texas, Southwestern

Wendell Wall, Jr., M.D.
University of North Dakota

Limited Enrollment: **OBSTETRICS AND GYNECOLOGY REVIEW REGISTRATION**

Name _____

Address _____

City/State/Zip _____

Phone _____

For:
- ☐ Nov. 27-Dec. 6, 1989 — Chicago
- ☐ Spring 1990 — Site to be Chosen
- ☐ Fall 1990 — Site to be Chosen
- ☐ Check enclosed for $_____
- ☐ Please send more information

Mail today to:

1094 Dawn Lane, Dept. A8
P.O. Box 2218
Terre Haute, IN 47802

*"Accommodations were comfortable...."**

GOALS AND LOCATION: This course for residents and practicing gynecologists is a comprehensive review and update offered in the spring and fall with the fall course more clinically oriented. The meeting hotels will be the best combination of good study environment and bargain rates. Our experience shows that patient negotiation gives our participants the best value. Please wait for instruction before buying travel tickets.

*"...and those little extras...."**

LOWEST AIR FARES: Please call toll-free 1-800-548-8185 and identify yourself with this Osler course.

*"...remarkably complete and pleasant."**

PLACEMENT SERVICE: For a practice opportunity write or call 1-800-356-7537.

*"...the most education for the money."**

FEES AND C.M.E. CREDITS:
- **C.M.E. Credit: 60 Hours**
- Practicing Gynecologists: $660
- Residents and Fellows: $440
- Repeating seminar within 3 years: half price
- Optional extra day, Dec. 3 (more questions, answers and pathology laboratory): $150
- **Add 10% within 10 days of the course.**
- Attendees not in course hotel add $8/day.
- Fees do not include lodging or meals.
- Deposit of $50.00 will reserve your position.
- Most home study materials will be mailed after half the registration fee is received.

*"...home study material was extremely helpful."**

REFUNDS: Subject to a $50 fee, refunds will be made until the seminar begins.
- Cancellation after mailing home study material requires retention of half the fee.

*"I feel [the course] helped me pass...."**

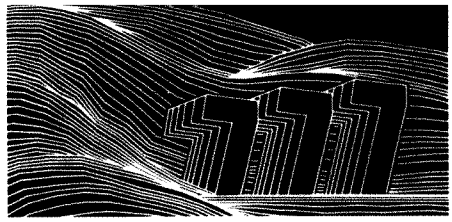
INFORMATION:
Joseph H. Selliken, Jr., M.D.
THE OSLER INSTITUTE
1094 Dawn Lane, P.O. Box 2218
Terre Haute, IN 47802
(800) 356-7537 or (812) 299-5658

* *Comments by participants*

21- and 28-day regimens

ORTHO-NOVUM® 777 Tablets

norethindrone/ethinyl estradiol



Each white tablet contains 0.5 mg norethindrone and 0.035 mg ethinyl estradiol. Each light peach tablet contains 0.75 mg norethindrone and 0.035 mg ethinyl estradiol. Each peach tablet contains 1 mg norethindrone and 0.035 mg ethinyl estradiol. Each green tablet in 28-day regimen contains inert ingredients.

IMPORTANT NOTE—This information is a BRIEF SUMMARY of the complete prescribing information provided with the product and therefore should not be used as the basis for prescribing the product. This summary was prepared by deleting from the complete prescribing information certain text, tables and references. The physician should be thoroughly familiar with the complete prescribing information before prescribing the product.

INDICATIONS AND USAGE: PREVENTION OF PREGNANCY.

CONTRAINDICATIONS: Oral contraceptives should not be used in women who currently have the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Carcinoma of the endometrium or other known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Cholestatic jaundice of pregnancy or jaundice with prior pill use. 8. Hepatic adenomas or carcinomas. 9. Known or suspected pregnancy.

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia, and gallbladder disease, although the risk of serious morbidity or mortality is very small in healthy women without underlying risk factors. The risk of morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, obesity and diabetes. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks. The information contained in this brief summary is principally based on studies carried out in patients who used oral contraceptives with higher formulations of estrogens and progestogens than those in common use today. The effect of long term use of the oral contraceptives with lower formulations of both estrogens and progestogens remains to be determined. Throughout this brief summary epidemiological studies reported are of two types: retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of a disease, namely a *ratio* of the incidence of a disease among oral contraceptive users to that among nonusers. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide a measure of attributable risk, which is the *difference* in the incidence of disease between oral contraceptive users and nonusers. The attributable risk does provide information about the actual occurrence of a disease in the population. For further information, the reader is referred to a text on epidemiological methods. 1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. a. Myocardial Infarction. An increased risk of myocardial infarction has been associated with oral contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary artery disease such as hypertension, hypercholesterolemia, morbid obesity, and diabetes. The relative risk of heart attack for current oral contraceptive users has been estimated to be two to six. The risk is very low under the age of 30. Smoking in combination with oral contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women in their mid-thirties or older with smoking accounting for the majority of excess cases. Mortality rates associated with circulatory disease have been shown to increase substantially in smokers, especially in those 36 years of age and older among women who use oral contraceptives. Oral contraceptives may compound the effects of well-known risk factors, such as hypertension, diabetes, hyperlipidemias, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and cause glucose intolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see section 9 in WARNINGS). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors. b. Thromboembolism. An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to non-users to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. Cohort studies have shown the relative risk to be somewhat lower, about 3 for new cases and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease associated with oral contraceptives is not related to length of use and disappears after pill use is stopped. A two- to four-fold increase in relative risk of post-operative thromboembolic complications has been reported with the use of oral contraceptives. The relative risk of venous thrombosis in women who have predisposing conditions is twice that of women without such medical conditions. If feasible, oral contraceptives should be discontinued at least four weeks prior to and for two weeks after elective surgery of a type associated with an increase in risk of thromboembolism and during and following prolonged immobilization. Since the immediate postpartum period is also associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than four weeks after delivery in women who elect not to breast feed. c. Cerebrovascular diseases. Oral contraceptives have been shown to increase both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes), although, in general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and non-users, for both types of strokes, and smoking interacted to increase the risk of stroke. In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for nonsmokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk is also greater in older women. d. Dose-related risk of vascular disease from oral contraceptives. A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high density lipoproteins (HDL) has been reported with many progestational agents. A decline in serum high density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the activity of the progestogen used in the contraceptive. The activity and amount of both hormones should be considered in the choice of an oral contraceptive. Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral contraceptive agents should be started on preparations containing 0.035 mg or less of estrogen. e. Persistence of risk of vascular disease. There are two studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40-49 years who had used oral contraceptives for five or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. However, both studies were performed with oral contraceptive formulations containing 50 micrograms or higher of estrogens. 2. ESTIMATES OF MORTALITY FROM CONTRACEPTIVE USE. One study gathered data from a variety of sources which have estimated the mortality rate associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is low and below that associated with childbirth. However, smokers 35 and older and non-smokers 40 and older who use oral contraceptives have a significant increase in mortality higher than those using other methods of birth control. These facts must be weighed in conjunction with failure rates for other methods and the risk associated with subsequent pregnancy. 3. CARCINOMA OF THE REPRODUCTIVE ORGANS. Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. While there are conflicting reports the overall evidence in the literature suggests that use of oral contraceptives is not associated with an increase in the risk of developing breast cancer. The Cancer and Steroid Hormone (CASH) study also showed no latent effect on the risk of breast cancer for at least a decade following long term use. Some studies have shown a slightly increased relative risk of developing breast cancer. Some studies suggest that oral contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors. In spite of many studies of the relationship between oral contraceptive use and breast and cervical cancers, a cause and effect relationship has not been established. 4. HEPATIC NEOPLASIA. Benign hepatic adenomas are associated with oral contraceptive use, although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases/100,000 for users, a risk that increases after four or more years of use especially with oral contraceptives of higher dose. Rupture of benign hepatic adenomas may cause death through intra-abdominal hemorrhage. Studies from Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (>8 years) oral contraceptive users. However, these cancers are rare in the U.S. and the attributable risk (the excess incidence) of liver cancers in oral contraceptive users approaches less than one per million users. 5. OCULAR LESIONS. There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately. 6. ORAL CONTRACEPTIVE USE BEFORE OR DURING EARLY PREGNANCY. RISK OF BIRTH DEFECTS. Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. The majority of recent studies also do not indicate a teratogenic effect, particularly in so far as cardiac anomalies and limb reduction defects are concerned, when taken inadvertently during early pregnancy. The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion. It is recommended that for any patient who has missed two consecutive periods pregnancy should be ruled out before continuing oral contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral contraceptive use should be discontinued until pregnancy is ruled out. 7. GALLBLADDER DISEASE. Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral contraceptive formulations containing lower hormonal doses of estrogens and progestogens. 8. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. Oral contraceptives have been shown to cause a decrease in glucose tolerance in a significant percentage of users. This effect has been shown to be directly related to estrogen dose. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the non-diabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects prediabetic and diabetic women in particular should be carefully monitored while taking oral contraceptives. A small proportion of women will have persistent hypertriglyceridemia while on the pill. As discussed earlier (see WARNINGS 1a and 1d), changes in serum triglycerides and lipoprotein levels have been reported in oral contraceptive users. 9. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in women taking oral contraceptives, and this increase is more likely in older oral contraceptive users and with extended duration of use. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing progestational activity. Women with a history of hypertension or hypertension-related diseases, or renal disease, should be encouraged to use another method of contraception. If women elect to use oral contraceptives, they should be monitored closely and if significant elevation of blood pressure occurs, oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives, and there is no difference in the occurrence of hypertension between former and never users. 10. HEADACHE. The onset or exacerbation of migraine or development of headache with a new pattern which is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause. 11. BLEEDING IRREGULARITIES. Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first three months of use. Non-hormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out. Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was preexistent. 12. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. **PRECAUTIONS:** 1. PHYSICAL EXAMINATION AND FOLLOW UP. A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology, and relevant laboratory tests. In case of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care. 2. LIPID DISORDERS. Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult. 3. LIVER FUNCTION. If jaundice develops in any woman receiving such drugs, the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function. 4. FLUID RETENTION. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention. 5. EMOTIONAL DISORDERS. Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 6. CONTACT LENSES. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist. 7. DRUG INTERACTIONS. Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar association, though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines. 8. INTERACTIONS WITH LABORATORY TESTS. Certain endocrine and liver function tests and blood components may be affected by oral contraceptives: a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. b. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radio-immunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. c. Other binding proteins may be elevated in serum. d. Sex-binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged. e. Triglycerides may be increased. f. Glucose tolerance may be decreased. g. Serum folate levels may be depressed by oral contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives. 9. CARCINOGENESIS. See WARNINGS section. 10. PREGNANCY. Pregnancy Category X. See CONTRAINDICATIONS and WARNINGS sections. 11. NURSING MOTHERS. Small amounts of oral contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child. **INFORMATION FOR THE PATIENT: See Patient Package Insert. ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS section): Thrombophlebitis and venous thrombosis with or without embolism. Arterial thromboembolism. Pulmonary embolism. Myocardial infarction. Cerebral hemorrhage. Cerebral thrombosis. Hypertension. Gallbladder disease. Hepatic adenomas or benign liver tumors. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea. Vomiting. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Amenorrhea. Temporary infertility after discontinuation of treatment. Edema. Melasma which may persist. Breast changes: tenderness, enlargement, secretion. Change in weight (increase or decrease). Change in cervical erosion and secretion. Diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted: Pre-menstrual syndrome. Cataracts. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. Acne. Changes in libido. Colitis. **OVERDOSAGE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea and withdrawal bleeding may occur in females.

Revised 11/88

ORTHO PHARMACEUTICAL CORPORATION
Raritan, NJ 08869-0602

ORTHO

Low rates of breakthrough bleeding and nuisance side effects†

High levels of patient satisfaction



21- and 28-day regimens

ORTHO-NOVUM® 777 Tablets

norethindrone/ethinyl estradiol

†Serious as well as minor side effects have been reported with the use of oral contraceptives. The physician should remain alert to the earliest manifestations of any symptoms of serious disease and discontinue oral contraceptive therapy when appropriate.

Please see brief summary of complete Prescribing Information on the preceding page.

ORTHO PHARMACEUTICAL CORPORATION
Raritan, New Jersey 08869-0602

© OPC 1988

OJ-376R

11. 12. 90

September 1989
volume 161, *number* 3

UCM

American Journal of OBSTETRICS AND GYNECOLOGY

Copyright © 1989 by The C.V. Mosby Company

Editor in Chief
JOHN I. BREWER

Editors
FREDERICK P. ZUSPAN • E. J. QUILLIGAN

Associate Editor
ALBERT B. GERBIE

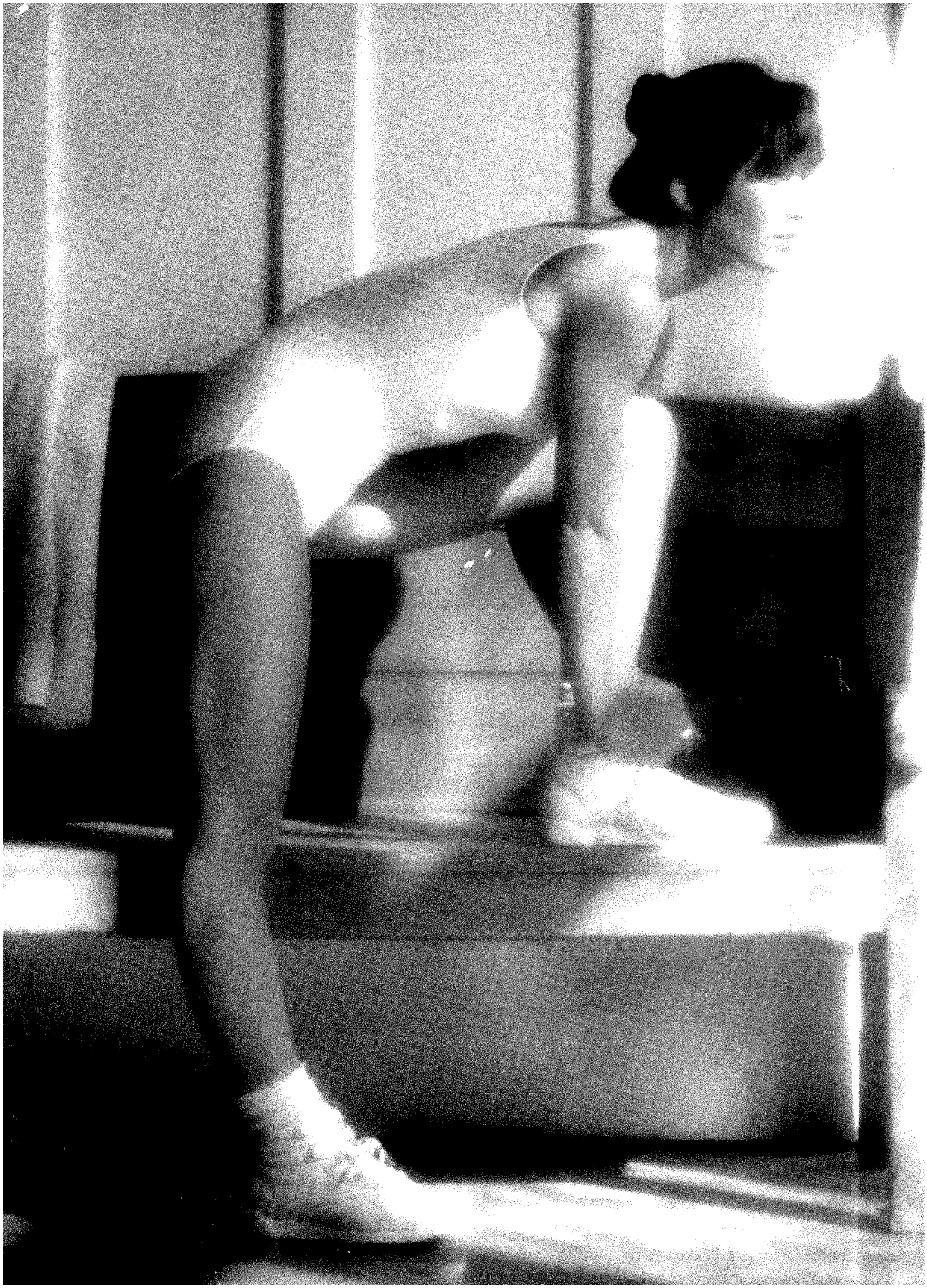
Official Publication

AMERICAN GYNECOLOGICAL AND OBSTETRICAL SOCIETY
CENTRAL ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS
SOUTH ATLANTIC ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS
PACIFIC COAST OBSTETRICAL AND GYNECOLOGICAL SOCIETY
AMERICAN BOARD OF OBSTETRICS AND GYNECOLOGY
SOCIETY FOR GYNECOLOGIC INVESTIGATION
SOCIETY OF PERINATAL OBSTETRICIANS

Published by

THE C.V. MOSBY COMPANY
St. Louis, Missouri 63146-3318

ISSN 0002-9378





Even at midcycle, she wears white with confidence

Only Tri-Norinyl provides a full 1 mg of progestin at the midcycle (days 8–16), when clinical data indicate that breakthrough bleeding and spotting are most likely to occur.[1]

To help control midcycle breakthrough bleeding: make the midcycle the high point of protection.

Extra midcycle progestin to reduce midcycle breakthrough bleeding

9 DAYS
1.0 mg norethindrone

7 DAYS
0.5 mg norethindrone

5 DAYS
0.5 mg norethindrone

21 DAYS *0.035 mg ethinyl estradiol*

TRI-NORINYL®

(NORETHINDRONE AND ETHINYL ESTRADIOL) TABLETS

1. Data available from Syntex Laboratories, Inc., document #92363.

Serious as well as minor side effects have been reported following the use of all oral contraceptives. These include thromboembolic disease. Before prescribing Tri-Norinyl, please see brief summary of prescribing information on following page.

© 1989 Syntex Laboratories, Inc.

SYNTEX

SYNTEX LABORATORIES, INC.
PALO ALTO, CA 94304

TRI-NORINYL®

(NORETHINDRONE AND ETHINYL ESTRADIOL) TABLETS

TRI-NORINYL® 21-Day Tablets (7 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg, 9 green tablets of norethindrone 1.0 mg with ethinyl estradiol 0.035 mg, 5 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg)
TRI-NORINYL® 28-Day Tablets (7 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg, 9 green tablets of norethindrone 1.0 mg with ethinyl estradiol 0.035 mg, 5 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg; 7 inert tablets)
BREVICON® 21-Day Tablets (norethindrone 0.5 mg with ethinyl estradiol 0.035 mg)
BREVICON® 28-Day Tablets (21 norethindrone 0.5 mg with ethinyl estradiol 0.035 mg tablets followed by 7 inert tablets)
NORINYL® 1 + 35 21-Day Tablets (norethindrone 1 mg with ethinyl estradiol 0.035 mg)
NORINYL® 1 + 35 28-Day Tablets (21 norethindrone 1 mg with ethinyl estradiol 0.035 mg tablets followed by 7 inert tablets)
NORINYL® 1 + 50 21-Day Tablets (norethindrone 1 mg with mestranol 0.05 mg)
NORINYL® 1 + 50 28-Day Tablets (21 norethindrone 1 mg with mestranol 0.05 mg tablets followed by 7 inert tablets)
NORINYL® 1 + 80 21-Day Tablets (norethindrone 1 mg with mestranol 0.08 mg)
NORINYL® 1 + 80 28-Day Tablets (21 norethindrone 1 mg with mestranol 0.08 mg tablets followed by 7 inert tablets)
NORINYL® 2 mg Tablets (norethindrone 2 mg with mestranol 0.1 mg)
NOR-Q.D.® (norethindrone) Tablets 0.35 mg

ORAL CONTRACEPTIVE (O.C.) AGENTS

Indications Prevention of pregnancy. *DOSE-RELATED RISK OF THROMBOEMBOLISM.* Because studies have shown a positive association between OC estrogen dose and risk of thromboembolism, it is prudent to minimize estrogen exposure. Prescribe an OC with the least amount of estrogen compatible with an acceptable pregnancy rate and patient acceptance. Start new users on OCs containing 0.05 mg or less of estrogen.

Contraindications 1. Known or suspected pregnancy (see Warning #5). 2. Thrombophlebitis or thromboembolic disorders. 3. Past history of deep vein thrombophlebitis or thromboembolic disorders. 4. Undiagnosed abnormal genital bleeding. 5. OCs should not be used by women who have or have had any of the following: a. cerebral vascular or coronary artery disease, including myocardial infarction. b. known or suspected carcinoma of the breast. c. known or suspected estrogen dependent neoplasia. d. benign or malignant liver tumor that developed during use of OCs or other estrogen containing products.

WARNINGS: Cigarette smoking increases the risk of serious cardiovascular side effects from OC use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use OCs should be strongly advised not to smoke.

The use of OCs is associated with increased risk of several serious conditions including thromboembolism, stroke, myocardial infarction, liver tumor, gall bladder disease, visual disturbances, fetal abnormalities, and hypertension. Practitioners prescribing OCs should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with OC use is established. One study demonstrated an increased relative risk for fatal venous thromboembolism and several studies demonstrated it for non-fatal venous thromboembolism. They estimate that OC users are 4-11 times more likely than nonusers to develop these diseases without evident cause. One British study reported an excess death rate of 40% in OC users, most of which resulted from cardiovascular disease. Another British study showed a lower death rate in OC users than controls; an increase in cardiovascular deaths was seen but was not statistically significant. A U.S. prospective study failed to disclose increased mortality rates from cardiovascular disorders, but a subset analyzed as a retrospective, case-control study showed significant increases in venous thromboembolism. CEREBROVASCULAR DISORDERS. Two American studies demonstrated an increased relative risk for stroke not shown in prior British studies. In an American study of cerebrovascular disorders in women with and without predisposing causes, relative risk of hemorrhagic stroke was estimated as 2.0 times greater and thrombotic stroke as 4-9.5 times greater in users than nonusers. A British long-term follow-up study reported in 1976 a highly significant association between OC use and stroke. Another study had suggested an association in 1974, but the number of cases was too small to estimate the risk. Subarachnoid hemorrhage has been shown to be increased by OC use in British and American studies. Smoking alone increases incidence of these accidents; smoking and pill use appear to increase risk more than either alone.

MYOCARDIAL INFARCTION (MI). Increased relative risk of MI associated with OC use has been reported. One British study found that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing MI, regardless of OC use. OCs were an additional risk factor. In terms of relative risk, it has been estimated that nonsmoking OC users (smoking is considered a major predisposing condition to MI) are twice as likely to have a fatal MI as nonsmoking nonusers. OC users who are smokers have a 5-fold increased risk of fatal infarction compared to nonsmoking users, and a 10-12-fold increased risk compared to nonsmoking nonusers. The number of cigarettes smoked is important. In determining the importance of these relative risks, baseline rates for various age groups must be considered. (Estimates are based on British vital statistics which show acute MI death rates 2-3 times less than in the U.S. so U.S. death rates could be higher.) Importance of other predisposing conditions in determining relative and absolute risks has not been quantified; other synergistic actions may exist. RISK OF DOSE. Using data from several national adverse reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to estrogen dose in OCs. OCs with 0.1 mg or more of estrogen were associated with a higher risk of thromboembolism than those containing 0.05-0.08 mg but quantity of estrogen may not be the sole factor. This was supported by a U.S. study. A British study found a positive association between dose of progestogen or estrogen and certain thromboembolic conditions. Swedish authorities noted decreased reporting of thromboembolic episodes when higher estrogen preparations were no longer prescribed. Careful epidemiological studies to determine degree of thromboembolic disease risk associated with progestogen-only OCs have not been done. Thromboembolic disease has been reported in women using these products, and they should not be considered free of excess risk. PERSISTENCE OF RISK. Two studies have suggested an increased risk may persist for 6 years after discontinuation of OC use for cerebrovascular disease and 9 years for MI; another study suggested the persistence of risk for subarachnoid hemorrhage. ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES. A large British prospective study estimated mortality rate per 100,000 women per year from circulatory system diseases for OC users and nonusers according to age, smoking habits, and duration of use. The overall annual excess death rate for OC users was estimated to be 20/100,000 (ages 15-34–5/100,000; ages 35-44–33/100,000; ages 45-49–140/100,000). Risk is concentrated in long-term users and in smokers, and may persist after OC discontinuation. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all occurred in women 35 or older. An update provided the following rates: ages 15-34–1/6700 for nonsmokers and 1/2000 for smokers; ages 45 and over–1/2500 for nonsmokers and 1/500 for smokers. Risk appeared to increase with parity, but not with duration of use. Until more women under 35 with continuous use for 5 or more years are available, it is not possible to assess relative risk for this age group. Data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. Estimates include combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease for OCs) plus risk attributable to pregnancy or abortion if the method fails (which varies with the effectiveness of the contraceptive method). The study concluded that mortality associated with all contraceptive methods is below that of childbirth, except for OCs in women over 40 who smoke. (Rates given for pill only/smokers for each age are for smokers as a class. For "heavy" smokers (more than 15 cigarettes a day), the rates would be about double; for "light" smokers (less than 15), about half.) The lowest mortality is with the condom or diaphragm backed up by early abortion. The study also concluded that OC users who smoke, especially over 30, have greater mortality risk than OC users who do not smoke.

Risk of thromboembolic and thrombotic disease associated with OCs increases with age after 30 and for MI is further increased by hypertension, hyperlipidemias, obesity, diabetes, or history of preeclamptic toxemia, and especially by smoking. The following chart gives a gross estimate of risk of death from circulatory disorders associated with OC use.

SMOKING HABITS AND OTHER PREDISPOSING CONDITIONS–RISK ASSOCIATED WITH USE OF OCs

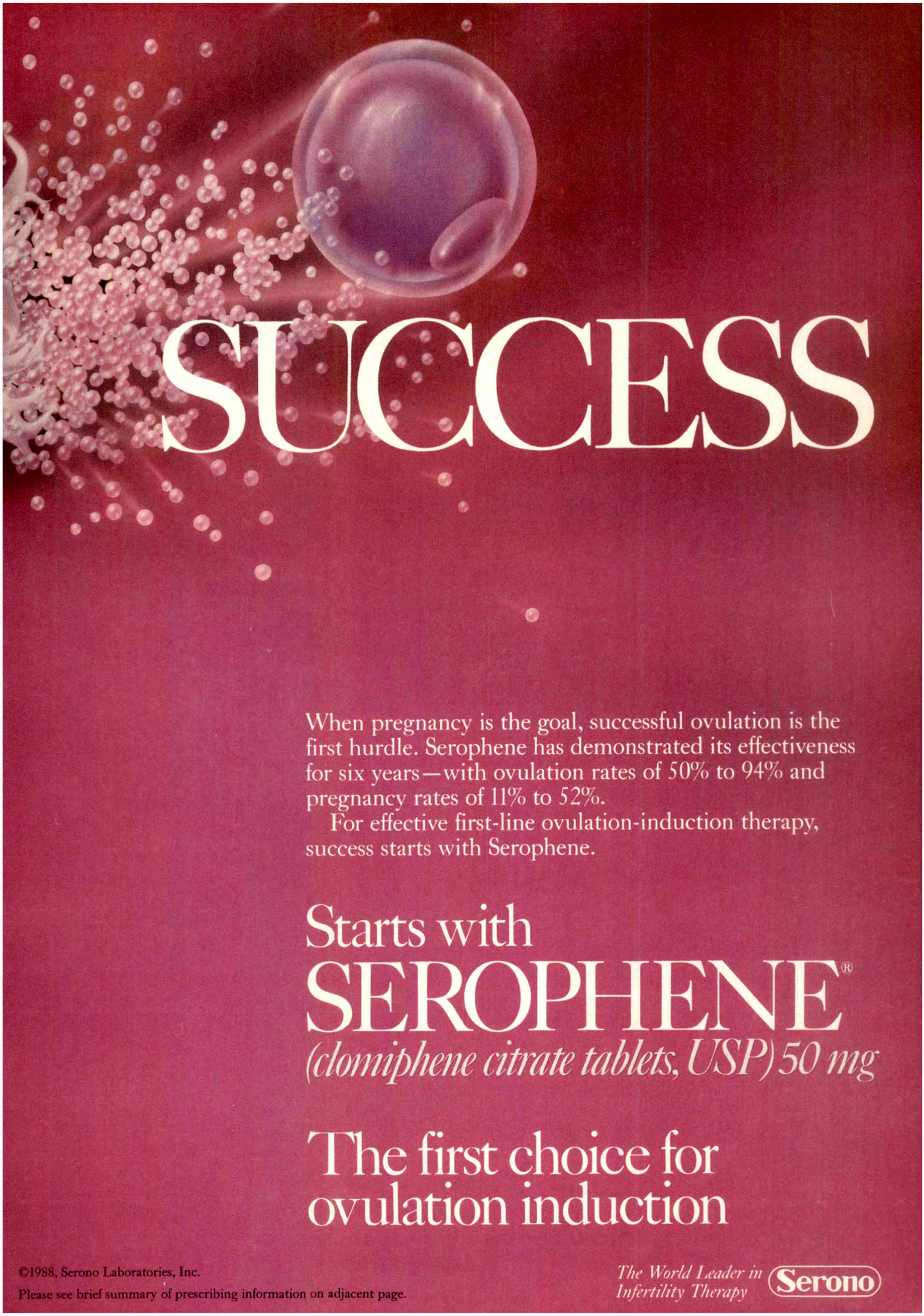
Age	Below 30	30-39	40 +
Heavy smokers	C	B	A
Light smokers	D	C	B
Nonsmokers (no predisposing conditions)	D	C,D	C
Nonsmokers (other predisposing conditions)	C	C,B	B,A

A–Use associated with very high risk.
B–Use associated with high risk.
C–Use associated with moderate risk.
D–Use associated with low risk.

Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, discontinue OC immediately. A 4-6 fold increased risk of post-surgery thromboembolic complications has been reported in OC users. If feasible, discontinue OCs at least 4 weeks before surgery associated with increased risk of thromboembolism or prolonged immobilization. Before resuming OC after major surgery or bedrest, balance risks of post-surgery thromboembolic complications with contraceptive needs. Data suggest varicose veins substantially increase risk of superficial venous thrombosis of the leg, the risk depending on the severity of the varicosities. 2. *Ocular Lesions:* Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OC use. Discontinue OC if there is unexplained, sudden, or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions; and institute appropriate diagnostic and therapeutic measures. 3. *Carcinoma:* Long-term continuous administration of natural or synthetic estrogen in certain animals increases certain tumors, benign or malignant, such as breast, cervix, vagina, uterus, ovary, pituitary and liver. Certain synthetic progestogens, none currently in OCs, increase the incidence of mammary nodules, benign and malignant, in dogs. Several retrospective case-control studies reported an increased relative risk (3.1-13.9 times) associating endometrial carcinoma with the prolonged use of estrogens in postmenopausal women. One publication reported the first 30 cases submitted to the registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the adenocarcinomas found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding when OCs were first given, polycystic ovaries), nearly all occurred in women who had used sequential OCs, which are no longer marketed. No statistical association has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs, although individual cases have been reported. Studies have shown no increased risk of breast cancer to OC or estrogen users. One study found no overall increased risk of breast cancer in OC users but a greater risk was suggested for OC users with documented benign breast disease and for long-term (2-4 years) users. Another study found a history of breast cancer among grandmothers or aunts was significantly more frequent among breast cancer patients who had used an OC continuously for one or more years than among nonusers with breast cancer. One other study indicated an increasing risk of breast cancer in women taking menopausal estrogens, which increased with duration of follow-up. One author suggests that extended (over 6 years) OC use prior to first full term pregnancy was associated with a significant relative risk of breast cancer, and other studies suggested an increased risk of breast cancer with long-term OC use before age 25. A reduced occurrence of benign breast tumors in OC users has been well documented. One study reported malignant melanoma more frequently in OC users than controls and suggests an increased incidence of urinary tract and thyroid cancers. A prospective study of women with cervical dysplasia found an increase in severity and conversion to cancer *in situ* in OC users compared to nonusers. This became statistically significant after 3-4 years of use. Nonreversal of dysplasia within the first 6 months of pill use was suggested to predict progression after prolonged exposure. One study disclosed an increased risk of cancer of the cervix (largely carcinoma *in situ*) in OC users under 40, particularly those who had used OCs over 4 years. Two other studies suggested an increasing incidence of cervical neoplasms with longer duration of OC use. There have been other reports of microglandular hyperplasia of the cervix in OC users. One study reported an association between OC use and endocervical adenocarcinoma. In summary, there is no confirmed evidence from human studies of increased risk of cancer associated with OCs. Close clinical surveillance of all OC users is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, take appropriate diagnostic measures to rule out malignancy. Monitor OC users with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms with particular care. (See also Warnings, 4. *Liver Tumors.*) 4. *Liver Tumors:* Sudden severe abdominal pain or shock may be due to rupture and hemorrhage of a liver tumor. There have been reports associating benign or malignant liver tumors with short-term and long-term OC use. One study reported use of OCs with high hormonal potency and age over 30 may further increase risk of hepatocellular adenoma. Two studies relate risk with duration of use, risk being much greater after 4 or more years of use. Long-term OC users have an estimated annual incidence of hepatocellular adenoma of 3-4/100,000. Although an uncommon lesion, it should be considered in women presenting with an "acute abdomen". The tumor may cause serious or fatal hemorrhage. Patients with liver tumors have demonstrated variable clinical features which may make preoperative diagnosis difficult. Some cases presented because of right upper quadrant masses, while most had signs and symptoms of acute intraperitoneal hemorrhage. Routine radiological and laboratory studies may not be helpful. Liver scans may show a focal defect. Hepatic arteriography may be useful in diagnosing primary liver neoplasm. Cases of hepatocellular carcinoma have been reported in long-term users of OCs.[65-167] An epidemiological study suggested a 4-fold increase in relative risk for hepatocellular carcinoma in OC users for 8 years or more, which increased to 7 in women with markers of hepatitis B. A second study suggested a risk of about 20 for use in excess of 8 years. 5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring:* Use of female sex hormones–estrogenic and progestational agents–during early pregnancy may seriously damage the offspring. Females exposed *in utero* to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1/1000 exposures or less. Although there is no evidence that OCs further enhance the risk of developing this type of malignancy, such OC users should be monitored with particular care. A high percentage of women exposed to diethylstilbestrol (30-90%) have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are a precursor of vaginal malignancy. Male children so exposed may develop urogenital tract abnormalities. Although similar data are not available on other estrogens, it cannot be presumed that they would not induce similar changes. Increased risk of congenital anomalies, including heart and limb defects, has been reported following use of sex hormones, including OCs, in pregnancy. One case-control study estimated a 4.7-fold increased relative risk of limb-reduction defects in infants exposed *in utero* to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some exposures involved only a few days of treatment. Data suggest risk of limb-reduction defects in exposed fetuses is somewhat less than 1/1000 live births. In a large prospective study, cardiovascular defects in children born to women who received female hormones, including OCs, during early pregnancy occurred at 18.2/1000 births, compared to 7.8/1000 for children not so exposed *in utero.* These results are statistically significant. A Welsh study found a statistically significant excess of neural tube defects among offspring of prior OC users (within 3 months) than among controls. The incidence of twin births may be increased for women who conceive shortly after discontinuing OC use. In the past, female sex hormones were used during pregnancy in an attempt to treat threatened or habitual abortion. There is evidence that estrogens are ineffective, and there is no evidence from well controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. If the patient has not adhered to the prescribed schedule, consider possible pregnancy at the first missed period (or 45 days from the last menstrual period if progestogen-only OCs are used) and discontinue OC use until pregnancy has been ruled out. For any patient who has missed two consecutive periods, rule out pregnancy before continuing the OC. If pregnancy is confirmed, tell the patient about potential risks to the fetus and discuss advisability of continuing the pregnancy. Women who discontinue OCs to become pregnant should use an alternate form of contraception for a period of time before attempting to conceive. A 3-month period is supported by a study suggesting increased frequency of neural tube defects in women impregnated during the first 3 months after cessation of OC use. Do not use progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding as a pregnancy test. 6. *Gall Bladder Disease:* Studies report increased risk of gall bladder disease in OC or estrogen users. In one study, an increased risk appeared after 2 years of use and doubled after 4-5 years. In another study, an increased risk was apparent between 6 and 12 months. 7. *Carbohydrate and Lipid Metabolic Effects:* Because a decrease in glucose tolerance has been observed in a significant percentage of patients on OCs, prediabetic and diabetic OC users should be carefully observed. An increase in triglycerides and total phospholipids has been observed in OC users but its clinical significance is unknown. 8. *Elevated Blood Pressure:* An increase in blood pressure has been reported with OC use. Hypertension may occur within a few months of beginning OCs. In the first year of use, incidence of hypertension may be no higher in OC users than in nonusers. Incidence in users increases with exposure and in the fifth year of use is 2.5-3 times that in the first year. Age is strongly correlated with hypertension in OC users. Women with a history of elevated blood pressure (hypertension), preexisting renal disease, history of toxemia or elevated blood pressure during pregnancy, familial tendency to hypertension or its consequences, or history of excessive weight gain or fluid retention during the menstrual cycle may be more likely to develop elevated blood pressure when given OCs and should be monitored closely. Even though elevated blood pressure may remain within the "normal" range, closely watch elevations, particularly for women with other risk factors for cardiovascular disease or stroke. High blood pressure may or may not persist after OC discontinuation. 9. *Headache:* Discontinue OC and evaluate the cause of onset or exacerbation of migraine or development of a new pattern of headache which is recurrent, persistent, or severe. 10. *Bleeding Irregularities:* Breakthrough bleeding, spotting, and missed menses often make users discontinue OCs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, consider nonfunctional causes. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, use adequate diagnostic measures to rule out pregnancy or malignancy. If pathology has been excluded, time or another formulation may solve the problem. While potentially useful in minimizing menstrual irregularity, change to an OC with a higher estrogen content only if necessary since this may increase the risk of thromboembolic disease. Women with a history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may tend to remain anovulatory or to become amenorrheic after OC discontinuation. Women with these preexisting problems should be advised of this and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may occur in women without previous irregularities. A higher incidence of galactorrhea and of pituitary tumors (e.g., adenomas) has been associated with amenorrhea in former users compared with nonusers. One study reported a 16-fold increased incidence of pituitary prolactin-secreting tumors among patients with postpill amenorrhea when galactorrhea was present. 11. *Infertility:* There is evidence of impairment of fertility in women discontinuing OCs in comparison with other contraceptive methods, which appears to be independent of duration of use. While impairment diminishes with time, there is an appreciable difference in the results in nulliparous women for OC and other groups 30 months after discontinuation of birth control. For parous women the difference is not apparent 30 months after cessation of contraception. One study suggests that the incidence of *Chlamydia trachomatis* infection, the leading cause of nongonococcal PI.D., is increased by OCs. 12. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in OC failure. In progestogen-only OC failures, the ratio of ectopic to intrauterine pregnancies is higher than in nonusers, since the drugs are more effective in preventing intrauterine than ectopic pregnancies. 13. *Breast Feeding:* OCs in the postpartum period may interfere with lactation by decreasing quantity and quality of breast milk. A small fraction of OC hormonal agents has been identified in milk of mothers receiving OCs. Effects, if any, on the breast-fed child have not been determined. If feasible, defer OC use until the infant has been weaned. **Precautions** *GENERAL* 1. Take a complete medical and family history before starting OCs. Pretreatment and periodic physical exams should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant lab tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may enlarge. 3. Observe patients with a history of psychic depression and discontinue OCs if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the OC and use an alternate method of contraception to determine whether the symptom is drug related. 4. OCs may cause some fluid retention. Prescribe with caution, and only with careful monitoring, in patients with conditions that might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma or cardiac, hepatic or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while using OCs. Discontinue OC if jaundice develops. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism that may result in a relative pyridoxine deficiency. The clinical significance is unknown. 8. Serum folate levels may be depressed by OC use. Since the pregnant woman is predisposed to folate deficiency and incidence of folate deficiency increases with increasing gestation, if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and related complications. 9. Advise the pathologist of OC use when relevant specimens are submitted. 10. Certain endocrine and liver function tests and blood components may be affected by estrogen-containing OCs. For example: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test. f. Increased phospholipids and triglycerides. g. Temporarily decreased glucose tolerance. 11. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist and temporary or permanent cessation of wear considered. DRUG INTERACTIONS. OCs may be less effective and there may be increased breakthrough bleeding from interactions with rifampin, isoniazid, ampicillin, tetracycline, neomycin, penicillin V, chloramphenicol, sulfonamides, nitrofurantoin, barbiturates, phenytoin, primidone, analgesics, tranquilizers, antimigraine preparations, antihistamines, and griseofulvin. OCs may alter effectiveness of such other drugs as oral anticoagulants, anticonvulsants, tricyclic antidepressants, antihypertensive agents (e.g., guanethidine), vitamins, hypoglycemic agents, tranquilizers, hypnotic preparations, theophylline, and beta blockers. Toxic liver effects have been reported with a combination of an OC and cyclosporin A. *CARCINOGENESIS* See Warnings. *PREGNANCY* Pregnancy category X. See Contraindications and Warnings. *NURSING MOTHERS* See Warnings. **Adverse Reactions** An increased risk of the following serious adverse reactions has been associated with OC use (see Warnings): thrombophlebitis, thrombosis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, mesenteric thrombosis, liver tumors, cerebral hemorrhage, hypertension, gall bladder disease, congenital anomalies, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis, Budd-Chiari syndrome, Raynaud's disease, arterial thromboembolism. The following adverse reactions have been reported in OC users and are believed to be drug related: bleeding irregularities (breakthrough bleeding, spotting, missed menses during treatment, amenorrhea after treatment), gastrointestinal symptoms (nausea, vomiting, bloating, abdominal cramps, colitis), dysmenorrhea, infertility after discontinuance of treatment, edema, chloasma or melasma which may persist after drug is discontinued, breast changes (tenderness, enlargement, and secretion), intolerance to contact lenses, changes in corneal curvature (steepening), change in weight (increase or decrease), change in cervical erosion and cervical secretion, possible diminution of lactation when given immediately postpartum, cholestatic jaundice, migraine, increase in size of uterine leiomyomata, rash (allergic), mental depression, reduced tolerance to carbohydrates, vaginal candidiasis, prolactin-secreting pituitary tumors, chilblains. The following adverse reactions have been reported in OC users and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of hair, erythema multiforme, erythema nodosum, hemorrhagic eruptions, vaginitis, porphyria, impaired renal function, malignant nephrosclerosis (hemolytic uremic syndrome), malignant hypertension, acute renal failure, premature supraventricular contractions. **Information for the Patient** (See Patient Package Insert).

Rev. April 1987

SYNTEX



When pregnancy is the goal, successful ovulation is the first hurdle. Serophene has demonstrated its effectiveness for six years—with ovulation rates of 50% to 94% and pregnancy rates of 11% to 52%.

For effective first-line ovulation-induction therapy, success starts with Serophene.

Starts with SEROPHENE®

(clomiphene citrate tablets, USP) 50 mg

The first choice for ovulation induction

©1988, Serono Laboratories, Inc.

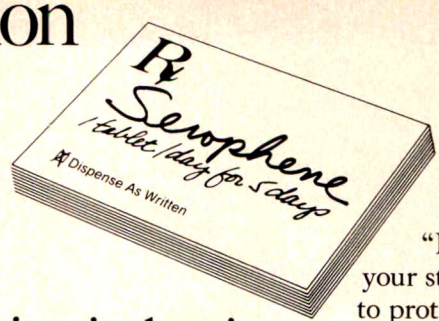
Please see brief summary of prescribing information on adjacent page.

The World Leader in Infertility Therapy

Serono

Successful ovulation starts with SEROPHENE®

(clomiphene citrate tablets, USP) 50 mg



Be sure your patients receive the drug you prescribe. Specify "Dispense as Written" or your state's approved wording to protect the integrity of your Serophene prescription.

The first choice for ovulation induction

Serophene®
(clomiphene citrate tablets, USP)
Brief Summary:
Indications:
Clomiphene citrate is indicated for the treatment of ovulatory failure in patients desiring pregnancy, and whose husbands are fertile and potent. Impediments to this goal must be excluded or adequately treated before beginning therapy. Administration of clomiphene citrate is indicated only in patients with demonstrated ovulatory dysfunction and in whom the following conditions apply:

1. Normal liver function.
2. Physiologic indications of normal endogenous estrogen (as estimated from vaginal smears, endometrial biopsy, assay of urinary estrogen, or from bleeding in response to progesterone). Reduced estrogen levels, while less favorable, do not prevent successful therapy.
3. Clomiphene citrate therapy is not effective for those patients with primary pituitary or ovarian failure. It cannot substitute for appropriate therapy of other disturbances leading to ovulatory dysfunction, e.g., diseases of the thyroid or adrenals.
4. Particularly careful evaluation prior to clomiphene citrate therapy should be done in patients with abnormal uterine bleeding. It is most important that neoplastic lesions are detected.

Contraindications:
Pregnancy
Although no direct effect of clomiphene citrate therapy on the human fetus has been seen, clomiphene citrate should not be administered in cases of suspected pregnancy as such effects have been reported in animals. To prevent inadvertent clomiphene citrate administration during early pregnancy, the basal body temperature should be recorded throughout all treatment cycles, and therapy should be discontinued if pregnancy is suspected. If the basal body temperature following clomiphene citrate is biphasic and is not followed by menses, the possibility of an ovarian cyst and/or pregnancy should be excluded. Until the correct diagnosis has been determined, the next course of therapy should be delayed.
Liver Disease
Patients with liver disease or a history of liver dysfunction should not receive clomiphene citrate therapy.
Abnormal Uterine Bleeding
Clomiphene citrate is contraindicated in patients with abnormal uterine bleeding.
Warnings:
Visual Symptoms
Patients should be warned that blurring and/or other visual symptoms may occur occasionally with clomiphene citrate therapy. These may make activities such as driving or operating machinery more hazardous than usual, particularly under conditions of variable lighting. While their significance is not yet understood (see ADVERSE REACTIONS), patients having any visual symptoms, should discontinue treatment and have a complete ophthalmologic evaluation.
Precautions:
Diagnosis Prior to Clomiphene Citrate Therapy
Careful evaluation should be given to candidates for clomiphene citrate therapy. A complete pelvic examination should be performed prior to treatment and repeated before each subsequent course. Clomiphene citrate should not be given to patients with an ovarian cyst, as further ovarian enlargement may result.
Since the incidence of endometrial carcinoma and of ovulatory disorders increases with age, endometrial biopsy should always exclude the former as causative in such patients. If abnormal uterine bleeding is present, full diagnostic measures are necessary.
Ovarian Overstimulation During Treatment with Clomiphene Citrate
To minimize the hazard associated with the occasional abnormal ovarian enlargement during clomiphene citrate therapy (see ADVERSE REACTIONS), the lowest dose producing good results should be chosen. Some patients with polycystic ovary syndrome are unusually sensitive to gonadotropin and may have an exaggerated response to usual doses of clomiphene citrate. Maximal enlargement of the ovary, whether abnormal or physiologic, does not occur until several days after discontinuation of clomiphene citrate. The patient complaining of pelvic pains after receiving clomiphene citrate should be examined carefully. If enlargement of the ovary occurs, clomiphene citrate therapy should be withheld until the ovaries have returned to pretreatment size, and the dosage or duration of the next course should be reduced. The ovarian enlargement and cyst formation following clomiphene citrate therapy regress spontaneously within a few days or weeks after discontinuing treatment. Therefore, unless a strong indication for laparotomy exists, such cystic enlargement always should be managed conservatively.
Multiple Pregnancy
In the reviewed publications, the incidence of multiple pregnancies was increased during those cycles in which clomiphene citrate was given. Among the 1803 pregnancies on which the outcome was reported, 90% were single and 10% twins. Less than 1% of the reported deliveries resulted in triplets or more.
Of these multiple pregnancies, 96-99% resulted in the births of live infants. The patient and her husband should be advised of the frequency and potential hazards of multiple pregnancy before starting treatment.
Adverse Reactions:
Symptoms
Side effects are not prominent at the recommended dosage of clomiphene citrate, and infrequently interfere with treatment. Side effects tend to occur more frequently at higher doses and in the longer treatment courses used in some early studies. The more common side effects and the percent of patients experiencing them include vasomotor flushes (11%), abdominal discomfort (7.4%), abnormal uterine bleeding (0.5%), ovarian enlargement (14%), breast tenderness (2.1%), and visual symptoms (1.6%). The vasomotor symptoms resemble menopausal "hot flushes," and are not usually severe. They promptly disappear after treatment is discontinued. Abdominal discomfort may resemble ovulatory (mittelschmerz) or premenstrual phenomena, or that due to ovarian enlargement. In addition, nausea and vomiting (2.1%), nervousness and insomnia (1.9%), headache (1%), dizziness and lightheadedness (1%), increased urination (0.9%), depression and fatigue (0.8%), urticaria and allergic dermatitis (0.6%), weight gain (0.4%), and reversible hair loss (0.3%) have been reported.
When clomiphene citrate is administered at the recommended dose, abnormal ovarian enlargement (see PRECAUTIONS) is infrequent, although the usual cyclic variation in ovarian size may be exaggerated. Similarly, mid-cycle ovarian pain (mittelschmerz) may be accentuated. With prolonged or higher dosage, ovarian enlargement and cyst formation (usually luteal) may occur more often and the luteal phase of the cycle may be prolonged. Patients with polycystic ovary disease may be unusually sensitive to clomiphene therapy. Rare occurrences of massive ovarian enlargement have been reported, for example, in a patient with polycystic ovary syndrome whose clomiphene citrate therapy consisted of 100 mg daily for 14 days. Since abnormal ovarian enlargement usually regresses spontaneously, most of these patients should be treated conservatively.
The incidence of visual symptoms (see WARNINGS for further recommendations), usually described as "blurring" or spots or flashes (scintillating scotomata), correlates with increasing total dose. The symptoms disappear within a few days or weeks after clomiphene citrate is discontinued. This may be due to intensification and/or prolongation of after-images. Symptoms often appear first, or are accentuated, upon exposure to a more brightly lit environment. While measured visual acuity has not generally been affected, in one patient taking 200 mg daily, visual blurring developed on the seventh day of treatment, and progressed to severe diminution of visual acuity by the tenth day. No other abnormality was coincident, and the visual acuity was normal by the third day after treatment was stopped. Ophthalmologically definable scotomata and electroretinographic retinal function changes have also been reported.
BSP Laboratory Studies
Greater than 5% retention of sulfobromophthalein (BSP) has been reported in approximately 10 to 20% of patients in whom it was measured. Retention was usually minimal but was elevated during prolonged clomiphene citrate administration or with apparently unrelated liver disease. In some patients, pre-existing BSP retention decreased even though clomiphene citrate therapy was continued. Other liver function tests were usually normal.
Other Laboratory Studies
Clomiphene citrate has not been reported to cause a significant abnormality in hematologic or renal tests, in protein bound iodine, or in serum cholesterol levels.
Birth Defects
Of 1803 births following clomiphene citrate administration, 45 infants with birth defects were reported for a cumulative rate of 2.5%.
Six cases of Down's Syndrome, one neonatal death with multiple malformations and one case each of the following were reported: extropia, club foot, tibial torsion, blocked tear duct and hemangioma. The other congenital abnormalities were not described. The investigators did not report that these were presumed to be due to therapy. The cumulative rate of congenital abnormalities does not exceed that reported in the general population.
Available only on prescription
Manufactured by Ikapharm Ltd, Kfar Sava, Israel.
Distributed by: Serono Laboratories, Inc., 280 Pond Street, Randolph, MA 02368.
© Serono Laboratories, Inc.

SLSJA 1/88

The World Leader in Infertility Therapy

Serono

Toll-free: 800-225-5185
In Mass: 617-963-8154

Serono Laboratories, Inc.
280 Pond Street
Randolph, MA 02368

Mosby

American Journal of Obstetrics and Gynecology

Founded in 1920

Copyright © 1989 by The C.V. Mosby Company

Contents

September
1989

This issue contains Transactions of the Fifty-first Annual Meeting of the South Atlantic Association of Obstetricians and Gynecologists and Transactions of the Ninth Annual Meeting of the Society of Perinatal Obstetricians beginning on page 593.

TRANSACTIONS OF THE FIFTY-FIRST ANNUAL MEETING OF THE SOUTH ATLANTIC ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS

The challenge of change revisited 505
Frank C. Greiss, Jr., MD
Winston-Salem, North Carolina

Effects of terbutaline sulfate on fetal cardiac function 509
Ruth Petersen, MD, Laurence S. Carter, BS, Nancy C. Chescheir, MD, Vern L. Katz, MD, and Robert C. Cefalo, MD, PhD
Chapel Hill, North Carolina

After long-term exposure to terbutaline, guinea pig fetuses demonstrate myocardial adaptations that predispose them to impaired cardiac function.

Analysis of fetal loss after transcervical chorionic villus sampling—A review of 719 patients 513
Ronald V. Wade, MD, and S. Robert Young, PhD
Columbia, South Carolina

In a study of 714 patients, risk of fetal loss after transcervical chorionic villus sampling is associated with the increasing number of catheter insertions.

(Contents continued on page 7A)

Vol. 161, No. 3, September 1989. The American Journal of Obstetrics and Gynecology (ISSN 0002-9378) is published monthly (six issues per volume, two volumes per year) by The C.V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318. Second-class postage paid at St. Louis, Missouri, and additional mailing offices. POSTMASTER: Send address changes to The C.V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318, (314) 872-8370, ext. 351. Annual subscription rates for 1989: domestic, $65.50 for individuals and $118.00 for institutions. Printed in the U.S.A. Copyright © 1989 by The C.V. Mosby Company.

Satisfaction realized:



87% patient continuation rate† with Ortho ALL-FLEX* Arcing Spring Diaphragm.

Easy for patient to insert—
the Ortho ALL-FLEX* Diaphragm folds easily.

Easy for patient to correctly position in vagina—
the Ortho ALL-FLEX* Diaphragm can be compressed at any two opposing points around the rim.

Easy to use with **GYNOL II* (2% nonoxynol-9) Contraceptive Jelly**—
greaseless, odorless spermicide.

Easy to clean—one-step molding process eliminates gap between spring and dome so jelly or secretions can't accumulate.

Ortho ALL-FLEX*
DIAPHRAGM

†Percentage of patients continuing the method for 12 consecutive months.

Consult the revised patient brochure for new information on Toxic Shock Syndrome (TSS) and wearing time.

ORTHO PHARMACEUTICAL CORPORATION
Raritan, New Jersey 08869-0602

© OPC 1987 OJ-899 *Trademark

Contents continued from page 5A

The role of ultrasonography and amniocentesis in the evaluation of pregnancies at risk for neural tube defects 520

William A. Hogge, MD, Siva Thiagarajah, MD, James E. Ferguson II, MD, Patricia T. Schnatterly, MS, and Guy M. Harbert, Jr., MD

Charlottesville, Virginia

A study of 225 pregnancies suggests that targeted ultrasonography alone may be a reasonable evaluation alternative to amniocentesis in patients at risk for neural tube defects.

The effects of vibroacoustic stimulation on baseline heart rate, breathing activity, and body movements of normal term fetuses 524

Lawrence D. Devoe, MD, Nancy A. Searle, BA, David A. Ruedrich, MD, Ramon A. Castillo, MD, and William P. Metheny, PhD

Augusta, Georgia

Vibroacoustic stimulation of normal term fetuses results in brief elevation of fetal heart rate baseline without significant long-term effects on breathing or body movements.

Seminoma in pubertal patient with androgen insensitivity syndrome 530

W. Glenn Hurt, MD, Joann N. Bodurtha, MD, Janice B. McCall, MD, and M. Moinuddin Ali, MD

Richmond, Virginia

Complete androgen insensitivity and metastatic seminoma in a pubertal patient is reported.

Successful pregnancy after a liver transplant 532

Cynthia J. Sims, MD, Kathy B. Porter, MD, and Robert A. Knuppel, MD, MPH

Tampa, Florida

Uncomplicated pregnancy and delivery in a patient 35 months after liver transplantation are described.

(Contents continued on page 9A)

1989 Annual subscription rates	U.S.A.	International (surface mail) All regions	International (airmail)° Canada	Mexico	Region 1	Region 2	Region 3
Institutions†	$118.00	$142.50	$170.00	$176.25	$188.50	$230.25	$272.00
Individuals‡	$ 65.50	$ 90.00	$117.50	$123.75	$136.00	$177.75	$219.50
Students, residents‡	$ 38.00	$ 62.50	$ 90.00	$ 96.25	$108.50	$150.25	$192.00

Subscription rates include supplements. Single copies are $7.00. Remittances should be made by check, draft, or post office or express money order, in U.S. funds drawn through a U.S. bank, payable to this JOURNAL. Claims for missing issues will be serviced only within six months of cover date. Single copy prices will be charged on missing issue claims older than six months from cover date. Back issues generally are available for the previous five years. Contact the Publisher to confirm availability of specific issues.

°*Airmail breakdown—Domestic:* First-class and Priority rates for the U.S. and possessions are available upon request. *Canada:* All provinces of Canada. *Mexico:* All localities within Mexico. *Region 1:* Colombia, Venezuela, Central America, Caribbean Islands, Bahamas, Bermuda, and St. Pierre and Miquelon (also from American Samoa to Western Samoa and from Guam to the Philippines). *Region 2:* South America (except Colombia and Venezuela), Europe (except Estonia, Latvia, Lithuania, and U.S.S.R), and North Africa (Morocco, Algeria, Tunisia, Libya, and Egypt). *Region 3:* Estonia, Latvia, Lithuania, U.S.S.R., Asia, Australia, New Zealand, Pacific Ocean Islands, Africa (other than North Africa), Indian Ocean Islands, and the Middle East.

†Institutional (multiple-reader) subscriptions are available to public and private libraries, schools, hospitals, and clinics; city, county, state, provincial, and national government bureaus and departments; and all commercial and private institutions and organizations.

‡Individual subscriptions and all student-rate subscriptions must be in the names of, billed to, and paid by individuals. All student-rate requests must indicate training status and name of institution.

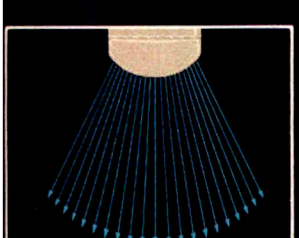
Subscriptions may begin at any time.

Corometrics puts the power o convex technolog in your han



The Aloka 620...
Outstanding image quality and more useful clinical information!

Practitioner tested and proven, convex array technology eliminates the problem of a narrow near field of view as seen with conventional sector scanners. A full wide aperture is maintained throughout the entire field of view, preserving resolution even at the sides of the sector scan.



Convex scan shows more clinical information.

A wide range of convex transducers is available, including our unique endovaginal probe designed for ease of use, unsurpassed control, and superior image quality. Advanced software includes an on-screen report page. The Aloka 620 — it all adds up to a new level of power and versatility for your office.



For more information or a demonstration call 1-800-624-7265, ext. 453 or 515.
In Connecticut call 265-5631.

COROMETRICS MEDICAL SYSTEMS, INC.
ULTRASOUND

Contents continued from page 7A

A clinical-pathologic study of mixed müllerian tumors of the uterus over a 16-year period—The Medical College of Georgia experience 533
Donald G. Gallup, MD, Donna S. Gable, MD, O. Eduardo Talledo, MD, and Luther B. Otken, Jr., MD
Augusta, Georgia

The outcome of 40 patients with mixed müllerian tumors of the uterus is correlated with demographic data, pathology, and treatment.

Evaluation of surgical staples in a cesarean section 540
Gene Burkett, MD, L. P. Jensen, MD, A. Lai, MD, M. J. O'Sullivan, MD, S. Yasin, MD, S. Beydoun, MD, and A. G. W. McLeod, MD
Miami, Florida

Absorbable staples reduced postoperative morbidity in cesarean section, but their use was not time-saving.

Outpatient triplet management: A contemporary review 547
Roger B. Newman, MD, Cheryl Hamer, RN, BS, and M. Clinton Miller, PhD
Charleston, South Carolina

A total of 198 triplet gestations were managed primarily on an outpatient basis and were delivered between 1985 and 1988 with a perinatal survival rate of 95%.

Maternal mortality in North Carolina: A forty-year experience 555
W. Joseph May, MD, and Frank C. Greiss, Jr., MD
Winston-Salem, North Carolina

Although the decrease in pregnancy-related death rates continues in North Carolina, variable trends in causal groups emerge and nonwhite/white relative risk remains high.

Risk factors for intraamniotic infection: A prospective epidemiologic study 562
David E. Soper, MD. C. Glen Mayhall, MD, and Harry P. Dalton, PhD
Richmond, Virginia

Logistic regression analysis identified four variables independently associated with intraamniotic infection.

Short course of antibiotic therapy in treatment of postpartum endomyometritis 568
Walter J. Morales, MD, PhD, Evan M. Collins, MD, Jeffrey L. Angel, MD, and Robert A. Knuppel, MD, MPH
Orlando, Florida

Through a prospective randomized study, short-course antibiotic therapy with combination tobramycin-clinidamycin was found safe and efficacious in the treatment of postpartum endomyometritis.

(Contents continued on page 10A)

Contents continued from page 9A

Subsequent fertility in women who undergo cardiac surgery 573
Wallace C. Nunley, Jr., MD, Lisa A. Kolp, MD, Laura N. Dabinett, MD, James D. Kitchin III, MD, Bruce G. Bateman, MD, and George B. Craddock, Jr., MD
Charlottesville, Virginia

There was no specific fertility problem identified in women who attempted conception after open heart surgery.

Fertility after ectopic pregnancy 576
Dorothy E. Mitchell, MD, Helen F. McSwain, MD, and Herbert B. Peterson, MD
Atlanta, Georgia

Clinical circumstances, including initial surgical procedure and postoperative hysterosalpingogram, are correlated with subsequent fertility.

Effects of clomiphene citrate on episodic luteinizing hormone secretion throughout the menstrual cycle 581
David F. Archer, MD, Glen Hofmann, MD, PhD, Robert Brzyski, MD, PhD, Barbara Ross, RN, BSN, Richard T. Scott, Jr., MD, Christine B. Philput, PhD, Sergio Oehninger, MD, and J. Catesby Ware, PhD
Norfolk, Virginia

Clomiphene citrate increased pituitary LH secretion in the follicular phase of normal women resulting in an increased serum LH level; the increased LH secretion was associated with an increased amplitude of each identified pulse but without a significant change in pituitary LH pulse frequency.

TRANSACTIONS OF THE NINTH ANNUAL MEETING OF THE SOCIETY OF PERINATAL OBSTETRICIANS

Management of women with one abnormal oral glucose tolerance test value reduces adverse outcome in pregnancy 593
Oded Langer, MD, Akolisa Anyaegbunam, MD, Lois Brustman, MD, and Michael Divon, MD
Bronx, New York

Treatment of women with one abnormal value on the oral glucose tolerance test reduced fetal macrosomia and neonatal metabolic complications to an incidence comparable to that in the normal population.

Routine umbilical cord blood gas determinations? 600
James A. Thorp, MD, Jone E. Sampson, MD, Valerie M. Parisi, MD, MPH, and Robert K. Creasy, MD
Houston, Texas

Results of umbilical cord blood gas determinations in newborns of 1924 nulliparous women are presented and used as a basis for considering obtaining cord arterial pH values at all deliveries.

Contents

Comparison of six different ultrasonographic methods for predicting lethal fetal pulmonary hypoplasia 606

Anthony M. Vintzileos, MD, Winston A. Campbell, MD, John F. Rodis, MD, David J. Nochimson, MD, Michael G. Pinette, MD, and Boris M. Petrikovsky, MD

Farmington, Connecticut

Of six different ultrasonographic methods, the chest area minus heart area/chest area ratio was found to have the best diagnostic accuracy in predicting lethal pulmonary hypoplasia.

Prophylactic amnioinfusion during labor complicated by meconium: A preliminary report 613

Yoel Sadovsky, MD, Erol Amon, MD, Mary E. Bade, BSMT, and Roy H. Petrie, MD, ScD

St. Louis, Missouri

Amnioinfusion decreases the frequency of thick meconium, neonatal acidemia, meconium below the vocal cords, and requirement for positive pressure ventilation during labor complicated by meconium.

Lack of evidence of a vasodepressor role for relaxin in spontaneously hypertensive and normotensive pregnant rats 618

Robert A. Ahokas, PhD, Baha M. Sibai, MD, and Garland D. Anderson, MD

Memphis, Tennessee

Bilateral ovariectomy (removal of the source of relaxin) does not prevent attenuation of angiotensin II pressor responsiveness and vasodilation in pregnant hypertensive or normotensive rats.

Perinatal factors influencing atrial natriuretic peptide levels in umbilical arterial plasma at the time of delivery 623

Lony C. Castro, MD, Chander P. Arora, PhD, Kay E. Roll, RN, Deborah A. Sassoon, MD, and Calvin J. Hobel, MD

Los Angeles, California

Atrial natriuretic peptide levels were higher in the umbilical artery than in the vein and were elevated in cases of preeclampsia and fetal distress.

Impact of antenatal dexamethasone administration on respiratory distress syndrome in surfactant-treated infants 628

Elaine E. Farrell, MD, Richard K. Silver, MD, Lois V. Kimberlin, RN, Eileen S. Wolf, RN, and Joanne M. Dusik, RN

Evanston, Illinois

An additive effect between antenatal dexamethasone and exogenous surfactant that reduced the incidence and severity of RDS was observed in this study; neonatal characteristics associated with a reduced incidence of RDS included singleton birth, delivery between 28 and 32 weeks' gestation, and male gender.

(Contents continued on page 12A)

Contents continued from page 11A

Cocaine abuse during pregnancy: Peripartum prevalence and perinatal outcome 633
Mark G. Neerhof, DO, Scott N. MacGregor, DO, Sandy S. Retzky, DO, and Terence P. Sullivan, DO
Chicago, Illinois

In this study peripartum cocaine abuse was common, increased during the 2-year study period, and was associated with increased perinatal morbidity.

Do the current standards for glucose tolerance testing in pregnancy represent a valid conversion of O'Sullivan's original criteria? 638
David A. Sacks, MD, Salim Abu-Fadil, MD, Jeffrey S. Greenspoon, MD, and Nick Fotheringham, PhD
Bellflower and Los Angeles, California

A formula converting whole blood glucose estimates by the Somogyi-Nelson method to plasma concentrations by the glucose oxidase method for use during pregnancy was empirically derived.

How reliable is the fifty-gram, one-hour glucose screening test? 642
David A. Sacks, MD, Salim Abu-Fadil, MD, Jeffrey S. Greenspoon, MD, and Nick Fotheringham, PhD
Bellflower and Los Angeles, California

A substantial proportion of gestational diabetes may go undiagnosed if reliance is placed on a single 1-hour, 50 gm glucose screening test.

Glycemic control in gestational diabetes mellitus—How tight is tight enough: Small for gestational age versus large for gestational age? 646
Oded Langer, MD, Judith Levy, MD, Lois Brustman, MD, Akolisa Anyaegbunam, MD, Ruth Merkatz, RN, and Michael Divon, MD
Bronx, New York

Targeting the level of glycemic control to an optimal range between 87 and 104 mg/dl in the management of women with gestational diabetes mellitus will result in neonatal size comparable to the general newborn population; thus undertreating and overtreating can result in intrauterine growth retardation and macrosomic fetuses.

Maternal hemoglobin F levels may have an adverse effect on neonatal birth weight in pregnancies with sickle cell disease 654
Akolisa Anyaegbunam, MD, Henny H. Billett, MD, Oded Langer, MD, Lois Brustman, MD, Claudia Berger, BA, Linda Wyse, MD, Ronald L. Nagel, MD, and Irwin R. Merkatz, MD
Bronx, New York

Although high maternal hemoglobin levels may be beneficial to the fetus, higher maternal hemoglobin F levels may have an adverse effect of neonatal birth weight.

Pneumonia during pregnancy: Has modern technology improved maternal and fetal outcome? 657
Nancy E. Madinger, MD, Jeffrey S. Greenspoon, MD, and A. Gray Ellrodt, MD
Los Angeles, California

A retrospective study of pneumonia during pregnancy revealed that maternal morbidity and preterm labor were frequent complications and that the rate of preterm delivery has not decreased in comparison with rates of earlier studies.

Contents

Comparison of predelivery versus postdelivery Kleihauer-Betke stains in cases of fetal death 663

John Owen, MD, Charles M. Stedman, MD, and Tommy L. Tucker, MT(ASCP)

Birmingham, Alabama

The delivery process does not simulate a massive fetomaternal hemorrhage in cases of fetal death.

Uterine rupture: Are intrauterine pressure catheters useful in the diagnosis? 666

M. Hellen Rodriguez, MD, Damon I. Masaki, MD, Jeffrey P. Phelan, MD, and Fidelia G. Diaz, MA

Los Angeles, California

In reviewing 76 cases, the intrauterine pressure catheter was not found to be useful in the diagnosis of uterine rupture.

The effect of continuous epidural analgesia on cesarean section for dystocia in nulliparous women 670

James A. Thorp, MD, Valerie M. Parisi, MD, MPH, Peter C. Boylan, MB, and Dennis A. Johnston, PhD

Houston, Texas

Epidural analgesia in labor may increase the incidence of cesarean section.

Can platelet volume predict progressive hypertensive disease in pregnancy? 676

James J. Walker, MB, ChB, Alan D. Cameron, MB, ChB, Steingrimur Bjornsson, MD, Charles R. J. Singer, MB, ChB, and Carol Fraser, FMILS

Glasgow, Scotland

This study shows that the use of mean platelet volume as a screening test for development of pregnancy-induced hypertension is of no value in the low-risk population, but a rise of mean platelet volume of at least 1 fl does appear to predict patients within a high-risk population who will progress to severe preeclampsia.

Measurement of cardiac output in pregnancy by thoracic electrical bioimpedance and thermodilution 680

Damon I. Masaki, MD, Jeffrey S. Greenspoon, MD, and Joseph G. Ouzounian, BS

Los Angeles, California

Thoracic electrical bioimpedance and thermodilution estimates of cardiac output correlated well (+20%) in eight of nine peripartum patients for whom comparison was valid.

In utero cocaine exposure: Observations of fetal behavioral state may predict neonatal outcome 685

Roderick F. Hume, Jr., MD, Maj, MC, USA, Karen J. O'Donnell, PhD, Cathi L. Stanger, RN, Allen P. Killam, MD, and Jeannine L. Gingras, MD

Durham, North Carolina

Ultrasonographic observation of altered fetal behavioral state organization and regulation successfully predicted abnormal neonatal behavior in perinates who were exposed to cocaine in utero.

(Contents continued on page 14A)

Contents continued from page 13A

In utero diagnosis of benign fetal macrocephaly 690
Robert DeRosa, MD, Roger R. Lenke, MD, Thaddeus W. Kurczynski, MD, Wayne H. Persutte, BS, RDMS, and Joanne M. Nemes, RNC, BSN
Toledo, Ohio

A case of benign familial macrocephaly was diagnosed prenatally by serial ultrasonographic evaluations and family history.

Rapid determination of fetal sex by deoxyribonucleic acid amplification of Y chromosome-specific sequences 693
Thomas L. Pinckert, MD, Roger V. Lebo, PhD, and Mitchell S. Golbus, MD
San Francisco, California

The polymerase chain reaction sex test amplifies and identifies male-specific sequences to quickly and correctly determine fetal sex.

"Possibly" de novo translocations: Prenatal risk counseling 698
E. Robert Wassman, MD, Debra L. Cheyovich, DrPH, and Yasunori Nakahara, MD
Pasadena, California

Apparently balanced translocations found incidentally at prenatal diagnosis in a large series show little difference in outcome from normal, but conservative counseling should be done.

Amniotic fluid acetylcholinesterase ratios in prenatal diagnosis of fetal abnormalities 703
JoAnn C. Kelly, PhD, Elizabeth Petrocik, and E. Robert Wassman, MD
Pasadena, California

The ratio of amniotic fluid acetylcholinesterase in pseudocholinesterase is useful in differentiating pregnancies with neural tube defects, ventral wall defects, blood contamination, and other problems.

Effect of diagnostic and therapeutic cordocentesis on maternal serum α-fetoprotein concentration 706
Carl Weiner, MD, Stanley Grant, RN, Jerry Hudson, PhD, Roger Williamson, MD, and Katharine Wenstrom, MD
Iowa City, Iowa

Maternal serum α-fetoprotein rose after cordocentesis to a similar degree for that reported after amniocentesis.

Two hundred intrauterine exchange transfusions in severe blood incompatibilities 709
Marie-Héléne Poissonnier, MD, Yves Brossard, MD, Nicole Demedeiros, MD, Juliana Vassileva, MD, Françoise Parnet, MD, Marc Larsen, MD, Monique Gosset, Jacques Chavinie, MD, and Jacques Huchet, MD
Paris, France

Sixty severely anemic fetuses and 47 with hydrops were treated with a success rate of 91.6% and 61.7%, respectively.

Contents

Normal values for human umbilical venous and amniotic fluid pressures and their alteration by fetal disease 714

Carl P. Weiner, MD, Joni Heilskov, RN, Gay Pelzer, RN, Stanley Grant, RN, Katharine Wenstrom, MD, and Roger A. Williamson, MD

Iowa City, Iowa

Human amniotic fluid and fetal umbilical venous pressure are altered by a variety of spontaneously occurring disorders.

Ovine fetal cardiorespiratory response to nicardipine 718

R. Harold Holbrook, Jr., MD, Ellen M. Voss, BS, and Ronald N. Gibson, BS

Stanford, California

Direct administration of the calcium antagonist nicardipine to the ovine fetus was not associated with the significant cardiorespiratory changes previously reported with maternal administration.

Effect of prolonged hypoxemia on fetal heart rate accelerations and decelerations in sheep 722

Alan D. Bocking, MD, Susan White, BSc, Robert Gagnon, MD, and Howard Hansford

London, Ontario, Canada

Hypoxemia was maintained for 24 hours in fetal sheep by restricting uterine blood flow, which led to an initial increase in the number of fetal heart rate accelerations and decelarations followed by a return to normal patterns by 12 to 16 hours.

Prolonged hyperinsulinemia reduces plasma fatty acid levels in the major lipid groups in fetal sheep 728

Paul L. Ogburn, Jr., MD, Marshall Goldstein, MD, Jane Walker, BS, and Barbara S. Stonestreet, MD

Providence, Rhode Island

Prolonged infusion of insulin into fetal sheep lowered the major plasma fatty acid levels (with the exception of linoleic acid) in triglycerides, phospholipids, and nonesterified fatty acids.

Pharmacokinetics of azidothymidine during late pregnancy in Long-Evans rats 732

B. B. Little, MA, PhD, R. E. Bawdon, PhD, J. T. Christmas, MD, S. Sobhi, BS, and L. C. Gilstrap III, MD

Dallas, Texas

Placental transfer of azidothymidine was studied in pregnant rats and was found to equilibrate rapidly into the fetal compartment but at levels significantly lower than initial maternal levels.

Ultrastructural aspects of preeclampsia. I. Placental bed and uterine boundary vessels 735

Douglas R. Shanklin, MD, and Baha M. Sibai, MD

Memphis, Tennessee

Direct-vision uterine biopsies obtained at cesarean section or from section hysterectomy showed extensive ultrastructural endothelial injury in patients with preeclampsia but not in normotensive patients.

(Contents continued on page 16A)

Contents continued from page 15A

Birth weight prediction from remote ultrasonographic examination 742
Joseph A. Spinnato, MD, Robert D. Allen, and Hiram W. Mendenhall, MD
Mobile, Alabama

Five existing static equations were remodeled to permit birth weight estimates from remote ultrasonographic parameters.

Dating the early pregnancy by sequential appearance of embryonic structures 747
Wendy B. Warren, MD, Ilan Timor-Tritsch, MD, David B. Peisner, MD, Sashi Raju, and Mortimer G. Rosen, MD
New York, New York

The transvaginal sonographic appearance of embryonic structures was used as a method of dating the early first-trimester pregnancy.

Macrosomia in the postdate pregnancy: Is routine ultrasonographic screening indicated? 753
Judith L. Chervenak, MD, Michael Y. Divon, MD, Jordan Hirsch, MD, Barbara A. Girz, DO, and Oded Langer, MD
Bronx, New York, and Newark, New Jersey

Ultrasonographic screening for macrosomia may be a valuable adjunct to current fetal surveillance protocols used in the postdate pregnancy.

Discordant twins—A prospective study of the diagnostic value of real-time ultrasonography combined with umbilical artery velocimetry 757
Michael Y. Divon, MD, Barbara A. Girz, DO, Avi Sklar, MD, Denise A. Guidetti, MD, and Oded Langer, MD
Bronx, New York

The presence of either a 15% difference in ultrasonographic estimates of fetal weight or a 15% difference in systolic/diastolic ratios correctly identified 78% of discordant pairs.

Doppler umbilical artery waveform indices—Normal values from fourteen to forty-two weeks 761
Susan K. Hendricks, MD, Tanya K. Sorensen, MD, Keith Y. Wang, MD, PhD, Jeanette M. Bushnell, MSN, Elaine M. Seguin, RNC, and Rosalee W. Zingheim, BSN
Seattle, Washington

Fetal velocimetry was performed in 590 low-risk cases and normal values are reported for 14 to 42 weeks' gestation.

Screening with Doppler velocimetry in labor 765
Leonard Feinkind, MD, Ovadia Abulafia, MD, Isaac Delke, MD, Joseph Feldman, MD, and Howard Minkoff, MD
Brooklyn, New York

Doppler velocimetry in 273 laboring patients was correlated with the nonstress test result on presentation and was predictive of fetal outcome.

Contents

The effect of magnesium sulfate tocolysis on the fetal biophysical profile 771
Alan M. Peaceman, MD, Bruce A. Meyer, MD, James A. Thorp, MD, Valerie M. Parisi, MD, MPH, and Robert K. Creasy, MD
Houston, Texas

Magnesium sulfate tocolysis causes nonreactive nonstress testing and cessation of breathing movements in a significant number of fetuses, without affecting tone, movements, or amniotic fluid volume.

Controlled trial of hydration and bed rest versus bed rest alone in the evaluation of preterm uterine contractions 775
Richard A. Pircon, MD, Howard T. Strassner, MD, Donna S. Kirz, MD, and Craig V. Towers, MD
Orange and Long Beach, California, and Chicago, Illinois

The use of hydration as a therapy to differentiate those with true preterm labor from those with false preterm labor could not be supported.

Fetal heart rate patterns in the small-for-gestational-age human fetus 779
Robert Gagnon, MD, Cora Hunse, RN, and Alan D. Bocking, MD
London, Ontario, Canada

Currently used criteria for nonstress testing may not be clinically applicable in growth-retarded human fetuses.

Impact of residual amniotic fluid volume in patients receiving parenteral tocolysis after premature rupture of the membranes 784
Richard K. Silver, MD, Scott N. MacGregor, DO, and Edward D. Hobart
Evanston, Illinois

Diminished residual amniotic fluid volume in patients with premature membrane rupture who are receiving tocolytic therapy is associated with a significant decrease in latency.

Cardiovascular and metabolic effects associated with nifedipine and ritodrine tocolysis 788
James E. Ferguson II, MD, Donald C. Dyson, MD, R. Harold Holbrook, Jr., MD, Thomas Schutz, BA, and David K. Stevenson, MD
Santa Clara and Stanford, California

Cardiovascular and metabolic effects of nifedipine or ritodrine tocolysis were compared; minimal effects were noted when nifedipine was used.

Prolactin levels in umbilical cord blood of human infants: Relation to gestational age, maternal complications, and neonatal lung function 795
C. Richard Parker, Jr., PhD, Paul C. MacDonald, MD, David S. Guzick, MD, John C. Porter, PhD, Charles R. Rosenfeld, MD, and John C. Hauth, MD
Birmingham, Alabama, and Dallas, Texas

In a study involving 543 infants, 361 of whom were preterm, subnormal serum prolactin levels were found in infants who developed respiratory distress syndrome.

(Contents continued on page 18A)

Contents continued from page 17A

Amniotic fluid leukotaxis assay as an early indicator of chorioamnionitis 802
Glenn A. Pankuch, BS, Peter H. Cherouny, MD, John J. Botti, and Peter C. Appelbaum, MD, PhD
Hershey, Pennsylvania

Detection of leukoattractants in amniotic fluid correlates more accurately with histologic chorioamnionitis than culture, gas-liquid chromatography, and Gram stain.

Evidence relating bacterial vaginosis to intraamniotic infection 808
Helayne M. Silver, MD, Rhoda S. Sperling, MD, Patricia J. St. Clair, BA, and Ronald S. Gibbs, MD
San Antonio, Texas

In a two-part study, we provide evidence that links bacterial vaginosis with clinically evident intraamniotic infection.

The incidence of positive amniotic fluid cultures in patients in preterm labor with intact membranes 813
M. Amanda Skoll, MD, Michael L. Moretti, MD, and Baha M. Sibai, MD
Memphis, Tennessee

Amniotic fluid cultures were performed on 127 women in preterm labor with intact membranes; positive cultures were found in 5.5%.

Infection and labor. V. Prevalence, microbiology, and clinical significance of intraamniotic infection in women with preterm labor and intact membranes 817
Roberto Romero, MD, Marina Sirtori, MD, Enrique Oyarzun, MD, Cecilia Avila, MD, Moshe Mazor, MD, Robert Callahan, BS, Virginia Sabo, MSN, Apostolos Athanassiadis, MD, and John C. Hobbins, MD
New Haven, Connecticut

Intraamniotic infection is present in one of every five women with preterm labor and intact membranes who is delivered of a preterm newborn.

Diagnosis of birth asphyxia based on fetal pH, Apgar score, and newborn cerebral dysfunction 825
Larry C. Gilstrap III, MD, Kenneth J. Leveno, MD, Jody Burris, RN, M. Lynne Williams, RN, and Bertis B. Little, PhD
Dallas, Texas

It is inappropriate to diagnose birth affixia unless the infant is severely depressed (1- and 5-minute Apgar scores of ≤ 3) and has severe metabolic acidosis at birth (umbilical artery blood pH <7.000).

First-trimester midgut herniation: A high-frequency transvaginal sonographic study 831
Ilan E. Trimor-Tritsch, MD, Wendy B. Warren, MD, David B. Peisner, MD, and Exenia Pirrone, LPN
New York, New York

The physiologic midgut herniation can reliably be detected between 8 and 12 menstrual weeks by high-frequency transvaginal sonography.

Contents

CORRESPONDENCE

Calcium intake and pregnancy-induced hypertension Stefan Semchyshyn, MD *Livingston, New Jersey* 834

Reply José M. Belizán, MD, and José Villar, MD *Rosario, Argentina, and Bethesda, Maryland* 834

Analytical and clinical sensitivity and specificity in pregnancy testing R. J. Norman, MD *Woodville, South Australia* 835

Reply Kenneth Emancipator, MD *Bethesda, Maryland* 835

Neonatal seizures and cesarean section Peter Boylan, MD, *Dublin, Ireland* 836

Reply William N. Spellacy, MD *Tampa, Florida* 837

Is central venous pressure monitoring "contraindicated" in patients with severe preeclampsia? David G. Woodward, MD, and Mark E. Romanoff, MD *San Antonio, Texas* 837

Reply Steven L. Clark, MD, and David B. Cotton, MD *Provo, Utah, and Houston, Texas* 838

Recurrence of functional ovarian cysts after laparoscopic fenestration Rudy L. DeWilde, MD *Wuppertal, West Germany* 839

Criticism of publication of pharmaceutical-supported research results draws response William J. Ledger, MD *New York, New York* 839

Reply The Editors 840

BOOKS

Books 841

ITEMS

Items 842

CORRECTIONS

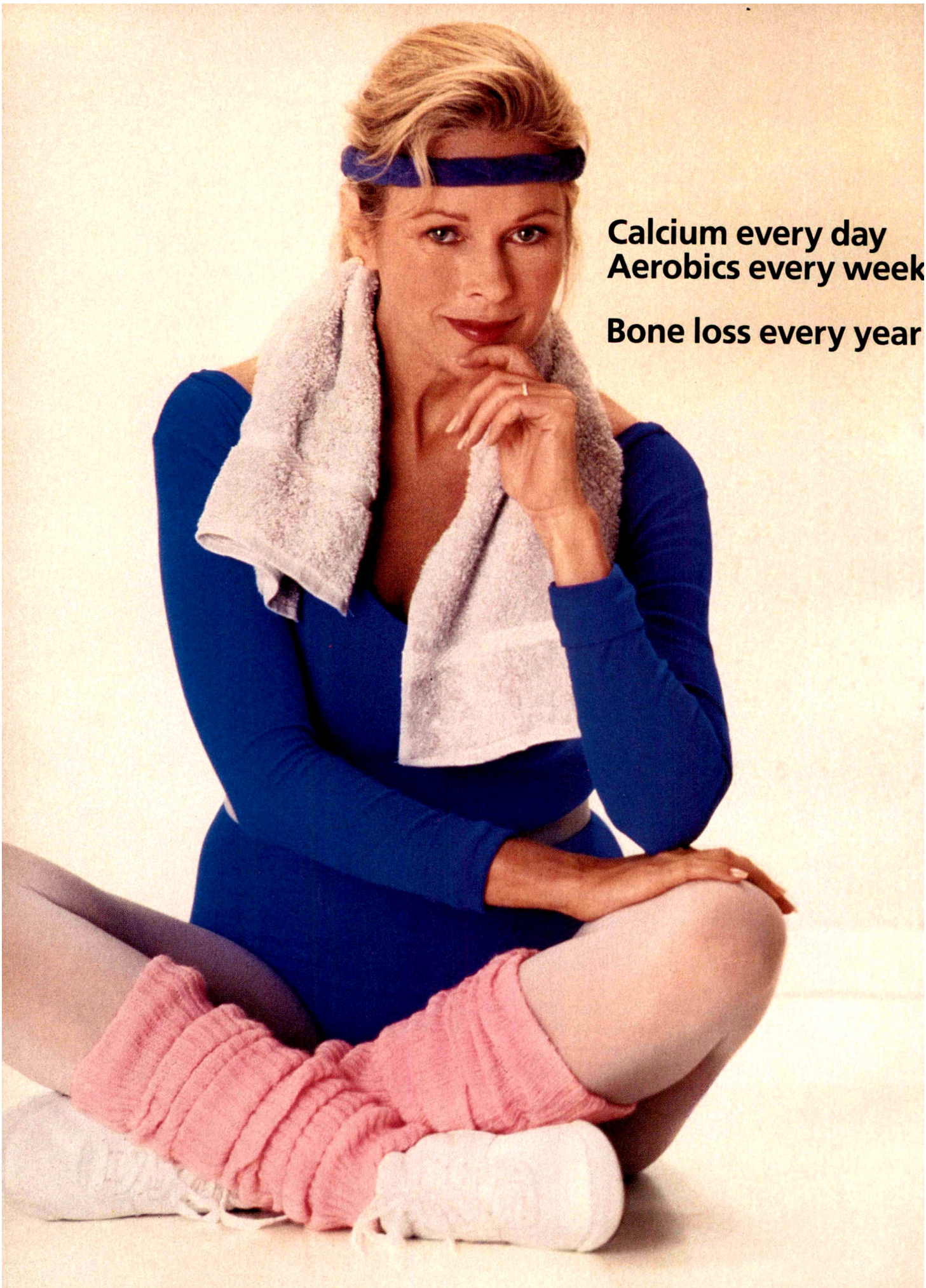
Effects of regular and decaffeinated coffee on fetal breathing and heart rate 669
(Salvador HS, Koos BJ. 1989;160:1043-7.)

Identification of a deoxyribonucleic acid allelic variant for β1-4 galactosyltransferase expression associated with male sperm binding/penetration infertility 816
(Humphreys-Beher MG, Blackwell RE. 1989;160:1160-5.)

INFORMATION FOR AUTHORS

Information for authors 25A

Index to advertisers on page 58A


Calcium every day
Aerobics every week
Bone loss every year

She needs PREMARIN to help prevent further bone loss

Calcium[1] and exercise are not enough to prevent post-menopausal osteoporosis, since estrogen deficiency is the primary cause.[2] PREMARIN is the only brand of estrogen indicated to prevent further bone loss and in a recently published study has been shown to reduce the risk of hip fractures by as much as 66%.[3]

Early PREMARIN therapy offers the best protection

The most significant rate of bone loss occurs in early menopause. PREMARIN can help maintain bone mass, preventing the progression of osteoporosis and its consequences.

Protection continues as long as estrogen therapy continues

Clinical studies show that within three years of menopause when therapy is withdrawn, bone loss begins again.[4]

PREMARIN®

(conjugated estrogens tablets)

Proven benefits for menopause and beyond

See important information on adjacent page.

For moderate-to-severe vasomotor symptoms and for osteoporosis

PREMARIN®
(conjugated estrogens tablets)



0.3 mg 0.625 mg 0.9 mg 1.25 mg 2.5 mg

The appearance of these tablets is a trademark of Wyeth-Ayerst Laboratories.

For atrophic vaginitis

PREMARIN®
(conjugated estrogens)

Vaginal Cream

0.625 mg/g



BRIEF SUMMARY *(FOR FULL PRESCRIBING INFORMATION AND PATIENT INFORMATION, SEE PACKAGE CIRCULARS.)*

PREMARIN® Brand of conjugated estrogens tablets, USP
PREMARIN® Brand of conjugated estrogens Vaginal Cream, in a nonliquefying base

1. ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.
Three independent, case-controlled studies have reported an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for more than one year. This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population-based cancer reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last decade. The three case-controlled studies reported that the risk of endometrial cancer in estrogen users was about 4.5 to 13.9 times greater than in nonusers. The risk appears to depend on both duration of treatment and on estrogen dose. In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semi-annual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration; it therefore appears prudent to utilize such a regimen. Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy. There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equi-estrogenic doses.
2. ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.
The use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing, in later life, a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated as not greater than 4 per 1,000 exposures. Furthermore, a high percentage of such exposed women (from 30% to 90%) have been found to have vaginal adenosis, epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are precursors of malignancy. Although similar data are not available with the use of other estrogens, it cannot be presumed they would not induce similar changes. Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies, including congenital heart defects and limb-reduction defects. One case-controlled study estimated a 4.7-fold increased risk of limb-reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than 1 per 1,000. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. If PREMARIN is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus, and the advisability of pregnancy continuation.

DESCRIPTION: PREMARIN (conjugated estrogens, USP) contains a mixture of estrogens, obtained exclusively from natural sources, blended to represent the average composition of material derived from pregnant mares' urine. It contains estrone, equilin, and 17α-dihydroequilin, together with smaller amounts of 17α-estradiol, equilenin, and 17α-dihydroequilenin as salts of their sulfate esters. Tablets are available in 0.3 mg, 0.625 mg, 0.9 mg, 1.25 mg, and 2.5 mg strengths of conjugated estrogens. Cream is available as 0.625 mg conjugated estrogens per gram.

INDICATIONS AND USAGE: PREMARIN (conjugated estrogens tablets, USP): Moderate-to-severe *vasomotor* symptoms associated with the menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression without associated vasomotor symptoms and they should not be used to treat such conditions.) Osteoporosis (abnormally low bone mass). Atrophic vaginitis. Kraurosis vulvae. Female castration.

PREMARIN (conjugated estrogens) Vaginal Cream is indicated in the treatment of atrophic vaginitis and kraurosis vulvae.

PREMARIN HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETUS (SEE BOXED WARNING).

CONTRAINDICATIONS: Estrogens should not be used in women (or men) with any of the following conditions: 1. Known or suspected cancer of the breast except in appropriately selected patients being treated for metastatic disease. 2. Known or suspected estrogen-dependent neoplasia. 3. Known or suspected pregnancy (see Boxed Warning). 4. Undiagnosed abnormal genital bleeding. 5. Active thrombophlebitis or thromboembolic disorders. 6. A past history of thrombophlebitis, thrombosis, or thromboembolic disorders associated with previous estrogen use (except when used in treatment of breast or prostatic malignancy).

WARNINGS: Estrogens have been reported to increase the risk of endometrial carcinoma (see Boxed Warning). However, a recent large, case-controlled study indicated no increase in risk of breast cancer in postmenopausal women. A recent study has reported a 2- to 3-fold increase in the risk of surgically confirmed gallbladder disease in women receiving postmenopausal estrogens.

Adverse effects of oral contraceptives may be expected at the larger doses of estrogen used to treat prostatic or breast cancer or postpartum breast engorgement; it has been shown that there is an increased risk of thrombosis in men receiving estrogens for prostatic cancer and women for postpartum breast engorgement. Users of oral contraceptives have an increased risk of diseases, such as thrombophlebitis, pulmonary embolism, stroke, and myocardial infarction. Cases of retinal thrombosis, mesenteric thrombosis, and optic neuritis have been reported in oral contraceptive users. An increased risk of postsurgery thromboembolic complications has also been reported in users of oral contraceptives. If feasible, estrogen should be discontinued at least 4 weeks before surgery of the type associated with an increased risk of thromboembolism, or during periods of prolonged immobilization. Estrogens should not be used in persons with active thrombophlebitis, thromboembolic disorders, or in persons with a history of such disorders in association with estrogen use. They should be used with caution in patients with cerebral vascular or coronary artery disease. Large doses (5 mg conjugated estrogens per day), comparable to those used to treat cancer of the prostate and breast, have been shown to increase the risk of nonfatal myocardial infarction, pulmonary embolism, and thrombophlebitis. When doses of this size are used, any of the thromboembolic and thrombotic adverse effects should be considered a clear risk.

Benign hepatic adenomas should be considered in estrogen users having abdominal pain and tenderness, abdominal mass, or hypovolemic shock. Hepatocellular carcinoma has been reported in women taking estrogen-containing oral contraceptives. Increased blood pressure may occur with use of estrogens in the menopause and blood pressure should be monitored with estrogen use. A worsening of glucose tolerance has been observed in patients on estrogen-containing oral contraceptives. For this reason, diabetic patients should be carefully observed. Estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases.

PRECAUTIONS: Physical examination and a complete medical and family history should be taken prior to the initiation of any estrogen therapy with special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolaou smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed. Conditions influenced by fluid retention, such as asthma, epilepsy, migraine, and cardiac or renal dysfunction, require careful observation. Certain patients may develop manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, etc. Prolonged administration of unopposed estrogen therapy has been reported to increase the risk of endometrial hyperplasia in some patients. Oral contraceptives appear to be associated with an increased incidence of mental depression. Patients with a history of depression should be carefully observed. Pre-existing uterine leiomyomata may increase in size during estrogen use. The pathologist should be advised of estrogen therapy when relevant specimens are submitted. If jaundice develops in any patient receiving estrogen, the medication should be discontinued while the cause is investigated. Estrogens should be used with care in patients with impaired liver function, renal insufficiency, metabolic bone diseases associated with hypercalcemia, or in young patients in whom bone growth is not yet complete. If concomitant progestin therapy is used, potential risks may include adverse effects on carbohydrate and lipid metabolism.

Concomitant Progestin Use: The lowest effective dose appropriate for the specific indication should be utilized. Studies of the addition of a progestin for 7 or more days of a cycle of estrogen administration have reported a lowered incidence of endometrial hyperplasia. Morphological and biochemical studies of the endometrium suggest that 10 to 13 days of progestin are needed to provide maximal maturation of the endometrium and to eliminate any hyperplastic changes. Whether this will provide protection from endometrial carcinoma has not been clearly established. There are possible additional risks which may be associated with the inclusion of progestin in estrogen replacement regimens. The choice of progestin and dosage may be important in minimizing possible adverse effects.

The following changes may be expected with larger doses of estrogen:

a. Increased sulfobromophthalein retention.

b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.

c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by PBI, T_4 by column, or T_4 by radioimmunoassay. Free T_3 resin uptake is decreased, reflecting the elevated TBG; free T_4 concentration is unaltered.

d. Impaired glucose tolerance.

e. Decreased pregnanediol excretion.

f. Reduced response to metyrapone test.

g. Reduced serum folate concentration.

h. Increased serum triglyceride and phospholipid concentration.

As a general principle, the administration of any drug to nursing mothers should be done only when clearly necessary since many drugs are excreted in human milk.

Long-term, continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver. However, in a recent, large case-controlled study of postmenopausal women there was no increase in risk of breast cancer with use of conjugated estrogens.

ADVERSE REACTIONS: The following have been reported with estrogenic therapy, including oral contraceptives: breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea; premenstrual-like syndrome; amenorrhea during and after treatment; increase in size of uterine fibromyomata; vaginal candidiasis; change in cervical erosion and in degree of cervical secretion; cystitis-like syndrome; tenderness, enlargement, secretion (of breasts); nausea, vomiting, abdominal cramps, bloating; cholestatic jaundice; chloasma or melasma which may persist when drug is discontinued; erythema multiforme; erythema nodosum; hemorrhagic eruption; loss of scalp hair; hirsutism; steepening of corneal curvature; intolerance to contact lenses; headache, migraine, dizziness, mental depression, chorea; increase or decrease in weight; reduced carbohydrate tolerance; aggravation of porphyria; edema; changes in libido.

ACUTE OVERDOSAGE: May cause nausea, and withdrawal bleeding may occur in females.

DOSAGE AND ADMINISTRATION:

PREMARIN® Brand of conjugated estrogens tablets, USP

1. *Given cyclically for short-term use only.* For treatment of moderate-to-severe *vasomotor* symptoms, atrophic vaginitis, or kraurosis vulvae associated with the menopause (0.3 mg to 1.25 mg or more daily). The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible. Administration should be cyclic (eg, three weeks on and one week off). Attempts to discontinue or taper medication should be made at three- to six-month intervals.

2. *Given cyclically:* Osteoporosis. Female castration. Osteoporosis —0.625 mg daily. Administration should be cyclic (eg, three weeks on and one week off). Female castration—1.25 mg daily, cyclically. Adjust upward or downward according to response of the patient. For maintenance, adjust dosage to lowest level that will provide effective control.

Patients with an intact uterus should be monitored for signs of endometrial cancer and appropriate measures taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

PREMARIN® Brand of conjugated estrogens Vaginal Cream

Given cyclically for short-term use only. For treatment of atrophic vaginitis or kraurosis vulvae.

The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible.

Administration should be cyclic (eg, three weeks on and one week off).

Attempts to discontinue or taper medication should be made at three- to six-month intervals.

Usual dosage range: 2 g to 4 g daily, intravaginally, depending on the severity of the condition.

Treated patients with an intact uterus should be monitored closely for signs of endometrial cancer and appropriate diagnostic measures should be taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

Revised October 1987

References:

1. Nilas L, Christiansen C, Rødbro P: Calcium supplementation and postmenopausal bone loss. *Br Med J* 1984; 289:1103-1106. **2.** Riggs BL: Research Directions in Osteoporosis. Scientific Workshop, National Institutes of Health, February 9-11, 1987 **3.** Kiel DP, Felson DT, Anderson JJ, et al: Hip fracture and the use of estrogens in postmenopausal women. *N Engl J Med* 1987;317:1169-1174. **4.** Christiansen C, Christensen MS, Transbøl I: Bone mass in postmenopausal women after withdrawal of oestrogen/gestagen replacement therapy. *Lancet* 1981;1:459-461.

© 1989 Wyeth-Ayerst Laboratories

WYETH-AYERST LABORATORIES
Philadelphia, PA 19101

American Journal of Obstetrics and Gynecology

Editors

JOHN I. BREWER, *Editor in Chief*
Sandra Fernandez, *Senior Editorial Assistant*

FREDERICK P. ZUSPAN, *Editor*
Donna Peterson, *Editorial Assistant*

E. J. QUILLIGAN, *Editor*
Sherry A. Merryman, *Editorial Assistant*

ALBERT B. GERBIE, *Associate Editor*

Advisory committee on policy

Garland Anderson
William R. Anderson
Mason C. Andrews
E. Forrest Boyd
David C. Figge
A. Jerome Freeland
Edgar O. Horger III
W. Glenn Hurt
James M. Ingram
Walter S. Keifer
Emmet J. Lamb
Roger A. Lobo
Douglas J. Marchant
James H. Maxwell
Arthur M. McCausland
Allan G. W. McLeod
James A. Merrill
Daniel R. Mishell, Jr.
John C. Morrison
Carl J. Pauerstein
Richard Paul
Karl C. Podratz
John A. Read
Ruth W. Schwartz
Henry A. Thiede
Carol Trumbold

Board of corresponding editors

Luigi Carenza, *Rome, Italy*
T. K. A. B. Eskes, *Nijmegen, The Netherlands*
Wolfgang Künzel, *Giessen, West Germany*
Matsuto Mochizuki, *Kobe, Japan*
M. Reuben Peyser, *Tel Aviv, Israel*
Nils-Otto Sjöberg, *Malmö, Sweden*
Gordon M. Stirrat, *Bristol, England*
Claude Sureau, *Paris, France*
Carl Wood, *Melbourne, Victoria, Australia*
Olavi Ylikorkala, *Helsinki, Finland*

Publisher
THE C.V. MOSBY COMPANY, 11830 Westline Industrial Drive,
St. Louis, Missouri 63146-3318
Kay G. Goehler, *Senior Manuscript Editor*

American Journal of Obstetrics and Gynecology

Societies

in addition to those listed on the front cover
the Journal is the official publication
of the following societies

NEW YORK OBSTETRICAL SOCIETY

OBSTETRICAL SOCIETY OF PHILADELPHIA

BROOKLYN GYNECOLOGICAL SOCIETY

ST. LOUIS GYNECOLOGICAL SOCIETY

NEW ORLEANS GYNECOLOGICAL AND OBSTETRICAL SOCIETY

THE OBSTETRICAL AND GYNECOLOGICAL SOCIETY OF MARYLAND

CHICAGO GYNECOLOGICAL SOCIETY

CINCINNATI OBSTETRICAL AND GYNECOLOGICAL SOCIETY

WASHINGTON GYNECOLOGICAL SOCIETY

PITTSBURGH OBSTETRICAL AND GYNECOLOGICAL SOCIETY

BOSTON OBSTETRICAL SOCIETY

LOUISVILLE OBSTETRICAL AND GYNECOLOGICAL SOCIETY

SEATTLE GYNECOLOGICAL SOCIETY

ALABAMA ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS

AKRON OBSTETRICAL AND GYNECOLOGICAL SOCIETY

KANSAS CITY GYNECOLOGICAL SOCIETY

CENTRAL NEW YORK ASSOCIATION OF GYNECOLOGISTS
AND OBSTETRICIANS

NEW JERSEY OBSTETRICAL AND GYNECOLOGICAL SOCIETY

IOWA OBSTETRIC AND GYNECOLOGIC SOCIETY

THE TEXAS ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS

OKLAHOMA CITY OBSTETRICAL AND GYNECOLOGICAL SOCIETY

MEMPHIS OBSTETRICAL AND GYNECOLOGICAL SOCIETY

UTAH OBSTETRICAL AND GYNECOLOGICAL SOCIETY

ROCHESTER OBSTETRICAL AND GYNECOLOGICAL SOCIETY

ARKANSAS OBSTETRICAL AND GYNECOLOGICAL SOCIETY

TENNESSEE STATE OBSTETRICAL AND GYNECOLOGICAL SOCIETY

NEW YORK GYNECOLOGICAL SOCIETY

PACIFIC NORTHWEST OBSTETRICAL AND GYNECOLOGICAL ASSOCIATION

BUFFALO OBSTETRICAL AND GYNECOLOGICAL SOCIETY

SAN FRANCISCO GYNECOLOGICAL SOCIETY

JACKSON GYNECIC SOCIETY

INDIANA OBSTETRICAL AND GYNECOLOGICAL SOCIETY

THE MINNESOTA OBSTETRICAL AND GYNECOLOGICAL SOCIETY

COLUMBUS OBSTETRIC AND GYNECOLOGIC SOCIETY

GREATER HARTFORD OBSTETRICAL AND GYNECOLOGICAL SOCIETY

American Journal of Obstetrics and Gynecology

Copyright © 1989 by The C.V. Mosby Company

Information for Authors

Editors

John I. Brewer, *Editor in Chief*
710 North Fairbanks Court, Suite 8300,
Chicago, IL 60611

Sandra Fernandez, *Senior Editorial Assistant*
Tel.: (312) 908-5266

Frederick P. Zuspan, *Editor*
The Ohio State University, Fifth Floor, Means Hall,
1654 Upham Dr., Columbus, OH 43210

Donna Peterson, *Editorial Assistant*
Tel.: (614) 293-8798

E. J. Quilligan, *Editor*
University of California, Irvine, California College of Medicine, Med Surge 1, Room 109H, Irvine, CA 92717

Sherry A. Merryman, *Editorial Assistant*
Tel.: (714) 856-5092

Albert B. Gerbie, *Associate Editor*
710 North Fairbanks Court, Suite 8300,
Chicago, IL 60611

Publisher
THE C.V. MOSBY COMPANY, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318

Kay G. Goehler, *Senior Manuscript Editor*
Tel.: (314) 872-8370 (ext. 591)

INFORMATION FOR AUTHORS

Editorial policies

The requirements for manuscripts submitted to the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY conform to the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" established by the International Committee of Medical Journal Editors and published in *Annals of Internal Medicine* 1988;108:258-65. Certain requirements unique to our JOURNAL are provided in Information for Authors, published in each issue of the JOURNAL, and in more detail in the *Guide to Writing for the American Journal of Obstetrics and Gynecology*. The latter may be obtained from The C.V. Mosby Company or the Editors on request.

Manuscript submission. Manuscripts should be submitted to one of the three Editors as follows:

1. **Dr. Brewer**—all manuscripts originating from the southeastern quadrant of the United States or Canada and those presented before one of the official sponsoring societies, except the Society for Gynecologic Investigation and The Society of Perinatal Obstetricians.

2. **Dr. Zuspan**—manuscripts from the northeastern quadrant of the United States and from Japan, Israel, Italy, and England, manuscripts written for Clinical Opinion and Current Development, and Letters to the Editors. Dr. Zuspan is responsible for manuscripts from The Society of Perinatal Obstetricians. Manuscripts from The Society of Perinatal Obstetricians should be submitted to Dr. John A. Read, 9102 Lake Steilacoom Point Road, S.W., Tacoma, WA 98498.

3. **Dr. Quilligan**—manuscripts from the north central states (including Ohio), states west of the Mississippi River, Hawaii, Alaska, and abroad (except Japan, Israel, Italy, and England). Dr. Quilligan is responsible for manuscripts from the Society for Gynecologic Investigation. Manuscripts from the Society for Gynecologic Investigation should be submitted to Dr. Roger A. Lobo, Women's Hospital, 1240 North Mission Road, Room 1M2, Los Angeles, CA 90033.

Author's designation of reviewers. When authors submit their manuscripts, they may provide the names and addresses of three reviewers for consideration by the Editors.

Copyright statement. Effective July 1, 1988, all manuscripts must be accompanied by the following written statement, signed by *all* authors: "The undersigned author(s) transfers all copyright ownership of the manuscript [title of article] to The C.V. Mosby Company in the event the work is published. The undersigned author(s) warrants that the article is original; is not under consideration by another publication; and its essential substance, tables, or figures have not been previously published. This restriction does not apply to abstracts or press reports published in connection with scientific meetings. The author(s) confirms the final manuscript has been read and each author's contribution has been approved by the appropriate author. The author(s) responsible for the manuscript must be identified."

Previous publication. If a report by the same author(s) has been previously published in any medium that deals in any respect whatever with the same patients, same animals, same laboratory experiments, or same data, in part or in full, as those reported in the manuscript being submitted, two reprints of the article or two copies of the manuscript, be it a full-length report or an abstract, must be submitted with the manuscript. The author(s) should inform the Editor of the circumstances of the two reports. This requirement also applies to the submission of a manuscript in which a few different patients, animals, laboratory experiments, or data were added to those reported in a previous publication or in a submitted or accepted manuscript. Articles previously published in another language will not be considered.

Human and animal experimentation. It is assumed by the Editors that manuscripts emanating from a particular

institution are submitted with the approval of the requisite authority. Human experimentation that requires local institutional approval must have this approval *before the experiment is started* and approval must be so indicated in the Methods section of the submitted manuscript. Reports of experiments on animals must *state in the Methods section of the manuscript* that the guidelines for the care and use of the animals *approved by the local institution* were followed.

Authorship. For manuscripts with two or more authors, each author must qualify by having participated actively and sufficiently in the study that is being performed and reported. The inclusion of each author in the authorship list of a report is based only (1) on substantial contributions to (a) concept and design, or analysis and interpretation of data and (b) drafting the manuscript or revising it critically for important intellectual content; and (2) on final approval by each author of the version of the manuscript. Conditions 1 (a and b) and 2 must both be met. Others contributing to the work should be recognized separately in an Acknowledgment. In the covering letter that accompanies the submitted manuscript, it must be confirmed that all authors fulfilled both conditions.

Conflict of interest. Authors are expected to inform the Editor, in a letter accompanying the submitted manuscript, of any commercial association that might pose a conflict of interest, such as ownership, stock holdings, equity interests and consultant activities, or patent-licensing situations. Such information is confidential, is not given to the consultants, and does not play a part in the decision of the quality or timeliness of the manuscript. If the manuscript is accepted, the author and the Editor will determine how best to release the information. The usual and customary listing of sources of support and institutional affiliations on the title page is proper and does not imply a conflict of interest; only where there is a possible conflict of interest is the author(s) expected to inform the Editor.

Disclaimer. Statements and opinions expressed in articles and communications herein are those of the author(s) and not necessarily those of the Editor(s) or publisher, and the Editor(s) and publisher disclaim any responsibility or liability for such material. Neither the Editor(s) nor the publisher guarantees, warrants, or endorses any product or service advertised in this publication, nor do they guarantee any claim made by the manufacturer of such product or service.

General requirements for preparation of manuscripts

The original and two good-quality photocopies of the manuscript and three sets of glossy prints of illustrations are required.

Manuscripts must be typed double-spaced on one side only of 22 × 28 cm (8½ × 11 inch) white bond paper with 1-inch margins at top, bottom, and sides. Number pages consecutively in the upper right-hand corner in the following order: title page, condensation, abstract, body of text, acknowledgments, references, legends, and tables.

Title page. The title page (page 1) should contain in sequence the title (concise and suitable for indexing purposes); author line with first name, middle initial, and last name of each author and each author's highest academic degree (both MD and PhD are acceptable); city(ies), state(s) in which the study was conducted; divisional, or departmental, and institutional affiliations at the time the study was performed; source(s) of financial support; presented line, if applicable; disclaimers, if any; name, address, and business and home telephone numbers of author to whom requests for reprints should be addressed (if reprints will not be available, it should be so stated); and name, address, and business and home telephone numbers of author responsible for correspondence concerning the manuscript if different from author to whom reprint requests are addressed. At the bottom of the title page supply a short title for the running head not exceeding 52 characters (including word spaces).

Condensation. On page 2 of the manuscript provide a brief, concise condensation, typed double-spaced, that will appear with the title in publication of the Contents pages of the JOURNAL. It should be a single sentence, limited to a maximum of 25 words, delineating the essential point(s) made in the manuscript.

Abstract page and key words/phrases. On manuscript page 3 type the abstract, double-spaced, with the required margins and headed by the title of the article and name(s) of author(s). Abstracts for regular articles, Current Investigation, Clinical Opinion, and Current Development may not exceed 150 words. Abstracts for case reports and brief communications may not exceed 50 words. Below the abstract list 3 to 5 *key words* or short phrases for indexing purposes.

Text. Do not hesitate to write your manuscript in the first-person, active voice if it is more appropriate to the information you wish to convey. The passive voice is generally more effective for describing techniques or observations, since the emphasis is on the "action" rather than on the person performing the action.

Only standard abbreviations are to be used. Consult the *Council of Biology Editors Style Manual* or the AMA's *Manual for Authors and Editors*. Abbreviations in the title are not acceptable. They should be avoided, if possible, in the abstract. In the text they should be kept to a practical minimum. The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement.

Either the generic, chemical, or proprietary names of drugs may be used. If the generic or chemical name is used, authors may, if they desire, insert the proprietary name in parentheses after the first mention in the text, with the name of the manufacturer and city and state.

Regular articles are customarily organized into the following sections: an introduction and headings that identify Material and Methods, Results, and Comment. Authors may wish to summarize their findings in a short paragraph at the end of the Comment section. This format may not be appropriate for some types of articles.

In the introduction, state concisely the purpose and rationale for the study and cite only the most pertinent references as background.

In the *Material and Methods* section describe briefly (but in sufficient detail to permit other workers to evaluate and reproduce the results) the plan, patients and/or experimental animals and controls, methods and procedures utilized, and statistical method(s) employed.

In the *Results* section present the detailed findings. Include mentions of all tables and/or figures. Avoid duplication of text and supporting material. Emphasize only your important observations; do not compare your

Estimating length of manuscripts

The length of text material (introduction through Comment section) in regular manuscripts accepted for publication normally ranges from 750 to 4200 words (an average of 2000 words). A 4200-word text can seldom be accepted, especially if tables and figures are included. The average manuscript of 2000 words of text with abstract, 3 tables with captions, 2 figures with legends, and references makes a 5.7-page article in the JOURNAL. The 2000 words of text alone make approximately 8 pages of manuscript typed double-spaced with the required 1-inch margins (approximately 250 words per page). A table or figure that occupies both columns of half a JOURNAL page is equivalent to approximately 500 words in manuscript. Thus, if a greater number of illustrations and tables are used, the length of the text should be adjusted accordingly.

observations with those of others. Such comparisons and comments are reserved for the Comment section.

In the *Comment* section state the importance and significance of your findings but do not repeat the details given in the Results section. Limit your opinions to those strictly indicated by the facts in your report. Compare your findings with those of others. No new data should be presented in this section.

Acknowledgments. Acknowledge only persons who have made substantive contributions to the study.

References. A reasonable number are allowed, except in case reports and brief communications (limited to 2) and in manuscripts for the Current Development section (for which there is no limit). Number references consecutively in the order in which they are mentioned in the text. Use the format of the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" (Vancouver style) (Ann Intern Med 1988;108:258-65). Journal titles should conform to abbreviations used in *Cumulated Index Medicus*.

Examples (if six or fewer authors, list all; if seven or more authors, list three then et al.):

JOURNAL: Flamm BL, Fischermann E, Quilligan EJ, et al. Vaginal delivery following cesarean section—use of oxytocin augmentation and epidural anesthesia. AM J OBSTET GYNECOL 1984;148:759-63.

BOOKS: James VHT, Folkerd EJ, Bonney RC, Beranek PA, Reed MJ. Factors influencing estrogen production and metabolism in postmenopausal women with endocrine cancer. In: van Herendael HB, Riphagen FE, Goessens L, van der Pas H, eds. The climacteric, an update. Lancaster, England: MTP Press, 1983:29.

Personal communications and unpublished data, if essential, may be used but not as numbered references. If they are used, they are to be referred to, within parentheses, at the appropriate location in the text. If used, the author(s) must obtain written and signed permission for their use from the individual being quoted. This signed permission must accompany the manuscript when it is submitted to the Editor. Abstracts are not acceptable as numbered references.

Illustrations and tables. Illustrations and tables should supplement, not duplicate, the text; presentation of data in either one or the other will suffice.

A reasonable number of halftone and line illustrations will be reproduced without charge, but special arrangements must be made with the Editors for *color illustrations* at a cost of $525 per page (one side).

For *color photographs* submit original transparencies and two sets of unmounted prints on glossy (smooth-surface) paper. Polaroid prints are not acceptable. Color transparencies must have a color balance (consistency in lighting and film speed) that is acceptable to the author and Editors before acceptance for publication. Please note that 35 mm transparencies are enlarged to twice their original size. If it is important to deviate from this standard, please so indicate when the material is submitted. The *top, first author's last name,* and *figure number* must be indicated on the front of each transparency and the back of each print. Consistency in size of illustrations within the article is strongly preferred.

For *black-and-white illustrations* submit three sets of 3 × 4 inch (minimum) to 5 × 7 inch (maximum) unmounted, glossy photographic prints. All lettering must be done with commercially available paste-on letters (or numbers) or by a professional; typed or freehand lettering is not acceptable. All lettering must be in proportion to the drawing, graph, or photograph. Original drawings, appropriately done in black India ink, roentgenograms, and other material must be submitted as glossy photographic prints with good black-and-white contrast. Consistency in size within the article is strongly preferred. Any special instructions regarding sizing should be clearly noted.

Do not use paper clips or mar the surface of prints in any way.

Figures must be cited consecutively in the text in Arabic numerals and identified thusly on the back of the print (gummed label with): author(s) name(s), title of article, number, and top marked clearly.

Figures will be returned only on request by the author.

Tables should be typed on separate sheets of paper, one table to a page, and included at the end of the text. They should be numbered in Roman numerals. Each table must be cited in sequence at an appropriate point in the text. Captions should be brief yet indicate clearly the purpose or content of each table, and each column should be precisely defined by headings. Abbreviations and special designations should be explained in a footnote to the table. *If a table or any part thereof has been taken from copyrighted material, a legend to the table must give full credit to the original source.* Special arrangements must be made with the Editors for elaborate tables because of space limitations.

Legends to illustrations. Legends for all figures must be typed double-spaced on paper separate from the text of the manuscript, and these pages must be numbered in sequence after the references. Titles should be included in

the legend, *not* on the print. Original magnifications should be provided. *If an illustration has been taken from copyrighted material, the legend must give full credit to the original source.*

Computer-generated illustrations. *Black-and-white illustrations* submitted must be legible and clearly printed in jet-black ink on heavy coated paper with either a glossy or dull finish. Any patterns or shadings must be dark enough for reproduction and must be distinguishable from each other. Lines, symbols, and letters should be both smooth and complete. The legend for the illustration should not appear on the print. On the back of each print the name of the first author and the figure number should be given and the top indicated. Original individual laser or plotter prints are to be submitted *unmounted* with the manuscript. Laser prints should be full size at 300 dots per inch (DPI) or greater full-page resolution; multiple illustrations on a page cannot be accepted. Dot matrix prints and photographic halftones are not acceptable. *Color illustrations* are acceptable, but special arrangements must be made with the Editors. The colors used must be dark enough and of sufficient contrast for reproduction. With the exception of fluorescent colors, all colors can be reproduced in four-color illustrations. The preparation and submitting of color prints should follow the preceding guidelines for black-and-white computer-generated illustrations.

Permissions. Direct quotations, tables, or illustrations that have appeared in copyrighted material must be accompanied by *written permission* for their use from the copyright owner and original author along with complete information as to source. Photographs of identifiable persons must be accompanied by signed releases or else all recognizable features masked.

Requirements for special sections

Case reports and brief clinical and basic science communications. Limit of 700 words, 2 references. Include abstract of 50 words maximum, 3 to 5 key words/phrases for indexing purposes, and short title. If tables and/or figures are used, an equivalent number of words must be deducted from the total (see "Estimating Length of Manuscript").

Current Investigation. Same requirements as for regular article.

Clinical Opinion. Limit of 3000 words. Include abstract of 50 to 150 words, 3 to 5 key words/phrases, and short title. Submit to Dr. Zuspan.

Current Development. Limit of 6000 words. Include abstract of 50 to 150 words, 3 to 5 key words/phrases, and short title. Submit to Dr. Zuspan.

Correspondence. Two types of correspondence will be considered for publication. (1) A Letter to the Editors commenting on an article that has appeared in the JOURNAL should be brief and directly related to the published article. The editorial staff reserves the right to shorten letters if necessary and to make minor editorial alterations without reference to the writer. Letters may be published together with a reply from the original author. If the original author does not respond, a notation indicating "Response declined" will be published. As space for letters is limited, only a selection of letters submitted may be published. (2) A brief case presentation or a short report of a pertinent observation in the form of a Letter to the Editors will be considered for publication. All letters should be typed double-spaced. The original and a good photocopy must be submitted. Letters should be sent to Dr. Zuspan.

Announcements. Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60 U.S., and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, The C.V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, MO 63146-3318.

Books. Books received will be listed in the JOURNAL. They should be sent to Dr. Gerbie. No books will be returned.

Reprints

Reprints of articles must be obtained from the author. The corresponding author will receive a price schedule and order form at the time of publication. Reprints in quantities must be ordered from the publisher with the author's consent.

Business communications

Communications of a business nature and all advertising communications should be addressed to: Journal Publisher, The C.V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, MO 63146-3318, or call Journal Advertising Production Manager (314) 872-8370.

Checklist

—Letter of submission
—Copyright transfer letter
—Copies of other manuscripts containing duplicated data (see paragraph "Previous Publication")
—Original and two photocopies of manuscript
—Title page
—Title of article
—Full name(s), highest academic degree(s), and affiliations of author(s)
—Line citing financial support
—Author to whom correspondence is to be sent, including address and business and home telephone numbers
—Reprint requests line or line stating reprints not available
—Short title
—Condensation (double-spaced)
—Abstract (double-spaced), 3 to 5 key words/phrases
—Article proper (double-spaced)
—References (double-spaced), on a separate sheet
—Legends (double-spaced), on a separate sheet
—Tables (double-spaced), on a separate sheet
—Illustrations, properly labeled (three sets of glossy prints)
—Permission to reproduce published material
—Informed consent for patient photographs

MATERNAL-FETAL MEDICINE OPPORTUNITIES

Representative sample of our ongoing opportunities nationwide

CALIFORNIA:
Private Practice Partnership

794 M/F - Outstanding partnership opportunity in central California to assist the current perinatologist in developing a regional perinatal center and network based at a modern 450-bed full service community hospital with over 5,000 deliveries annually. Candidates with a keen interest in ultrasound, genetic procedures, UBS, and teaching should explore this opportunity. There is a documented service area of over two million residents, and you are only one hour from Yosemite and a pleasant drive to the Pacific Shores.

COLORADO:
Hospital-Based/Private Practice

772 M/F - A 328-bed premier Denver area hospital is seeking a perinatologist to complement their 40 active OB/GYN's and full range of obstetrical services. Documented service area is over 800,000 with annual deliveries of 4,200. Level III NICU expanding to 20 beds. Coverage provided by other perinatologists with potential for formal associations. Amenities include the sophistication of a cosmopolitan city, yet minutes away from fantastic skiing, mountain climbing and unlimited water sports.

IDAHO:
Hospital-Based/Private Practice

631 M/F - Opportunity to become the second perinatologist in southeastern Idaho, and to affiliate with a 300-bed regional medical center (Level III NICU) which provides a statewide perinatal outreach program. Service area entails 400,000 with over 3,200 annual deliveries. If you enjoy snow skiing, hiking, white water rafting, and a quality of lifestyle like no other, please inquire.

MISSOURI:
Associate/Private Practice

898 M/F - A well-established perinatologist in southwestern Missouri is seeking an associate to join a thriving practice, affiliated with a state-of-the-art 505-bed regional referral center. Service area encompasses over 700,000. Facility has a 52-bed NICU staffed by four neonatologists and a neonatal transport is in place (over 700 admissions per year). Excellent compensation package with long-term growth potential.

OHIO:
Faculty Position/Hospital-Based

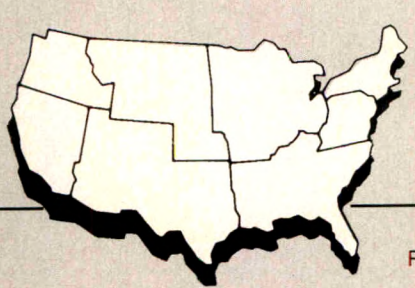
831 M/F - A 814-bed regional tertiary care center, in conjunction with the Medical College of Ohio, is seeking their second perinatologist. This is a salaried faculty appointment with the option of flexible private practice capability. The position provides an excellent opportunity to satisfy your professional academic desires within a modern Level III women and children's center, located in a stable northwestern Ohio lakeshore community.

MASSACHUSETTS:
Full-Time Faculty Position

973 OB/GYN/M/F - A 900-bed tertiary care regional referral center in Western Massachusetts is seeking their third perinatologist. This prestigious institution in the designated western campus of Tufts University School of Medicine. The facility has an encatchment area of over 1,000,000 people and it has the second largest maternity unit in the state with over 6,000 deliveries a year. There were 220 transfer deliveries last year and this service continues to grow rapidly. The OB/GYN department owns its own ultrasound equipment (ATL Mark 9) which is housed in the department, plus two full-time technicians and a computerized data base system. The 600 member medical staff is comprised of a full array of subspecialties plus nurse midwives and 20 OB/GYN residents. A new woman & children's center will be completed in early 1991. Competitive compensation with full benefit package is being offered.

Voluntary Hospitals of America

- Over 800 hospitals in 45 states
- Excellent financial and comprehensive benefit packages
- No cost or obligation for our services



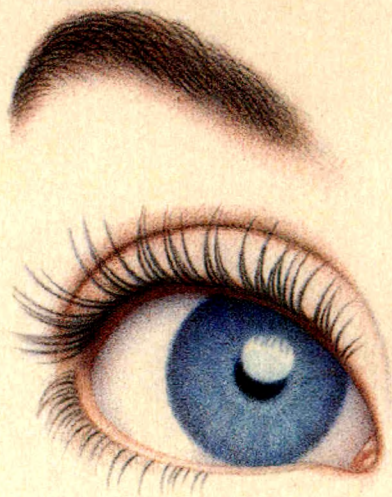
For further information, please forward your curriculum vitae and/or call the toll-free number listed below.

Susan Panther
Physician Placement Specialist
1-800-626-1857

VHA®

VHA PHYSICIAN PLACEMENT SERVICES
Recruitment • Consulting • Planning

Suite 250, Browenton Place • 2000 Warrington Way • Louisville, KY 40222
Phone (502) 423-9622 • Toll free numbers: 1-800-626-1857 • In Kentucky 1-800-292-1856

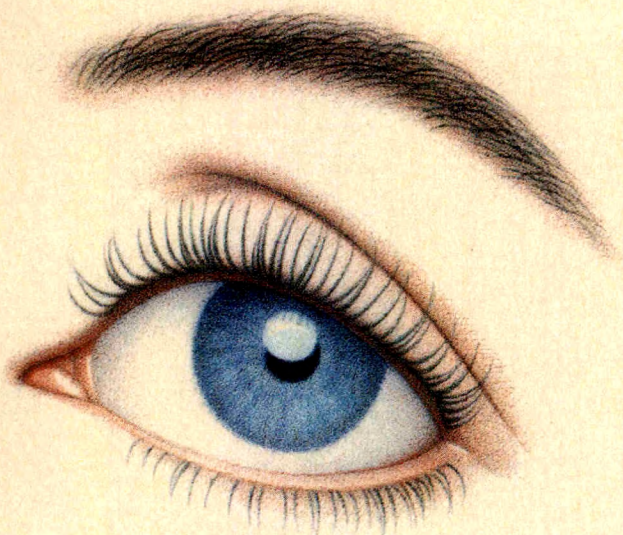


"When breakthrough bleeding happened to me I was scared, and that's why I called my doctor."

She deserves a







better start

TRI·LEVLEN®

Levonorgestrel and ethinyl estradiol tablets—Triphasic regimen

54%* less breakthrough bleeding* than Ortho-Novum® 7/7/7

Demonstrated in the largest parallel comparative trial of triphasic OCs to date†[1]

*Serious as well as minor side effects have been reported with the use of all oral contraceptives. The physician should remain alert to the earliest symptoms of serious disease and discontinue oral contraceptive therapy when appropriate. Please see full prescribing information, a brief summary of which follows.

†100 women randomly assigned to Tri-Levlen® tablets or Ortho-Novum® 7/7/7; 50 women placed on nonhormonal contraception as a control. Evaluations were made at baseline and once a month for 6 months.

One cycle of Tri-Levlen® tablets therapy provides: ethinyl estradiol–30 mcg/day for 6 days, 40 mcg/day for 5 days and 30 mcg/day for 10 days; levonorgestrel–50 mcg/day for 6 days, 75 mcg/day for 5 days and 125 mcg/day for 10 days. One cycle of Ortho-Novum® 7/7/7 therapy provides: ethinyl estradiol–35 mcg/day for 21 days; norethindrone–500 mcg/day for 7 days, 750 mcg/day for 7 days and 1000 mcg/day for 7 days.

Ortho-Novum® is a registered trademark of Ortho Pharmaceutical Corp.

BERLEX Dedicated to innovation in OB/GYN medicine

TRI-LEVLEN® 28 Levonorgestrel and ethinyl estradiol
TRI-LEVLEN® 21 tablets–Triphasic regimen

BRIEF SUMMARY
Tri-Levlen®—6 brown tablets, each containing 0.050 mg of levonorgestrel (d(-)-13 beta-ethyl-17-alpha-ethinyl-17-beta-hydroxygon-4-en-3-one), a totally synthetic progestogen, and 0.030 mg of ethinyl estradiol (19-nor-17α-pregna-1,3,5(10)-trien-20-yne-3, 17-diol); 5 white tablets, each containing 0.075 mg levonorgestrel and 0.040 mg ethinyl estradiol; 10 light-yellow tablets, each containing 0.125 mg levonorgestrel and 0.030 mg ethinyl estradiol (7 light-green tablets containing inert ingredients are included in the 28-day triphasic regimen).

Indications and Usage—Tri-Levlen Tablets are indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OCs) as a method of contraception.

Contraindications—OCs should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5). 8. Benign or malignant liver tumor which developed during the use of OCs or other estrogen-containing products.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.
The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems:* An increased risk of thromboembolic and thrombotic disease associated with the use of OCs is well established. Three principal studies in Great Britain and three in the United States have demonstrated an increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OCs are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.
CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.
MYOCARDIAL INFARCTION—An increased risk of myocardial infarction associated with the use of OCs has been reported, confirming a previously suspected association. These studies, conducted in the United Kingdom, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk of developing myocardial infarction, regardless of whether the patient was an OC user or not. OCs, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to myocardial infarction) are about twice as likely to have a fatal myocardial infarction as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal infarction compared to users who do not smoke but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, the amount of smoking is also an important factor. In determining the importance of these relative risks, however, the baseline rates for various age groups must be given serious consideration. The importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; it is quite likely that the same synergistic action exists, but perhaps to a lesser extent.
RISK OF DOSE—In an analysis of data derived from several national adverse reaction reporting systems, British investigators concluded that the risk of thromboembolism, including coronary thrombosis, is directly related to the dose of estrogen used in OCs. Preparations containing 100 mcg or more of estrogen were associated with a higher risk of thromboembolism than those containing 50-80 mcg of estrogen. Their analysis did suggest, however, that the quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the United States.
ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the U.K. estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OCs according to age, smoking habits and duration of use. The overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), the risk being concentrated in older women, in those with a long duration of use and in cigarette smokers. It was not possible, however, to examine the interrelationships of age, smoking and duration of use, nor to compare the effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all of these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess the magnitude of the relative risk for this younger age group. The available data from a variety of sources have been analyzed to estimate the risk of death associated with various methods of contraception. The estimates of risk of death for each method include the combined risk of the contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OCs) plus the risk attributable to pregnancy or abortion in the event of method failure. This latter risk varies with the effectiveness of the contraceptive method. The study concluded that the mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OCs in women over 40 who smoke. The lowest mortality is associated with the condom or diaphragm backed up by early abortion. The risk of thromboembolic and thrombotic disease associated with OCs increases with age after approximately age 30 and, for myocardial infarction, is further increased by hypertension, hypercholesterolemia, obesity, diabetes or history of preeclamptic toxemia and, especially, by cigarette smoking. The physician and the patient should be alert to the earliest manifestations of thrombophlebitis and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A four-to-six-fold increased risk of postsurgery thromboembolic complications has been reported in OC users. If feasible, OCs should be discontinued at least 4 weeks before surgery of a type associated with an increased risk of thromboembolism or prolonged immobilization.
PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Great Britain involving cerebrovascular disease and another study in the United States concerning myocardial infarction suggest that an increased risk of these conditions in users of OCs persists after discontinuation of the OC. In the British study, the risk of cerebrovascular disease remained elevated in former OC users for at least six years after discontinuation. In the U.S. study, an increased risk of myocardial infarction persisted for at least 9 years in women 40- to 49-years-old who had used OCs for five or more years. The findings in both these studies require confirmation since they are inconsistent with other published information.
2. *Ocular Lesions:* There have been reports of neuro-ocular lesions, such as optic neuritis or retinal thrombosis, associated with the use of OCs. Discontinue OC medication if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.
3. *Carcinoma:* Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases the frequency of carcinoma of the breast, cervix, vagina and liver. Certain synthetic progestogens, none currently contained in OCs, have been noted to increase the incidence of mammary nodules, benign and malignant, in dogs. In humans, three case-control studies have reported an increased risk of endometrial carcinoma associated with the prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OCs. Of the cases found in women without predisposing risk factors for adenocarcinoma of the endometrium (e.g., irregular bleeding at the time OCs were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These products are no longer marketed. No evidence has been reported suggesting an increased risk of endometrial cancer in users of conventional combination or progestogen-only OCs. Several studies have found no increase in breast cancer in women taking OCs or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women treated with OCs, found an excess risk in the subgroups of OC users with documented benign breast disease. A reduced occurrence of benign breast tumors in users of OCs has been well-documented. In summary, there is at present no confirmed evidence from human studies of an increased risk of cancer associated with OCs. Close clinical surveillance of all women taking OCs is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules, fibrocystic disease or abnormal mammograms should be monitored with particular care if they elect to use OCs.
4. *Hepatic Tumors:* Benign hepatic adenomas have been found to be associated with the use of OCs. One study showed that OC formulations with high hormonal potency were associated with a higher risk than lower potency formulations. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users of OCs. Two studies relate risk with duration of use of OCs, the risk being much greater after 4 or more years of OC use. While hepatic adenoma is a rare lesion, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women taking OCs. The relationship of these drugs to this type of malignancy is not known at this time.
5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring and Malignancy in Female Offspring:* The use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing in later life a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence at the present time that OCs further enhance the risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OCs. Furthermore, a high percentage of such exposed women (from 30 to 90%) have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with the use of other estrogens, it cannot be presumed that they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with the use of sex hormones, including OCs, in pregnancy. One case-control study has estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OCs, hormonal withdrawal tests for pregnancy or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than one in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OCs. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OCs is unknown. It is recommended that, for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period and further use of OCs should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and the advisability of continuation of the pregnancy should be discussed in the light of these risks. It is also recommended that women who discontinue OCs with the intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this recommendation. The administration of progestogen-only or progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.
6. *Gallbladder Disease:* Studies report an increased risk of surgically confirmed gallbladder disease in users of OCs and estrogens. In one study, an increased risk appeared after 2 years of use and doubled after 4 or 5 years of use. In one of the other studies, an increased risk was apparent between 6 and 12 months of use.
7. *Carbohydrate and Lipid Metabolic Effects:* A decrease in glucose tolerance has been observed in a significant percentage of patients on OCs. For this reason, prediabetic and diabetic patients should be carefully observed while receiving OCs. An increase in triglycerides and total phospholipids has been observed in patients receiving OCs. Three studies have been performed with the Tri-Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets Triphasic Regimen) formulation and no significant alterations in lipid metabolism were noted, with the exception of a slight increase in triglyceride levels in one study. The clinical significance of these findings remains to be defined.
8. *Elevated Blood Pressure:* An increase in blood pressure has been reported in patients receiving OCs. In some women, hypertension may occur within a few months of beginning OC use. In the first year of use, the prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. The prevalence in users increases, however, with longer exposure, and in the fifth year of use is two-and-a-half to three times the reported prevalence in the first year. Age is also strongly correlated with the development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure when given OCs. Hypertension that develops as a result of taking OCs usually returns to normal after discontinuing the drug.
9. *Headache:* The onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent or severe, requires discontinuation of OCs and evaluation of the cause.
10. *Bleeding Irregularities:* Breakthrough bleeding, spotting and amenorrhea are frequent reasons for patients discontinuing OCs. In breakthrough bleeding, as in all cases of irregular bleeding from the vagina, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or a change to another formulation may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase the risk of thromboembolic disease. Women with a past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuation of OCs. Women with these preexisting problems should be advised of this possibility and encouraged to use other contraceptive methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.
11. *Ectopic Pregnancy:* Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.
12. *Breast-feeding:* OCs given in the postpartum period may interfere with lactation. There may be a decrease in the quantity and quality of the breast milk. Furthermore, a small fraction of the hormonal agents in OCs has been identified in the milk of mothers receiving these drugs. The effects, if any, on the breast-fed child have not been determined. If feasible, the use of OCs should be deferred until the infant has been weaned.

Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OCs. The pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Papanicolaou smear and relevant laboratory tests. As a general rule, OCs should not be prescribed for longer than 1 year without another physical examination and PAP smear being performed. 2. Under the influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with a history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while taking OCs should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug-related. 4. OCs may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence of jaundice while receiving OC therapy. If jaundice develops in any patient receiving such drugs, the medication should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution in such patients. 7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. The clinical significance of this is yet to be determined. 8. Serum folate levels may be depressed by OC therapy. Since the pregnant woman is predisposed to the development of folate deficiency and the incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OCs, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of OC therapy when relevant specimens are submitted. 10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OCs: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test.

Information for the Patient—See Patient Package Labeling
Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.
Carcinogenesis—See "Warnings" section for information on the carcinogenic potential of OCs.
Pregnancy—Pregnancy Category X. See "Contraindications" and "Warnings."
Nursing Mothers—See "Warnings."
Adverse Reactions—An increased risk of the following serious adverse reactions has been associated with the use of OCs (see "Warnings"): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebrai thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies.
There is evidence of an association between the following conditions and the use of OCs, although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.
The following adverse reactions have been reported in patients receiving OCs and are believed to be drug-related: Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally: gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; dysmenorrhea; amenorrhea during and after treatment; temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement and secretion; change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses.
The following adverse reactions have been reported in users of OCs, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, hemolytic uremic syndrome.
Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OCs by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.
Dosage and Administration—To achieve maximum contraceptive effectiveness, Tri-Levlen Tablets (Levonorgestrel and Ethinyl Estradiol Tablets—Triphasic Regimen) must be taken exactly as directed and at intervals not exceeding 24 hours. (If Tri-Levlen is first taken later than the first day of the first menstrual cycle of medication or postpartum, contraceptive reliance should not be placed on it until after the first 7 consecutive days of administration. The possibility of ovulation and conception prior to initiation of medication should be considered.) Any time the patient misses 1 or 2 brown, white or light-yellow tablets, she should also use another method of contraception until she has taken a tablet daily for 7 consecutive days.
For full details on dosage and administration see prescribing information in package insert. 60611-1 5/86

TRI·LEVLEN®
Levonorgestrel and ethinyl estradiol tablets–
Triphasic regimen

Reference: 1. Data on file, Berlex Laboratories, Inc.

TRI-LEVLEN is a registered trademark of Berlex Laboratories, Inc.

BERLEX™
Dedicated to innovation in OB/GYN medicine
BERLEX Laboratories, Inc., Wayne, New Jersey 07470
© 1989, Berlex Laboratories, Inc. All rights reserved. 430-118

American Journal of Obstetrics and Gynecology

Founded in 1920

volume 161 *number* 3 SEPTEMBER 1989

TRANSACTIONS OF THE FIFTY-FIRST ANNUAL MEETING OF THE SOUTH ATLANTIC ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS

The challenge of change revisited

Presidential address

Frank C. Greiss, Jr., MD
Winston-Salem, North Carolina

In his commencement address to the class of 1988 Thomas K. Hearn, Jr., President of Wake University, made the following statement: "We humans are forever in search of a permanent and unchanging reality, some state of being' to arrest the moving flow of 'becoming' in time. But our efforts to seize and hold a permanent order are in vain. Change is not merely Nature's first law. It is perhaps Nature's only law."

One could deem Dr. Hearn's statement as being so self-evident that it did not need to be said. But if so, consider the following: "The challenge faced by physicians and medical organizations in a world of increasing complexity and change threatens to confuse, bewilder, and overwhelm us. Today we live in a wilderness of new knowledge. It is a challenge to our society as a whole. This challenge of change is vastly greater than at any other time in history because the tempo is so much faster, the social complexities so much more subtle. We must constantly review our methods and traditions in the light of changing knowledge. We must submit to a searching self-analysis to establish the justification for our prejudices."[1] Does not this statement describe, in part, organized medicine's position in 1989? Certainly, but when we consider that it was made 25 years ago by Frank R. Lock, the 29th president of our Association, in his presidential address titled "The Challenge of Change" to the American College of Obstetricians and Gynecologists, it is obvious that Dr. Hearn's reminder did need to be said.

After illustration of how much our perceptions focus on our own memories and our own situations, I will now fall into the same trap and review how vastly greater things may seem to have changed in the past 25 years. In defense of this, I view the rate of change as similar to that of an asymptote, accelerating more rapidly from the abscissa in 1989 than in 1964.

The changes

Although medicine has made dramatic scientific and technologic progress in the past quarter of a century, the changes that have had the most impact on medicine as an institution have been in the attitudinal, social, financial, and ethical spheres.

Attitudinal changes. A general loss of public faith in major institutions in American society has occurred. Not only medicine but education in general, organized religion, government, Congress, the press, the legal profession, and others have experienced this loss of confidence in our nation's leaders and its institutions. This resulted from a series of major events in American life—the Vietnam war, the civil rights revolution, Watergate, the environmental, consumer, and women's liberation movements, the escalation of the national debt of the 1980s, and most recently the advent of leveraged corporate buyouts. Since 1976 medicine's relatively high standing has declined more than in other institutions so that in terms of public confidence we now

From the Department of Obstetrics and Gynecology, Bowman Gray School of Medicine of Wake Forest University.
Presented at the Fifty-first Annual Meeting of the South Atlantic Association of Obstetricians and Gynecologists, Hot Springs, Virginia, January 29–February 1, 1989.
Reprint requests: Frank C. Greiss, MD, Department of Obstetrics and Gynecology, Bowman Gray School of Medicine, 300 S. Hawthorne Road, Winston-Salem, NC 27103.
6/6/12991

rank behind the military and major educational institutions.

High levels of confidence in a particular institution are often associated with three factors: its degree of altruistic behavior and sense of social mission, its level of science, and the degree of nonpolitical behavior it displays. During the past decade there has been little change in our political activity and there has been a marked increase in our scientific knowledge and capabilities. One is left with the conclusion that medicine's declining level of public trust is associated with the growing commercialization of health care, a move away from a traditional image of altruistic behavior and a sense of social mission.

Perhaps of equal importance in changing public perceptions has been a progressive decrease in the value placed on a fundamental American characteristic—personal accountability. Journalist David Brinkley commented on this trend at a United Way luncheon in Winston-Salem, North Carolina, and noted that for the past 50 years the American people have been told by the federal government when things were not going right, "not to worry, we'll fix it for you"; hardly an atmosphere that promotes anything but dependency and an "It wasn't my fault" attitude.

Social and financial changes. Sweeping social and financial changes have occurred in medicine in the past 25 years. They are so interwoven that they must be discussed together. In 1965 Congress passed the Social Security amendments that created Medicare and Medicaid. Organized medicine actively opposed this legislation. After it was passed, physicians not illogically interpreted that the government had said that it would pay for the medical care of our nation's elderly and indigent. Why then should physicians continue to provide free care (perhaps the beginning erosion of our image)? Similarly, institutions like the Kate Bitting Reynolds Health Care Trust in North Carolina diverted hospital supplements for indigent care to other charitable purposes.

It did not take long for Congress to realize they had a tiger by the tail, one getting bigger all of the time because of the increasing cost of medical technology and hospital care. The general inflation rate of the 1970s and the lack of financial incentives to contain costs further accentuated the problem. Between 1965 and 1980 total expenditures increased at an average annual rate of 12.5%, an increase from 5.9% to 9.8% of the gross national product. Federal concern for rising health care costs appeared early in the 1970s through piece-meal legislation. Constraints included mandatory utilization review (1972), an attempt to offer competitive alternatives to fee-for-service practice (Health Maintenance Organization Act of 1974), capital expenditure controls through the Certificate of Need program (1974), and toward the end of the decade, restriction of Medicare payments to levels that were below actual cost. These approaches were largely ineffective and they increased administrative red tape enormously.

In the 1980s there have been implementation of diagnostic related groups to control hospital costs, initiation of second opinion programs, the advent of outpatient and same-day surgery, and increased attention to quality assurance programs, all of which tend to undermine physician-patient relationships. Many would agree that the publication of hospital mortality statistics and the proliferation of peer review organizations and functions have been more divisive than productive.

In 1989 the Hsiao resource-based relative value scale threatens to cap expenditures for physician services as the scenario of expanding Medicare benefits reaches new heights with implementation of the Catastrophic Health Care Bill of 1988. Coverage now extends into the private home, hospice, and nursing home settings and enters the untrodden area of payments for prescription drugs. Those of you who have read the fine print of this bill understand that Congress knows it will be expensive and has passed a significant part of the cost to the recipients. The estimated 40% of Medicare beneficiaries who earn enough to pay federal income taxes will be subject to a surcharge on their taxes up to a maximum of $1050 per person or $2100 per couple in 1993. This represents an unprecedented departure from traditional funding of public programs. For the first time, funding has been transferred within a specific user group to support the poor rather than spreading the cost over the general populace such as occurs with public education or public housing.

On this background, it is not surprising that since 1982 the annual Public Opinion Poll of the American Medical Association[2] has rated cost as the main problem (62% to 68%) that faces health care and medicine in the United States today. However, physician perspectives are changing as a result of a new boy on the street. Whereas 44% to 50% of physicians believed the main problem was cost related in the 1981 through 1984 polls, professional liability was defined for the first time in 1986 as the biggest problem that confronts American medicine. I am certain that many of you in this Association would concur because we as obstetricians-gynecologists have seen the malpractice problem escalate costs, compromise or preclude obstetric care, alter practice patterns, interfere with physician-patient relationships, and most unfortunately have divisive impact within the medical community.

Ethical changes. Time permits but a few comments about the ethical dilemmas presented by social change and scientific progress in our increasingly litigious society. The Supreme Court decision of 1973 that legalized abortion has been soul-wrenching to many and remains controversial. The cases of Karen Ann Quinlan

in New Jersey and Baby Doe in Indiana have been viewed by many as unnecessary legal incursions into family decisions that traditionally have been solved as atraumatically as possible within an acceptable ethic fostered by the physician-patient relationship. In our specialty we have the capability to fertilize anyone's ovum with anyone's sperm and to nourish it to maturity in anyone's uterus. The new ethical problems presented by this technology have already prompted an in-depth study by the American Fertility Society. Perhaps, however, an even greater ethical issue has not been directly addressed. Last year >11% of our gross national product was spent for health care, whereas more than an estimated 35 million Americans were medically uninsured.

Public perceptions

After review of an impressive variety of changes that have impacted on medical practices during the past 25 years, perhaps I should now direct attention to our current public image for it is not all bad in terms of the individual physician.

Physicians. In the average American home, a television set is on 7 hours and 38 minutes of each day.[3] Health-related information occurs many times each hour and there are many physicians. Inasmuch as television presents the most broadly shared images of medical sciences and physicians, we should simultaneously be flattered and concerned by what Americans think they know about physicians. Physicians can solve many problems, even medical. They display infinite accessibility, patience, wisdom, and a lack of material interest. They are authoritative and daring; 40% are engaged in an unusual practice yet to be proven and 13% actually disobey or violate a rule to do something chancy. And they are successful 90% of the time.

This overidealized image of the television physician leads to unrealistic expectations that are reflected in public opinion surveys. A total of 54% of Americans rate physicians high in honesty and ethics. They resent, however, when physicians are not unselfish as they are on television. Half or more also respond that physicians do not explain things well, do not care as much as they used to, do not charge reasonable fees. They also believe that people are beginning to lose faith in physicians. However, the negative feeling Americans express toward physicians to surveyed items are not unusual—they feel that way toward many other professionals as well. In relative terms, the public image of physicians is quite good. In the April 1986 survey of the National Opinion Research Center, 46% of people had a great deal of confidence in those who run medical institutions—No. 1 in comparison with other institutions.[2]

Professional liability. The public is increasingly cognizant of a general liability crisis and a substantial majority is now sympathetic with physicians on a number of specific issues related to malpractice insurance and lawsuits. A total of 67% of people believe physicians have to pay too much for malpractice insurance and between 43% and 50% think that people who file malpractice suits are just looking for an easy way to make money. Between 56% and 70% think there should be a limit on the amount of money that can be awarded to someone who sues because of malpractice, 79% favor setting limits on lawyer's fees in large awards, and 41% to 59% think jury awards are too high.

Problems facing medicine. In the 1986 American Medical Association Survey, >63% of the public believed that cost is the main problem that faces medicine today, exceeding other considerations by 50%. Paradoxically, the public will press for more health care, not less. A similar paradox exists in how Americans view their own health care. On one hand, they are highly critical of the U. S. health care system as a whole; on the other hand, most people are highly satisfied with the health care they receive. Americans are not in favor of giving up their personal physicians for care delivered by low-cost physician groups. However, they are enthusiastic about requiring low income persons to use less costly clinics or health maintenance organizations.

Our position in 1989

The foregoing should reassure today's physicians. Our greatest ally, the public, is basically supportive of their own health care and is generally sympathetic to their physician's problems. Although the public perceives the problem of cost, it desires increased care, and it defers the solution of the blight of the poor to the government. The government, on the other hand, is faced with a continuing budget deficit and the largest national debt in history. Because many would consider it to be political suicide to cap services, our legislatures are increasingly shifting the onus to the health care delivery system. And oftentimes physicians find themselves caught in the middle. Government policies of reactive rationing, as opposed to prospective allocation, daily threaten physicians as they attempt to cope with the complexities of cost control and quality assurance, the specter of malpractice, and the difficult task of monitoring physician activities in the office, in hospitals, and in the courts. Perhaps most threatening is the relative lack of input physicians have had in dealing with these problems for traditionally, medicine is used to "calling the shots." We have yet to come to grips with the fact that society is challenging a major tenet of our professionalism, its autonomy. Let us digress for a moment and consider this professionalism of ours.

Professionalism. All of us know or think we know what professionalism is and all of us think we are professionals. However, when I asked a number of people about the elements comprised by professionalism,

I was surprised how difficult it was for them to verbalize a consistent group. This prompted me to go back to the Oxford English Dictionary in which any one of a number of definitions could be found. My own biases led me to select one attributed to Maurice in 1839: "that kind of business which deals primarily with men as men, and is thus distinguished from a trade which provides for the external wants or occasions of men."[4] Such a definition is obviously vague in terms of the description of the elements of professionalism. In 1915 Abraham Flexner espoused more definitive criteria[5] and in 1987 Reed and Evans[6] described components of professionalism that embody those described by Flexner but put them in more currently applicable terms. Those that I think are pertinent to our discussion are as follows: (1) the members of the profession determine its standards of knowledge and expertise, (2) the profession is given authority to license practitioners by state boards made up largely of members of the profession, (3) social policy and legislation that relate to the profession are heavily influenced by members of the profession, (4) a profession has a code of ethics that governs practice, the tenets of which are more stringent than legal controls, (5) a service orientation supercedes the proprietary interest of professionals, and (6) a profession is largely free of lay control with its practitioners exercising a high degree of occupational autonomy.

Reed and Evans[6] point out that professional autonomy subsumes most of the other preconditions of professionalism and that autonomy and professionalism are crucially linked, such that when slippage in a profession's autonomy occurs, the profession itself is threatened. This is what has happened during the past quarter of a century. Changes in societal attitudes and demands have taken autonomy away from the medical profession in areas of the establishment of social policy, infringement on ethical issues, monitoring physcian and hospital practice, and the challenge of medical practice through the mechanism of malpractice litigation. This slippage in our autonomy, combined with the pervasive and inseparable influence of financial factors, has induced apathy and a sense of self-pity within the medical profession.

The future

There is no panacea and it is evident that the forces and systems that have evolved over the past 25 years will not change rapidly. However, I think that medicine as an institution must realistically assess its current situation and expend its efforts in ways and directions that will not only improve our public image but will have the greatest likelihood to achieve the goal of easily accessible, high quality, affordable medical care for all citizens. Virtually all recent health legislation has been patchwork to address short-term problems with quick fixes. None has addressed a consistent long-term policy. When we consider our political system with its changing leadership every 4 to 8 years and its sensitivity to special interest groups, this should not be a surprise. To really influence long-term health policy, a coalition that represents all of the players from the consumers to the payers to the providers must be developed that will be an objective sustaining force with sufficient clout to influence legislation from the broad societal view. Such a coalition, the North Carolina Institute of Medicine, has been in existence since 1983. Originally supported by the Kate Bitting Reynolds Health Care Trust, it now has state legislative dollars as well. Its members are drawn from leaders in government, education, business, industry, the health and legal professions, the hospital and health insurance sectors, and the public at large. Because of its broad membership base without a majority from any single professional or special interest group, it can objectively represent the public interest on health-related public policy issues. Already a number of health policy concerns that range from indigent care to cost containment to medical malpractice insurance have been identified and of particular interest to members of the Association, the first task force to reduce infant mortality and morbidity in North Carolina has completed its deliberations. To fully appreciate the potential of such an Institute, particularly as it applies to medicine, one must understand the process involved in the achievement of consensus. Initially this involves exchange of information. Problems within the medical profession as well as those that involve multiple constituencies are identified and solutions are developed on the basis of objective unbiased data. Common goals are identified and means to those ends are planned. In this process, medicine maintains its traditional role of advocacy for quality basic health care to all at a reasonable cost. And when physicians have taken leadership in efforts to control costs, not only is their credibility and public image improved, but there is a greater potential for physicians to retain professional satisfaction than when controls are imposed from the outside. It is critical to understand that the Institute of Medicine is not a lobbying force per se. However, by virtue of its diverse membership, each with organizations and contacts, a potentially huge lobbying force can be developed. In effect, medicine lets the others do the lobbying for itself.

The Institute I have described is unique among the states and its impact remains unproven. However, it is supported by the state legislature, its influence is growing, and the process is one that clearly provides significant input from the medical profession. All of you are aware of similar although not as comprehensive coalitions at community levels. In general, their information base is small and they have little clout, as compared

with the potential of a statewide organization. However, I encourage all of you to become active participants because mutual understanding is inevitably improved and this is the basis on which progress is made.

As we influence the implementation of a health delivery system that provides affordable and uniform quality of care, we must stop protecting the old ways. We must accept change and deal with it positively if the public is to maintain its trust in the medical profession. We must understand how we got to our present position and the current forces that impact on it. We must remain united for otherwise we lose our effectiveness. We must understand that government and business really do not want to run medicine unless there is a void. We must maintain our commitment to service, especially the medically indigent, and we must understand that in the eyes of the public, curing is not synonymous with caring. Finally, I remind you that the greatest reward we physicians have is the satisfaction of improving the quality of life of our fellow man. Our professionalism and especially our altruism must remain pervasive for if we surrender our obligations to legislative and commercial forces, who will remain the public's unbiased medical advocate?

REFERENCES

1. Lock FR. The challenge of change. Obstet Gynecol 1964; 24:481-7.
2. Seekins SV. AMA studies on public attitudes. In: Proceedings of the Medical Education Group conference. Los Angeles: Medical Education Group of the American Medical Association, 1988:155-67.
3. Gerbner G. Television and public perceptions of medicine. In: Proceedings of the Medical Education Group conference. Los Angeles: Medical Education Group of the American Medical Association, 1988:141-7.
4. Oxford English Dictionary. Oxford, England: Oxford University Press, 1961;8:1427.
5. Flexner A. Is social work a profession? In: Proceedings of the 42nd annual meeting of the National Conference of Charities and Correction. Baltimore: The National Conference of Charities and Correction. 1915:576-90.
6. Reed RR, Evans D. The deprofessionalization of medicine. JAMA 1987;258:3279-82.

Effects of terbutaline sulfate on fetal cardiac function

Ruth Petersen, MD, Laurence S. Carter, BS, Nancy C. Chescheir, MD, Vern L. Katz, MD, and Robert C. Cefalo, MD, PhD

Chapel Hill, North Carolina

An isolated heart preparation was used to study the effects of terbutaline sulfate (Brethine) on fetal cardiac function. Pregnant guinea pigs received daily subcutaneous injections of terbutaline for 10 day, whereas a control group received placebo injections. Fetal guinea pig hearts were evaluated for cariodynamic and pathologic differences. Fetuses exposed to terbutaline demonstrated a higher mean heart rate ($p < 0.01$), a higher mean heart weight ($p < 0.05$), a higher mean heart weight/body weight ratio ($p < 0.01$), and a trend toward higher left ventricular systolic pressure levels ($p < 0.1$). These hemodynamic responses in fetuses exposed to terbutaline may result in increased functional demands that may predispose myocardial tissue to damage. (Am J Obstet Gynecol 1989;161:509-12.)

Key words: Terbutaline sulfate, β-sympathomimetic drugs, fetal effects, fetal guinea pig heart, heart preparation

β-Sympathomimetic therapy has become a popular tocolytic agent for preterm labor. However, various maternal and fetal cardiovascular side effects must be balanced with the therapeutic advantages of a drug like terbutaline sulfate. Maternal effects include an increase in heart rate and systolic pressure,[1] an increased risk for pulmonary edema, and electrocardiograph changes consistent with myocardial ischemia.[2-4] Animal studies have shown fetal myocardial tissue damage after administration of β-sympathomimetic drugs both in vivo[5] and in vitro[6] as well as organ weight changes, notably cardiomegaly, in rabbit fetuses chronically exposed to tocolytic agents (unpublished observations). Postmortem examination that indicate changes consistent with myocardial infarction have been reported for human

From the Division of Maternal and Fetal Medicine, Department of Obstetrics and Gynecology, University of North Carolina.
John R. McCain Student Fellowship Presentation, presented at the Fifty-first Annual Meeting of the South Atlantic Association of Obstetricians and Gynecologists, Hot Springs, Virginia, January 29–February 1, 1989.
Reprint requests: Robert C. Cefalo, MD, PhD, University of North Carolina, Department of Obstetrics and Gynecology, CB 7570, 214 MacNider Building, Chapel Hill, NC 27599-7570.
6/6/13965

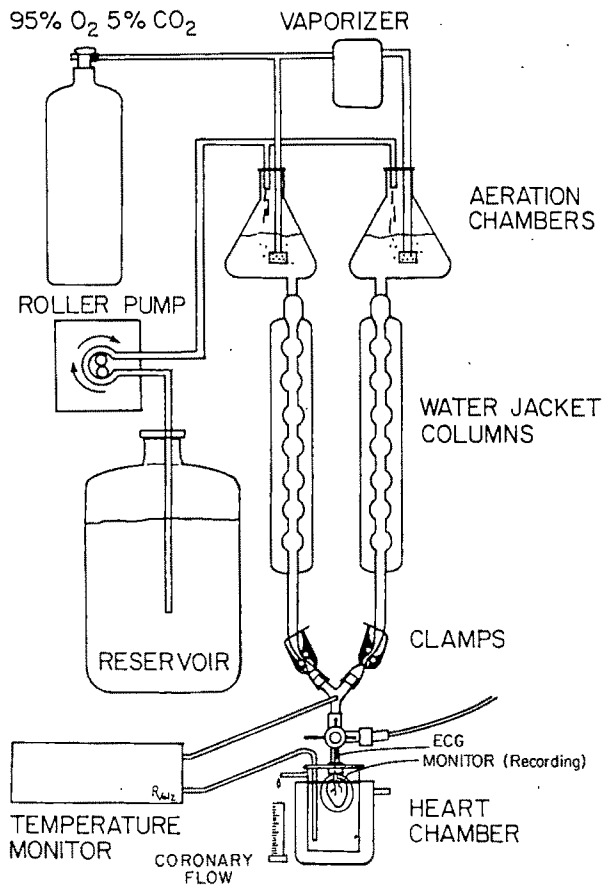


Fig. 1. Schematic representation of isolated heart perfusion on modified Langendorff apparatus.

infants chronically exposed to β-sympathomimetic drugs.[7,8] We studied fetal cardiac function and pathologic changes after chronic terbutaline therapy in the pregnant guinea pig.

Method and materials

This project was approved by the Committee on Animal Research at the University of North Carolina and all care and procedures were within their guidelines. Animals were given food and water as desired. Pregnant Hartley guinea pigs (1.1 to 1.36 kg, from Charles River Breeding Co, Waltham, Mass.) were randomly divided into two groups 11 days before estimated partus (normal gestation period = 65 days). The study group was given subcutaneous injections of 0.03 ml (1 mg/ml) of terbutaline sulfate (Brethine) with 0.04 ml of saline solution twice a day. This dose approximates the suggested therapeutic daily dose of terbutaline as a tocolytic agent for human beings on a weight basis. The control group was given 0.07 ml of saline solution subcutaneously twice a day.

One or two days before estimated term, the pregnant guinea pigs were anticoagulated with 1000 U of heparin intraperitoneally and anaesthetized with ether. The fetuses were removed and the hearts from two pups per litter were rapidly excised and placed in an ice-cold 0.9% saline solution. Almost immediately, the aortic root of the heart was secured to an 18-gauge needle and attached to a modified Langendorff apparatus (Fig. 1). The isolated fetal hearts were perfused with a Krebs-Henseleit solution that consisted of sodium chloride 118.5 mmol, sodium bicarbonate 25.0 mmol, dextrose 5.5 mmol, potassium chloride 4.4 mmol, calcium chloride 1.3 mmol, potassium monobasic phosphate 1.2 mmol, and magnesium sulfate 1.2 mmol. With the initiation of retrograde perfusion, the aortic valve closed and thereby channeled all the perfusion medium, aerated with 95% oxygen and 5% carbon dioxide, into the coronary arteries. Coronary flow was measured with a graduated cylinder and stopwatch as it exited the heart chamber (Fig. 1). Aortic perfusion pressure was maintained between 60 to 65 mm Hg. The perfusates entering and surrounding the heart were warmed by a water-jacketed system to 37° C.

A miniature latex balloon tip catheter filled with saline solution was inserted into the left ventricle through the mitral valve to measure fetal cardiac dynamics. Fetal heart rate (FHR), left ventricular systolic and end-diastolic pressures (maintained at 6 mm Hg) were continuously measured with the ventricular balloon. These data were recorded on a Hewlett-Packard 7754B four-channel system (Palo Alto, Calif.). From this, contractility, measured by the ratio of dP/dt, was determined from the slope of the left ventricular pressure waveform. Three electrodes that formed an Einthoven triangle were placed into the heart chamber to allow measurement of the PR, QRS, and RR intervals through this electrocardiogram configuration.

After initiation of aortic perfusion, placement of the left ventricular balloon and electrocardiogram electrodes, the hearts were allowed a 20-minute equilibration period. Readings of FHR, left ventricular systolic pressure, coronary flow, dP/dt, aortic perfusion pressure, and PR interval were taken at 20, 30 and 60 minutes.

Fetal hearts not used in the heart preparation and maternal hearts were extensively studied histologically with hematoxylin and eosin stain.

Differences between groups at 60 minutes were measured with the Student t test for unpaired observations. The p values <0.05 were statistically significant.

Results

From the seven pregnant guinea pigs that received terbutaline, 11 fetal hearts were evaluated with the isolated heart preparation. Eight fetal hearts from four pregnant guinea pigs injected with saline solution served as controls.

Table I lists fetal parameters measured including body weight, heart weight, FHR, left ventricular systolic pressure, coronary flow, dP/dt, and PR interval. Values are included for the control group ($n = 8$) and the group treated with terbutaline ($n = 11$). Fetuses in the group exposed to terbutaline showed a significant increase in FHR ($p < 0.01$) and fetal heart weight ($p < 0.05$), and a significant decrease in the PR interval ($p < 0.05$) relative to the control fetal values. In addition, there is a trend toward a higher left ventricular systolic pressure level ($p < 0.1$) for the treated fetal hearts.

Through evaluation of maternal and fetal body weights, heart weights, and litter size for the treated and control groups, the ratio of heart weight/body weight was found to be significantly greater ($p < 0.01$) for the treated fetal guinea pigs, compared with the controls. No similar difference existed between the treated mothers and control mothers.

Grossly, there were no identifiable differences in the fetal hearts of the treated group and the control group. Histologic examination of seven treated fetal hearts, and four control fetal hearts, and the maternal hearts did not reveal any evidence of myocardial necrosis. Ventricular dilation of one fetal heart in the treated group was seen.

Table I. Fetal weights and cardiodynamic measurements in control and terbutaline study groups

	Control ($n = 4$)	*Terbutaline ($n = 11$)*	*p Value*
Body weight (gm)	73 ± 3	78 ± 6	NS
Heart weight (mg)	325 ± 18	401 ± 31	<0.05
FHR (beats · min^{-1})	198 ± 4	228 ± 6	<0.01
Left ventricular systolic pressure (mm Hg)	42 ± 3	52 ± 4	<0.1
dP/dt (mm Hg · sec^{-1})	767 ± 57	870 ± 76	NS
Coronary flow (ml · min^{-1})	8.1 ± 0.4	8.0 ± 0.5	NS
PR interval	75 ± 4	63 + 2	<0.05
Heart weight/body weight × 100	0.446 ± 0.010	0.517 ± 0.015	<0.01

Comment

In the measurement of fetal cardiac parameters in the animals exposed to terbutaline, the effects of repeated administration of betamimetic drugs are noted through a higher mean heart rate and a trend toward a higher left ventricular systolic pressure level. An increase in both of these parameters results in a higher cardiac work load and a higher myocardial oxygen demand. As demonstrated by the fetuses exposed to terbutaline, adaptive responses to such chronically increased functional demands may lead to ventricular dilation and to a proportionately higher heart weight, suggestive of cardiac hypertrophy. The fact that fetal heart weight/body weight ratios in the treated group were higher than in the control group illustrates the possible hypertrophic response of the cardiac muscles exposed to chronic work loads.

As with any chronically stressed heart, there is a chance for eventual decompensation. The increased metabolic requirements placed on the fetal hearts exposed to betamimetic drugs, even with these minimal doses, may predispose these hearts to ischemia and infarction as myocardial tissue requirements exceed available supply. Explanations for our lack of microscopic myocardial damage may be related to the fact that we treated our animals with minimal therapeutic doses, unlike other investigators who approached much higher levels of β-sympathomimetic drugs.[5] Of importance is that even with these minimal doses we have demonstrated responses to β-sympathomimetic drugs are similar to those that predispose any myocardial tissue to acute and possibly lethal damage.

The decision to use long-term treatment with tocolytic agents requires the knowledge of both long-term and short-term maternal and fetal effects. We emphasize the importance of considering the impact such long-term treatment with tocolytic agents has on the fetal cardiac function. If β-sympathomimetic drugs are used chronically for tocolysis, these fetuses should be followed up carefully in utero for signs of decreasing or impaired cardiac function, such as tachycardia or fetal hydrops.

We thank Dr. F. G. Dalldorf for his assistance in the histologic review of our slides.

REFERENCES

1. Vojcek L, Lampe GL. Effects of a β-sympathiomimetic drug on the foetal and maternal cardiovascular system in a chronic sheep model. Acta Physiol Hung 1985; 65:511-6.
2. Ingemarsson I, Arulkumaran S, Kottegoda SR. Complications of beta-mimetic therapy in preterm labor. Aust NZ J Obstet Gynaecol 1985;25:182-9.
3. Katz M, Robertson PA, Creasy RK. Cardiovascular complications associated with terbutaline treatment for preterm labour. AM J OBSTET GYNECOL 1981;139:605-8.
4. Michalak D, Klein V, Marquette GP. Myocardial ischemia: a complication of ritodrine tocolysis. AM J OBSTET GYNECOL 1983;146:861-2.
5. Rona G, Chappel CI, Balazs T, et al. An infarct-like myocardial lesion and other toxic manifestations produced by isoproterenol in the rat. Arch Pathol 1959;67:443-5.
6. Weidinger H, Wiest W, Schleich A, et al. The effects of betamimetic drugs used for tocolysis on the fetal myocardium. J Perinat Med 1976;4:280-5.
7. Bohm N, Adlr CP. Focal necrosis, fatty degeneration and

subendocardial nuclear polyploidization of the myocardium in newborns after β-sympathicomimetic suppression of premature labor. Eur J Pediatr 1981;136:149-57.
8. Brossett P, Ronayette D, Pierre MC, et al. Cardiac complications of ritodrine in mother and baby. Lancet 1982; 1:1468.

Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. E. O. Horger III, Charleston, South Carolina. I offer my congratulations to Dr. Petersen and her associates for a very nice investigation of the effects of terbutaline sulfate on fetal cardiac function, for her winning the John R. McCain Student Fellowship Award, and for her presentation.

β-Sympathomimetic drugs are the most frequently used tocolytic agents. Whereas side effects of β-sympathomimetic therapy are not uncommon, they are usually well-tolerated. On the other hand, some side effects are potentially quite dangerous. Reported maternal hazards chiefly involve cardiovascular and metabolic problems including pulmonary edema, myocardial ischemia, arrhythmia, hyperglycemia, and hypokalemia. Cardiovascular side effects have been reported to occur in as many as 5% of these patients. Reported fetal and neonatal complications of note include cardiac toxicity and ischemia, hypoglycemia, hypocalcemia, and ileus. Although focal myocardial necrosis and fatty degeneration have been reported in the hearts of newborns whose mothers had been treated with β-sympathomimetic drugs, there has been no proof that such heart lesions were responsible for neonatal deaths.

Dr. Petersen and her associates studied the effects of terbutaline on cardiac function, weight, and histology in fetal guinea pigs after chronic maternal administration of terbutaline. Their findings included significant increases in heart rate and heart weight, a trend toward increasing left ventricular systolic pressure, and an absence of histologic changes.

Two important questions must be answered for those of us who use terbutaline and other β-sympathomimetics in the treatment and prevention of premature labor. First, why do these cardiac manifestations occur? And second, what is the real risk to the human fetus after the mother is treated with terbutaline? β-Sympathomimetic stimulation certainly increases heart rate in both mothers and fetuses, which generates an increased requirement of the myocardium for metabolic substrates. As the increased functional demands continue, myocardial hypertrophy occurs in response to the increased work. If the substrate demands exceed the supplies, the potential for myocardial ischemia certainly exists. This potential may be compounded by other factors that act in the situation of premature labor, such as hypoxia, acidosis, fever, and corticosteroids.

No histologic evidence of myocardial necrosis was found in the guinea pig fetal hearts. Possible explanations for this may lie in the duration of terbutaline treatment or in the dosage used. The guinea pigs were treated for the last 9 or 10 days of pregnancy, a span that runs approximately 14% to 15% of the guinea pig pregnancy. A similar proportion of human pregnancy would run 5 to 6 weeks, and we only infrequently manage to use β-sympathomimetics for such a long period of time. It was noted that the dosage used in the guinea pigs was low but approximated the suggested tocolytic dose of terbutaline for human beings. The guinea pig study used approximately 50 μg/kg terbutaline per day subcutaneously. Although this does run approximately equal to the recommended subcutaneous dosage in human beings, chronic administration in human patients is not continued subcutaneously. The usual oral dose in human patients runs considerably higher at approximately 200 to 400 μg/kg per day, but results in lower peak serum concentrations. The influence of these factors on fetal myocardial lesions has not been determined.

The risk to the human fetus cannot be answered by Dr. Petersen's study. Although other studies have shown similar changes in the hearts of human fetuses and newborns, the significance of these findings remains very uncertain. Nonetheless, the possibility is rather frightening.

Analysis of fetal loss after transcervical chorionic villus sampling—A review of 719 patients

Ronald V. Wade, MD, and S. Robert Young, PhD
Columbia, South Carolina

Chorionic villus sampling was performed under real-time ultrasonographic direction on a study group of 719 patients from September 9, 1985, through May 5, 1988. Follow-up of 714 of these patients who would have reached 28 weeks' gestation on September 1, 1988, revealed "an unintended" abortion rate of 4.1% by 20 weeks' gestation. Nine patients had a fetal loss <4 weeks after the procedure; 19 had losses up to 12 weeks after the procedure and before 20 weeks' gestation. This study revealed a significantly increased risk of fetal loss with an increase in the number of catheter insertions ($p < 0.001$). The risk of fetal loss after one sampling attempt was 3%, 7.8% after two attempts, and 14.3% after three attempts. (AM J OBSTET GYNECOL 1989;161:513-9.)

Key words: Chorionic villus sampling, spontaneous abortion, prenatal diagnosis, fetal loss

The safety of first-trimester fetal cytogenetic diagnosis by transcervical chorionic villus sampling is a matter of continued concern. The risk of the procedure is thought to be low, but it is difficult to delineate because of the methodologic problems in the establishment of the background loss rate in a comparable group of women.[1]

This analysis reviews multiple variables of patients who had fetal loss after transcervical chorionic villus sampling during the study period. The risk of the sampling procedure is assessed and the apparent pathophysiologic mechanisms of fetal loss are tabulated. The purpose of this study is to characterize specific determinants of increased fetal risk before the third trimester in women who undergo transcervical chorionic villus sampling. Ultimate pregnancy outcome and third-trimester pregnancy performance will constitute a separate publication at a later date.

Material and methods

Ultrasonography-directed transcervical chorionic villus sampling was performed on 719 patients in the Department of Obstetrics and Gynecology of the University of South Carolina School of Medicine and Richland Memorial Hospital between September 9, 1985, and May 5, 1988. Approval was obtained from the Institutional Review Committee of Richland Memorial Hospital and the Ethics Committee of the School of Medicine of the University of South Carolina to offer this method of first-trimester prenatal diagnosis. Follow-up data were obtained on 714 patients (99.3%) by direct patient and physician contact. The study group was defined as all patients with an estimated gestational age ≥28 weeks by September 1, 1988.

All chorionic villus sampling procedures were performed by a single physician (R. V. W.) with transabdominal real-time ultrasonography with guidance by either an Ultramark IV (Advanced Technology Laboratories, Inc., Bothell, Wash.) or a 4000 S/L (Advanced Diagnostic Research, Inc., Bothell, Wash.) ultrasound scanner with a 3.5 MHz transducer. Fetal gestational age was determined by fetal crown-rump length measurement according to the method of Robinson and Fleming.[2]

The cervix and vagina were cleansed with povidone-iodine solution and all samples were obtained by a polyethylene 16 gauge catheter (Intracath-Deseret Medical, Inc., Sandy, Utah) over a surgical wire stylet. If more than one attempt was required, the catheter and the operator's gloves were changed. Genital herpes virus infection or allergy to povidone-iodine were contraindications to performance of the procedure. The procedure was terminated after obtainment of an adequate volume of villi for diagnostic studies (>10 mg by visual estimate), after three attempts, or at the patient's request to discontinue the procedure.

The fetal losses were reviewed to determine the time interval from performance of the sampling procedure to fetal loss and were classified into groups as early, late, or remote. An early loss is defined as ≤4 weeks after the procedure; a late loss is defined as >4 weeks but ≤12 weeks after the procedure; and remote is defined as >12 weeks but ≤20 weeks after the procedure.

From the Department of Obstetrics and Gynecology, University of South Carolina School of Medicine.
Presented at the Fifty-first Annual Meeting of the South Atlantic Association of Obstetricians and Gynecologists, Hot Springs, Virginia, January 29–February 1, 1989.
Reprint requests: Ronald V. Wade, MD, Department of Obstetrics and Gynecology, Charlotte Memorial Hospital and Medical Center, Charlotte, NC 28232.
6/6/12989

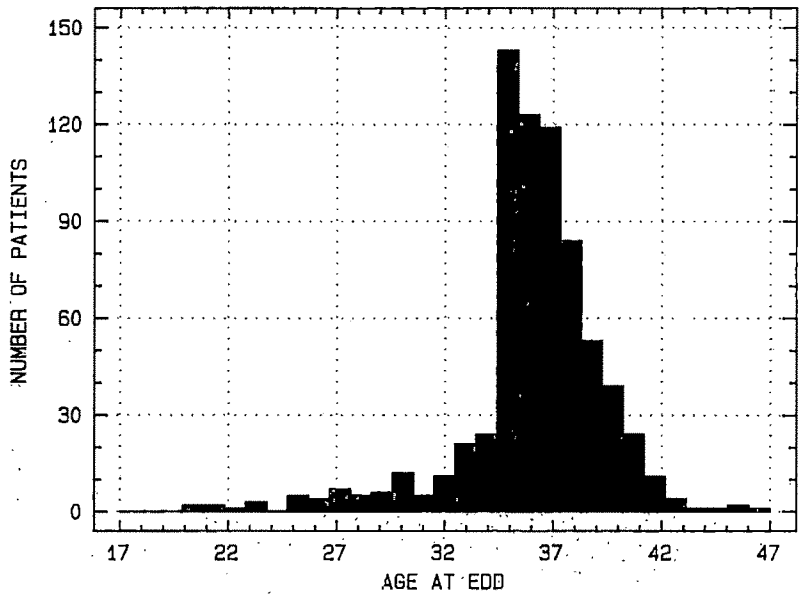


Fig. 1. Frequency of maternal age at time of estimated date of delivery (n = 714).

Table I. Mechanism of fetal loss by time interval from procedure to loss

Mechanism	*Early* $n = 9$	*Late* $n = 19$	*Remote* $n = 3$
Fetal death in utero	4	13	1
Spontaneous abortion	4	3	1
Septic abortion	1	0	0
Premature rupture of membranes	0	3	1

An effort was made to establish the underlying mechanism of fetal death.

All patients who had a fetal loss were analyzed by multiple criteria including maternal age, gravidity, estimated gestational age, quantity of the sample obtained, and number of sampling attempts. Particular emphasis was placed on review of the impact of multiple aspiration attempts, fetal karyotype, and volume of villi removed. The specific genetic findings and karyotype analysis are not evaluated in this study, but will be the subject of a future publication. In this study "abnormal karyotypes" refer to recognized aneuploidic conditions of clinical significance.

Continuous parameters were subjected to statistical tests by Student t test with results as mean ± SD. χ^2 analysis with Yate's correction was used to compare groups in two-by-two contingency tables. A p value <0.01 was considered to be significant. Tests for significance were performed and supported by Statgraphics (STSC, Rockville, Md.) and PC Statistician (Human Systems Dynamics, Northridge, Calif.) computer statistical software packages.

Results

Of the 719 patients who underwent chorionic villus sampling, 87% were at risk for fetal aneuploidy because of advanced maternal age. Age distribution is shown in Fig. 1. Of the 714 patients available for follow-up, 29 elected to interrupt their pregnancies for various reasons including abnormal karyotype, anencephaly, single gene defect, or other congenital conditions. A total of 20 patients (2.8%) had clinically abnormal karyotypes. An additional 20 patients had other cytogenetic findings such as balanced translocations and heterochromatin, but were included as "normal" patients in this analysis.

A total of 685 patients continued their pregnancies and formed the base study group for risk assessment. Fetal loss occurred before the beginning of the third trimester in 31 patients (4.5%); 28 (4.1%) of these patients had fetal loss before 20 weeks' gestation. Of the losses, nine were classified as early losses, 19 as late, and three as remote. The maximum gestational ages achieved by these groups were 16, 20, and 26 weeks, respectively. Table I indicates the mechanism of these losses.

Comparison of those patients with fetal losses to those who had no loss showed no significant difference for the parameters of maternal age, gravidity, quantity of villi aspirated, and gestational age, as shown in Table II. In addition, there was no difference between the group of patients who had fetal loss and those who had early, late, or remote losses when evaluated for these same variables by groups of one, two, or three sampling attempts. The mean number of catheter insertions dif-

Table II. Comparison of patients and procedural characteristics between those with continuing pregnancies and those with losses

Characteristic	Group			
	No loss $n = 654$	*Early* $n = 9$	*Late* $n = 19$	*Remote* $n = 3$
Maternal age	36.03 ± 3.47	36.44 ± 1.42	37.00 ± 2.79	34.00 ± 6.93
Gravidity	2.75 ± 1.29	3.10 ± 1.76	3.16 ± 1.34	2.33 ± 0.58
Quantity of villi	41.17 ± 15.36	36.10 ± 17.28	44.89 ± 16.22	25.00 ± 0.00
Gestational age	9.86 ± 0.86	10.06 ± 0.86	10.04 ± 0.86	9.17 ± 0.29

Data are mean ± SD.

fered between the control group without fetal loss and the loss groups (Table III). χ^2 analysis of the data in Table IV reveals an increasing risk of fetal loss with multiple attempts ($\chi^2 = 14.8914$; $p = 0.0006$). With one attempt, the risk as measured by relative frequency of a subsequent loss was 0.03 or 3%. After two attempts the risk rose to 0.078 or 7.8%, and at three attempts became .143 or 14.3%. When comparison was made of one attempt and two attempts, there was no significant difference ($p = 0.03$); significance was achieved when comparison was made of one attempt and three attempts ($p = 0.0017$). The significance level that reflects the increase in risk from two attempts to three attempts was weak at $p = 0.36$.

The proportion of abnormal karyotypes was not significantly different in those patients who had one, two, or three sampling attempts. Only three patients requested that the procedure be terminated. All of these requests were made after the first attempt and were secondary to cervical pain. Two patients who had previous cervical cryosurgery had severe pain when the catheter traversed the endocervix. In one patient an appropriate volume of villus tissue was obtained for diagnosis, in the other patient insufficient tissue was obtained. The third patient refused a second attempt because of the necessity of cervical traction with an Allis clamp.

The apparent lack of influence of operator experience on the frequency of the overall loss rate is shown in Table V. Of interest however, 36 of the 42 patients who had three sampling attempts occurred in the first 411 patients (i.e., subgroups 1 to 3). The six losses in the group of patients who had three attempts were distributed as follows: two losses in the first group, patients 1 to 137; three losses in patients with sequence numbers 275 to 411; and one in the last group with sequence numbers 549 to 685.

One patient had an obvious subchorionic bleeding episode then progressed to spontaneous abortion within 48 hours after the procedure. Another patient was seen 72 hours after the procedure with an incomplete abortion and had an oral temperature of 99.8° F. Both of these patients had one catheter insertion. The remaining early losses were characterized by silent fetal death or spontaneous abortion >7 days after the procedure. Women in the late group were more likely to have in utero death or premature rupture of the membranes.

Table III. Comparison of the number of catheter insertions between the control group without loss and the fetal loss groups by time interval from procedure to loss

Group	*Insertions*	*p Value**
No loss ($n = 654$)	1.27 ± 0.56	
Early loss ($n = 9$)	1.89 ± 0.78	0.001
Late loss ($n = 19$)	1.58 ± 0.77	0.018
Remote loss ($n = 3$)	1.67 ± 1.15	0.220

Data are mean ± SD.
*Student t test.

After an uncomplicated postprocedure course, three cases in the group with late loss had severe oligohydramnios, elevated maternal serum α-fetoprotein levels, and fetal death. The pathophysiology in these patients is uncertain. In none of these cases was there a history consistent with overt amniorrhexis, but two of the three patients did have prolonged bleeding near the time of fetal death, with the blood loss noted to be dark bloody fluid. In none of these patients was subchorionic hematoma noted by ultrasonography. All of these fetuses had normal renal development at postmortem examination.

Comment

Chorionic villus sampling continues to emerge as an increasingly acceptable means of fetal cytogenetic diagnosis in the first trimester.[3, 4] Many studies have detailed institutional experiences with this procedure, and reported loss rates from 1.9% to 5.8%.[3-9] Few studies to date have provided comprehensive follow-up on patients who undergo chorinoic villus sampling. This

Table IV. Fetal loss as a function of catheter insertions

	Catheter insertions						
	1		*2*		*3*		
Group	*n*	*%*	*n*	*%*	*n*	*%*	*Total*
No loss	512	97.0	106	92.2	36	85.7	654
Loss	16	3.0	9	7.8	6	14.3	31
TOTAL	528	100.0	115	100.0	42	100.0	685

$\chi^2 = 14.8914$; $p = 0.0006$.

Table V. Fetal loss as a function of physician experience

Chorionic villus sampling sequence	*Loss*	*No loss*
1-137	5	132
138-274	7	130
275-411	5	132
412-548	7	130
549-685	7	130

$\chi^2 = 0.811$.

analysis of 714 pregnancies that would have completed 28 weeks' gestation after transcervical chorionic villus sampling focuses on the risks of the procedure and the effect of the risk estimate on appropriate patient counseling.

The absolute increase in fetal loss after chorionic villus sampling is difficult to establish because of the problems involved with the definition of a comparable control group and background spontaneous abortion rate. The impact of the increase abortion rate as a function of maternal age must be considered. Several studies have been published in an effort to establish the background loss rate of ultrasonographically normal pregnancies at 8 to 12 weeks' gestation.[10-13] The overall loss rates in these studies have been noted to be in the 2.0% to 3.2% range. When corrected for maternal age in women older than 35 years, however, the spontaneous abortion rate up to 20 weeks' gestation is in the 4.3% range.[13] Comparison of losses after chorionic villus sampling with groups of women who have ultrasonographic scans at 8 to 12 weeks' gestation without instrumentation also ignores the spontaneous abortion of aneuploidic fetuses in the comparison group and the impact of elective interruption of aneuploidic pregnancies in the study group. Theoretically a randomized study is needed with a control group for women older than 35 years who did not undergo prenatal diagnosis, but such a study is not feasible at this time.

Comparison of the fetal losses in this study with a published transabdominal chorionic villus sampling study that reported a fetal loss rate of 3.2% reveals no significant difference in risks ($\chi^2 = 0.118$).[14] Large studies of transabdominal sampling should be reported to allow a more accurate comparison of the two methods.

Although data exists with regard to placental site, there is no information with regard to the position of the uterine fundus relative to the median axis of the pelvis (i.e., anteverted or retroverted). The placental site was therefore not evaluated as a potential variable because the important relationship is that of placental site relative to the position of the uterine fundus and accessibility through the endocervix.

It is difficult to define the impact of operator experience on the subsequent fetal loss rate from these data for two reasons. First, an extensive practice series of patients who requested elective pregnancy interruption (>100 samples) was perfomed by this team before institution of this series of ongoing pregnancies. Second, although the frequency of three insertions was greater in the first half of this series, the losses in these patients who underwent three sampling attempts was more evenly distributed. The latter fact must be viewed with caution, however, because of the few patients involved. The likelihood of a type II statistical error is significant when analyzing a loss group of only six patients.

The procedure-related loss rate is an extremely important statistic that should form the foundation of patient counseling. These data would suggest that patients should be informed of an increasing risk of fetal loss that reaches significance at three attempts. Reassessment of the maternal risk and the advisability of amniocentesis for diagnosis should be discussed if two sampling attempts do not provide an appropriate volume of villi for analysis (Fig. 2). Nonetheless, with the use of the spontaneous background rate of 4.3% in the group older than 35 years, the loss rate of 4.1% in our study group up to 20 weeks' gestation (28 losses out of 685 patients) compares favorably (Fig. 3). It would appear that the procedure-related risk in patients who had one catheter insertion is extraordinarily small and, indeed, may be negligible. Two insertions result in a loss rate higher than background, and three insertions further increase the risk of fetal loss. The loss rates of

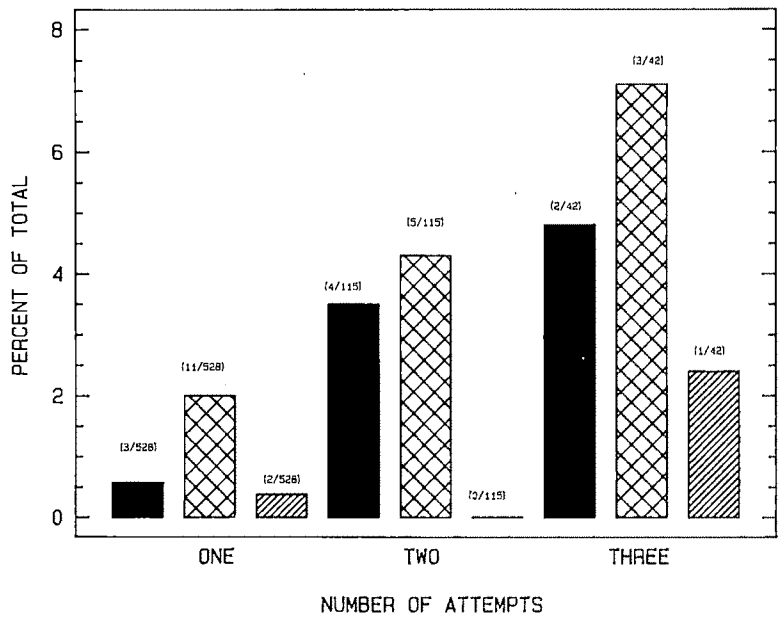


Fig. 2. Fetal losses as function of number of catheter insertions (attempts) expressed as percentage of total attempts. *Solid bar* indicates early loss; *cross-hatched bar* indicates late loss; *right diagonal bar* indicates remote loss.

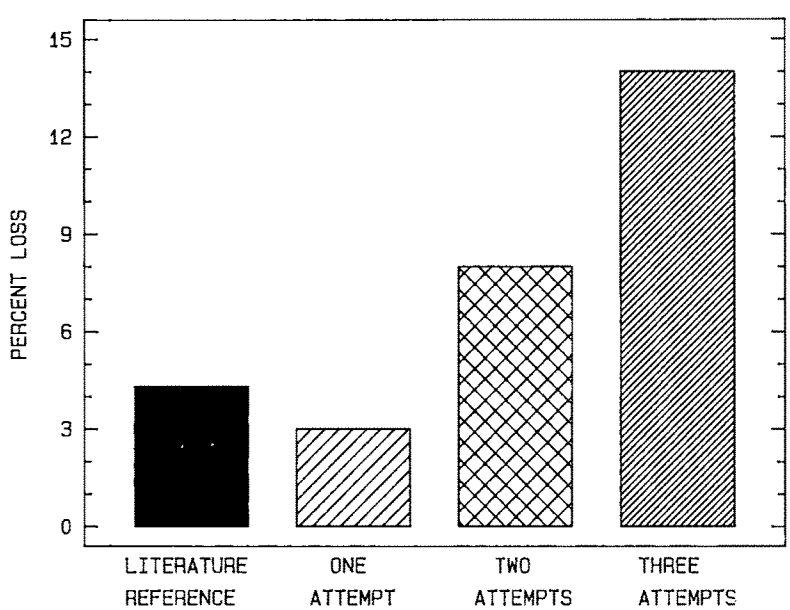


Fig. 3. Percentage of fetal loss expressed as function of catheter insertions (attempts) compared with background spontaneous loss data of Wilson et al.[13]

3% after one attempt, 7.8% after two attempts, and 14.3% after three attempts conform favorably to other studies that address this concern.[1, 8]

The designation of a particular fetal loss as "procedure related" or "not procedure related" is frequently speculative; pathophysiologic mechanisms responsible for fetal loss after chorionic villus sampling are clinically difficult to ascertain. Subchorionic hemorrhage appears to be the most obvious mechanism, but many losses remain idiopathic. Definition of a high-risk group is therefore difficult. However, it would seem prudent to approach with caution patients who have notable early pregnancy bleeding or subchorionic hematoma formation noted by ultrasonography before the procedure.

At the present time the explanation of the causes of

maternal serum α-fetoprotein levels elevated, oligohydramnios, and fetal death is unclear. This association of findings has been reported in noninstrumented pregnancies with a high incidence of renal abnormalities noted.[15] There was no perceptible loss of amniotic fluid in this group of patients but an imperceptible loss of fluid or other possible causes of placental failure as has been suggested by others as a possible mechanism of this symptom complex cannot be excluded.[16]

In summary, these data imply that much of the procedurally related risk of fetal loss after chorionic villus sampling may be a function of the number of catheter insertions with a significantly increased risk of fetal loss in those patients who have more than two sampling attempts. Counseling of patients should reflect this increase in risk.

REFERENCES

1. Special WHO report on risk evaluation in chorionic villus sampling. Prenat Diagn 1986;6:451-6.
2. Robinson HP, Fleming JEE. A critical evaluation of sonar crown-rump length measurements. Br J Obstet Gynaecol 1975;82:702-10.
3. Jackson LG. CVS latest newsletter. 1988 Aug 10:1-8.
4. Hogge WA, Schonberg SA, Golbus MS. Chorionic villus sampling: experience of the first 1000 cases. Am J Obstet Gynecol 1986;154:1249-52.
5. Martin AO, Simpson JL, Rosinsky BJ, Elias S. Chorionic villus sampling in continuing pregnancies. Am J Obstet Gynecol 1986;154:1353-62.
6. Simoni G, Gimelli G, Cuoro L, et al. First trimester fetal karyotyping: one thousand diagnoses. Hum Genet 1986; 72:203-9.
7. Leschot NJ, Wolf H, Verjaal M, et al. Chorionic villi sampling: cytogenetic and clinical findings in 500 pregnancies. Br Med J 1987;295:407-10.
8. Holzgreve W, Miny P. Chorionic villi sampling with an echogenic catheter: experiences of the first 500 cases. J Perinat Med 1987;15:244-50.
9. Green JE, Dorfmann A, Jones SL, Bender S, Patton L, Schulman JD. Chorionic villus sampling: experience with an initial 940 cases. Obstet Gynecol 1988;71:208-12.
10. Cashner KA, Christopher CR, Dysert GA. Spontaneous fetal loss after demonstration of a live fetus in the first trimester. Obstet Gynecol 1987;70:827-30.
11. Simpson JL, Mills JL, Holmes LB, et al. Low fetal loss rates after ultrasound-proved viability in early pregnancy. JAMA 1987;258:2555-7.
12. Mackenzie WE, Holmes DS, Newton JR. Spontaneous abortion rate in ultrasonographically viable pregnancies. Obstet Gynecol 1988;71:81-3.
13. Wilson RD, Kendrick V, Wittmann BK, McGillivray B. Spontaneous abortion and pregnancy outcome after normal first-trimester ultrasound examination. Obstet Gynecol 1986;67:352-5.
14. Smidt-Jensen S, Hahnemann N. Transabdominal chorionic villus sampling for fetal genetic diagnosis: technical and obstetrical evaluation of 100 cases. Prenat Diagn 1988;8:7-17.
15. Koontz WL, Seeds JW, Adams NJ, Johnson AM, Cefalo RC. Elevated maternal serum alpha-fetoprotein, second-trimester oligohydramnios, and pregnancy outcome. Obstet Gynecol 1983;62:301-4.
16. Jackson LG. CVS latest newsletter. 1986 Sept 12.

Editors' note: This manuscript was revised after the discussions were presented.

Discussion

Dr. George W. Cornell, Suffolk, Virginia (Official Guest). Since the introduction about 20 years ago of chorionic villus sampling as a tool for prenatal diagnosis of genetic and enzymatic fetal defects, there has been a persistent problem in the establishment of the safety of the procedure relative to amniocentesis and a background of spontaneous fetal wastage. A number of studies have been published that address this problem, and the present study adds to the entire body of knowledge on the subject.

This study benefits from its large sample size, good follow-up, and clearly defined parameters. The central point of the study is that the risk of the procedure increased with the numer of attempts, and is significantly increased when one versus three attempts are compared.

It would seem logical that operator experience would have a beneficial effect on sampling outcome and this has been suggested to be the case in some studies.[1] The present study, however, clearly shows that this is not the case. It also shows that maternal age does not affect the outcome. This is in contrast with a study from The Netherlands[2] that showed a statistically significant increase in risk with advancing maternal age, but not with the number of samplings. An earlier study[3] suggested that sampling was safest in gestational weeks 9 through 11, a finding not supported by the present study. Gravidity and quantity of villi aspirated are not shown to be statistically significant. A procedure-related loss of $<1\%$ is suggested, which agrees with loss rates in other studies.

A large randomized multicenter study[4] compared chorionic villus sampling at 9 to 12 weeks' gestation amniocentesis at 15 to 17 weeks' gestation. The results, although not statistically significant, suggested that the potential difference in loss rates between the two methods was not more than 2.4% for women ≥ 35 years of age. There was an unexplained tendency to later losses in the chorionic villus sampling group.

It is readily apparent from the literature that the original question of exactly what the risk of chorionic villus sampling is compared with amniocentesis and background loss is still now answered. In fact, it may never be answered because such a study would require that some study patients be randomized into a group in which nothing is done, which raises grave ethical concerns.

Transcervical chorionic villus sampling is more difficult when the placenta is in certain locations. The present paper does not correlate the number of attempts with placental location. This would be an interesting comparison. It might be possible to use transabdominal chorionic villus sampling technique on selected placental locations to further reduce fetal loss and thereby increase the safety of the procedure.

REFERENCES

1. Brambati B, Oldrini A, Ferrazzi E, Lanzani A. Chorionic villus sampling: an analysis of the obstetric experience of 1000 cases. Prenatal Diagnosis 1987;7:157.

2. Jahoda MGJ, Pijpers L, Reuss A, Vosters RPL, Wladimroff JW. The role of maternal age in the assessment of the abortion risk after first trimester prenatal diagnosis. Rotterdam, The Netherlands: Department of Obstetrics and Gynecology, Erasmus University.
3. Hogge WA, Schonberg SA, Golbus MS. Chorionic villus sampling: experience of the first 1000 cases. AM J OBSTET GYNECOL 1986;154:1249.
4. Multicenter randomized clinical trial of chorionic villus sampling and amniocentesis. Lancet 1989;1:1.

DR. WADE (Closing). I thank Dr. Cornell for his thoughtful comments, and I will elaborate on two aspects of his discussion.

First, the lack of a demonstrable effect of operator experience on the loss rate may be partially explained by the fact that the operator had performed >100 practice aspirations on >50 patients before beginning the series of patients with continuing pregnancies reported here. Second, these data included the location of the implantation site of the placenta, but did not include the relative position of the uterine fundus to the median axis of the pelvis. The most important relationship deals with the accessibility of the implantation site and therefore is a function of the relative implantation site orientation to the internal cervical os. We do not have data on the position of the uterine fundus in this data base. For this reason, placental location as a potential variable was not included.

The lack of impact of gestational age on ultimate outcome may well reflect our attempts to perform these procedures during a very narrow window of 9 to 11 weeks' gestational age when possible.

The role of ultrasonography and amniocentesis in the evaluation of pregnancies at risk for neural tube defects

William A. Hogge, MD, Siva Thiagarajah, MD, James E. Ferguson II, MD, Patricia T. Schnatterly, MS, and Guy M. Harbert, Jr., MD
Charlottesville, Virginia

To assess the relative efficacy of amniocentesis versus targeted (detailed) ultrasonography, 225 patients referred because of an elevated maternal serum α-fetoprotein level (79.6%) or a family history of neural tube defect (20.4%) were evaluated. Ultrasonographic examination alone detected all 26 fetal abnormalities (11 cases of anencephaly, 10 cases of open spina bifida, and five other anomalies). Twenty-eight patients declined amniocentesis; all had normal pregnancy outcomes. Of the 167 patients with apparently normal fetal anatomy by sonography, seven had elevated α-fetoprotein levels but no acetylcholinesterase in the amniotic fluid. Six of these pregnancies resulted in normal infants; one infant had congenital nephrosis. The remaining 160 patients had normal sonograms with normal amniotic fluid α-fetoprotein levels and no fetal malformations at delivery. Although these results suggest that targeted ultrasonography by experienced personnel is a reasonable alternative to amniocentesis in evaluations for neural tube defects, the availability, cost-effectiveness, and diagnostic accuracy of this approach must be well documented in large prospective studies. (Am J Obstet Gynecol 1989;161:520-4.)

Key words: Amniocentesis, ultrasonography, maternal serum α-fetoprotein, neural tube defects

Amniocentesis traditionally has been the procedure of choice for the evaluation of a pregnancy identified as being at risk for a fetus with a neural tube defect. These pregnancies usually were ascertained either because of an elevated maternal serum α-fetoprotein level or because of a family history of neural tube defects. The published management protocols recommend that a level I (basic) ultrasonographic evaluation be performed after two unexplained elevations of maternal serum α-fetoprotein.[1-3] If no explanation is found for the elevated level, an amniocentesis is performed. Between 5% and 10% of the population undergoing amniocentesis would be expected to have an elevated amniotic fluid α-fetoprotein level.[3] In this evaluation protocol, patients then would undergo a detailed (targeted) ultrasonographic examination to delineate the precise lesions, e.g., a neural tube defect, ventral wall defect, or other fetal anomaly.

With the advent of high-resolution ultrasonography, however, modifications to this protocol have been suggested, and the necessity of amniocentesis in all cases has been questioned.[4] In this study we have analyzed the experience in out prenatal diagnosis program and assessed the relative efficacy of amniocentesis versus targeted ultrasonography in pregnancies at risk for a fetus with a neural tube defect.

From the Departments of Obstetrics and Gynecology and Pediatrics, University of Virginia School of Medicine.
Presented as Official Guest at the Fifty-first Annual Meeting of the South Atlantic Association of Obstetricians and Gynecologists, Hot Springs, Virginia, January 29–February 1, 1989.
Reprint requests: W. Allen Hogge, MD, Department of Obstetrics and Gynecology, University of Virginia, Box 387, Charlottesville, VA 22908.
6/6/13966

Material and methods

From September 1985 through August 1988, 225 of the patients evaluated by the prenatal diagnosis program at the University of Virginia were referred because of an increased risk for a neural tube defect (79.6% because of an elevated maternal serum α-fetoprotein level and 20.4% because of a family history of neural tube defect). Patients were initially evaluated between 15 and 21 weeks' gestational age with a mean age of 17.9 weeks at the time of diagnosis. The criteria for referral were an initial maternal serum α-fetoprotein level >3.0 multiples of the median. If the initial value was 2.5 to 3.0 multiples of the median, the test was repeated, and evaluation undertaken if the second result was ≥2.5 multiples of the medians. Before referral the majority of patients had undergone a basic sonogram to exclude incorrect gestational age, multiple gestation, and fetal death. Each patient was counseled regarding the risk of an abnormality and the benefits and limitations of diagnostic ultrasonographic examination and amniocentesis in providing a diagnosis. A targeted ultrasonographic examination then was performed by physicians specifically trained in obstetric ultrasonography. The equipment used was a

General Electric RT3600 with either a 3.5 or 5.0 MHz linear array transducer. Amniocentesis for karyotype analysis and amniotic fluid α-fetoprotein determination thereafter was performed in all cases unless the patient declined further testing. Acetylcholinesterase was assayed on all fluid samples with an amniotic fluid α-fetoprotein level of ≥2.0 multiples of the median. All patients who declined amniocentesis were requested to return for a follow-up ultrasonographic examination four weeks later.

Data on pregnancy outcome were obtained through the ongoing surveillance program of the Prenatal Diagnosis Center. This information was determined by written questionnaires and telephone contact with referring physicians.

Results

Fetal malformations were detected in 26 cases (11.6% of patients evaluated) by the initial targeted ultrasonographic examination (Table I). The mean level of maternal serum α-fetoprotein was 9.0 multiples of the median with a range of 2.9 to 29.0 multiples of the median. Twenty-one of these malformations were neural tube defects (11 cases of anencephaly, 10 cases of myelomeningocele). Of the remaining five cases, four involved a fetus with omphalocele as one component of a multiple malformation syndrome. The fifth patient, referred for evaluation of an elevated maternal serum α-fetoprotein level (2.9 multiples of the median), had a fetus with a Dandy-Walker malformation of the posterior fossa, but there was no obvious defect to explain the laboratory finding. Amniotic fluid α-fetoprotein was 0.8 multiples of the median.

Abnormal ultrasonographic findings were present in three additional cases. These patients had oligohydramnios without identifiable fetal malformations. One spontaneously aborted a grossly normal fetus at 20 weeks' gestation, 2 weeks after the evaluation. Amniocentesis was not performed. The second patient was initially evaluated at 20 weeks' gestation and electively terminated her pregnancy when a follow-up sonogram 4 weeks later indicated persistent oligohydramnios. The third patient was first seen at 18 weeks' gestation and elected to continue the pregnancy. Serial ultrasonographic scans at 2- to 3-week intervals were performed with fetal microcephaly first noted at 27 weeks' gestational age with development of ventriculomegaly at 29 weeks. After delivery at term, clinical evaluation of the infant was suggestive of fetal infection. However, serologic studies and cultures were negative for toxoplasma, cytomegalovirus, rubella, herpes, syphilis, and enterovirus. These latter two patients both had amniocentesis with elevated amniotic fluid α-fetoprotein levels (3.8 and 8.9 multiples of the median, respectively). Electrophoresis for the presence of acetylcholinesterase was inconclusive in both fluid samples (a faint band in patient 3 and a "smear" pattern between the pseudocholinesterase and the acetylcholinesterase position in patient 2).

Table I. Abnormalities detected by ultrasonography

Diagnosis	*No. of cases*	*Maternal serum α-fetoprotein (multiples of median) (mean)*	*Gestational age (wk) (mean)*
Anencephaly	11	13.1	17.6
Open spina bifida	10	4.7	18.6
Omphalocele (with other abnormalities)	4	14.3	16.5
Dandy-Walker malformation	1	2.9	20

There were 28 patients who declined amniocentesis after targeted sonography demonstrated apparently normal fetal anatomy; all had normal pregnancy outcomes. One patient with a maternal serum α-fetoprotein level of 3.2 multiples of the median refused all testing except a basic sonogram for dates performed outside the prenatal diagnosis program. She subsequently was delivered of an infant with a 2 × 2 cm occipital encephalocele.

Of the 167 patients with normal fetal anatomy and amniotic fluid volume by sonography who underwent amniocentesis, seven had elevated amniotic fluid α-fetoprotein levels. Acetylcholinesterase was absent in all cases. Six of these pregnancies resulted in normal infants. The seventh infant had a presumptive diagnosis of congenital nephrosis on the basis of a maternal serum α-fetoprotein value of 19 multiples of the median with an amniotic fluid α-fetoprotein of 43 multiples of the median. This diagnosis was confirmed at birth. The remaining 160 patients had normal amniotic fluid α-fetoprotein levels and no fetal malformations at delivery. There were no spontaneous abortions in the patients who underwent amniocentesis.

Comment

The results of this study indicate that detailed ultrasonography is a reasonable alternative to amniocentesis in the evaluation of pregnancies at risk for a neural tube defect. All defects in our series were detected with ultrasonography before amniocentesis. The 28 patients who declined amniocentesis after a normal ultrasonographic examination were delivered of normal infants. In the remaining patients, except for the case of congenital nephrosis, amniocentesis did not provide any additional information to influence their management. In fact, reliance on the ultrasonographic findings

avoided intervention in the six pregnancies with elevated amniotic fluid α-fetoprotein levels and normal outcome.

The previous recommendations for evaluating gravid women thought to have a fetus at risk for a neural tube defect were based on an efficacy rate of 80% for the ultrasonographic diagnosis of spina bifida.[4, 5] However, during the last decade, advances in ultrasonographic technology and increased experience in the field of obstetric ultrasonography have combined to greatly improve the diagnostic accuracy for detection of fetal abnormalities. The early diagnosis of spina bifida has been enhanced by Nicolaides et al.,[6] who first drew attention to cranial abnormalities that are associated findings in these cases. These signs consisting of alteration in cranial contour ("lemon sign") and abnormalities of the cerebellum and posterior fossa facilitate the diagnosis of spina bifida between 16 and 24 weeks' gestation. Although no large study has yet documented how often a spinal defect will be missed when such signs are used in conjunction with the evaluation of the bony spine, the diagnostic accuracy should be higher than the previously reported 80%. Furthermore, there are defects other than neural tube defect that can cause maternal serum α-fetoprotein levels to be elevated, and discussions of risk on the basis of ultrasonographic diagnosis must include these defects as well.

However, there are a number a limitations to using sonography alone in the evaluation of these cases. The patients in this study were preselected to have a high prevalence of the disorder and were evaluated by examiners experienced at detecting fetal abnormalities. To achieve the necessary diagnostic sensitivity, experienced personnel and high-quality equipment are required. These features are frequently available only in centers with prenatal diagnosis programs. The diagnostic accuracy achieved in this study will not be expected with screening ultrasonographic examinations. Furthermore, even established programs have missed as many as 25% of fetal abnormalities on an initial ultrasonographic examination.[9] No defects were missed by ultrasonography in this study, but our numbers may be considered to be relatively small. Amniotic fluid analysis also provided reassurance in the few cases where ultrasonographic visualization was not fully conclusive. It is therefore imperative that, if fetal detail is not visualized unequivocally, amniocentesis be performed.

The risk of pregnancy loss attributed to amniocentesis is approximately 0.5%. Therefore any comparison of the diagnostic methods must weigh the incidence of procedure-related loss against the missed diagnosis rate. The increased loss rate is a significant consideration in view of the already high rate of fetal death before 20 weeks' gestation in patients with elevated maternal serum α-fetoprotein levels.[7, 8] Ultrasonography as the sole diagnostic modality offers the advantage of safety relative to amniocentesis.

This study confirms that ultrasonography is a very sensitive tool for detecting neural tube defects in a high-risk population. The data from this study, together with those of Drugan et al.[9] and Richards et al.,[4] indicate that the diagnosis would have been missed six times in 880 patients if ultrasonography were the only diagnostic tool. This failure rate of 0.7% is similar to the procedure-related risk of amniocentesis. Thus the preliminary information indicates that targeted ultrasonography alone may be a reasonable alternative when experienced personnel and high-quality equipment are available. However, until more data are available, we believe that the best approach in these patients is a targeted ultrasonographic examination followed by amniocentesis.

We wish to thank Pat Allinson, Joan Hogge, Lisa Kirson, and Mary Ann Shires who provided genetic counseling for the patients in this study, and Nancy Breeden for expert ultrasonographic technical support.

REFERENCES

1. Milunsky A, Alpert E. Results and benefits of a maternal serum alpha-fetoprotein screening program. JAMA 1984; 252:1438-42.
2. Burton BK, Sowers SG, Nelson LH. Maternal serum alpha-fetoprotein screening in North Carolina: experience with more than twelve thousand pregnancies. AM J OBSTET GYNECOL 1983;146:439-44.
3. Simpson JL, Nadler HL. Maternal serum alpha-fetoprotein screening in 1987. Obstet Gynecol 1987;69:134-5.
4. Richards DS, Seeds JW, Katz VL, Lingley LH, Albright SG, Cefalo RC. Elevated maternal serum alpha-fetoprotein with normal ultrasound: is amniocentesis always appropriate? A review of 26,069 screened patients. Obstet Gynecol 1988;71:203-7.
5. Roberts CJ, Hibbard BM, Roberts EE, Evans KT, Laurence KM, Robertson IB. Diagnostic effectiveness of ultrasound in detection of neural tube defects: the South Wales experience of 2509 scans (1977-1982) in high-risk mothers. Lancet 1983;2:1068-9.
6. Nicolaides KH, Campbell S, Gabbe SG, Guidetti R. Ultrasound screening for spina bifida: cranial and cerebellar signs. Lancet 1986;2:72-4.
7. Nelson LH, Bensen J, Burton BK. Outcomes in patients with unusually high maternal serum alpha-fetoprotein levels. AM J OBSTET GYNECOL 1987;157:572-6.
8. Haddow JE, Kloza EM, Smith DE, Knight GJ. Data from an alpha-fetoprotein pilot screening program in Maine. Obstet Gynecol 1983;62:556-60.
9. Drugan A, Zador IE, Syner FN, Sokol RJ, Sacks AJ, Evans MI. A normal ultrasound does not obviate the need for amniocentesis in patients with elevated serum alpha-fetoprotein. Obstet Gynecol 1988;72:627-30.

Editors' note: This manuscript was revised after these discussions were presented.

Table I. Patients offered amniocentesis: December 1987

No. screened	67,228
No. offered amniocentesis	1,156
No. declined	394

Table II. Outcomes in cases with amniocentesis declined

Amniocentesis declined		394 (34%)
Normal outcomes		333 (85%)
Neural tube defects		44 (11%)
On ultrasonography	40	
Diagnosed at delivery	4	
Other anomalies		17 (4.3%)
On ultrasonography	14	
Diagnosed at delivery	3	
Anomalies not detected before delivery		7/61 (11.5%)

Discussion

Dr. Lewis H. Nelson, Winston-Salem, North Carolina. At the Wake Forest Medical Center patients undergoing diagnostic amniocentesis, either early or routinely, are advised that the pregnancy loss rate from the procedure is 1/300. Risks to the pregnancy from diagnostic ultrasonography, independent of operator judgment, appear to be zero according to safety statements by the American Institute of Ultrasound in Medicine. Patients with an elevated maternal serum α-fetoprotein level above appropriate cutoffs have risks of open neural tube defects or other abnormalities that exceed the risk of an amniocentesis. The authors have presented their experience with the accuracy of ultrasonography, comparing a low-risk procedure with one of essentially no risk if one ignores the skill of the ultrasonographer. The issue of skill or operator effect is one of the two issues one must address in reviewing this presentation.

The authors have alluded to the other issue of small numbers in their study. If one assumes that amniocentesis has a sensitivity rate of 99.5% for the detection of open neural tube defects, then for a comparable sensitivity with 95% confidence limits the authors would have to study approximately 604 cases. In the current study with 26 abnormal cases, the 95% confidence levels give a sensitivity with a lower level of 89.1%, this is not as good as that for amniocentesis. We also must acknowledge that in studying the 604 cases there theoretically would have been two pregnancy losses. It is probably less hazardous legally, assuming good counseling before the procedure, to lose those two pregnancies than to miss the neural tube defect on ultrasonographic examination.

Ultrasonography is an extremely operator-dependent diagnostic procedure. Personnel working as technologists require different training than similar people working as radiology technicians. Although the American Board of Obstetrics and Gynecology has insisted that residents be trained in ultrasonography, each program provides a variable experience. Ultrasonographic training is also part of the fellowship in maternal-fetal medicine. Experience and skill, although less than in residencies, also are variable in those fellowships. The skill level of the authors is not stated in the article and the reader should not assume that it is equal among them. If one ignores the issue of skill, we see an experience as illustrated in Tables I and II. Not all patients were seen at Wake Forest Medical Center.

The authors also do not comment on the quality of the ultrasonographic examination. Are they willing to state that ". . . targeted ultrasonography alone is a reasonable evaluation alternative to amniocentesis . . ." in a patient of reasonable size whose fetus is in a back posterior position? The concluding sentence of the article is more realistic in the world of variable training and academic center egos. The authors state, "Although the preliminary information indicates that targeted ultrasonography may be a reasonable alternative, until more data are available, we believe that the best approach in these patients is a targeted ultrasonographic examination by experienced personnel, followed by amniocentesis." My fear is that the majority of readers will not be responsible to that statement and I strongly encourage the authors to emphasize the point more strongly in their article. It would be advantageous to have an alternative to amniocentesis, but we are far from offering ultrasonography as the alternative at this time.

Dr. Ronald Wade, Charlotte, North Carolina. Are any outcome data available for those with elevated serum α-fetoprotein levels and normal amniotic fluid α-fetoprotein levels? These patients are still at high risk and, even though they may have nonanomalous fetuses, those of us in high-risk obstetrics realize that we need to follow up these patients very closely.

Dr. Hogge (Closing). Dr. Wade, in response to your question, we did not look at that particular information in this study, but we certainly know from just anecdotal experience that there is, in fact, a much higher risk of pregnancy loss, early delivery, and small-for-gestational-age infants in patients with elevated serum levels of α-fetoprotein. There are a number of unusual things that will be coming out of the University of Virginia's prenatal diagnosis program in the form of case reports that have been related to high maternal seurm α-fetoprotein levels in the presence of normal amniotic fluid α-fetoprotein.

I would like to thank Dr. Nelson for his discussion because he said the kinds of things that I would have liked to say very strongly in our Comment section. I will revise it with his comments in mind. We wanted to prove that amniocentesis was, in fact, the best modality. Our data, as you see, led us to have to reassess that particular approach. Hopefully, with large numbers, we will have the information that is unavailable at the

Table I. Patients with normal sonograms selecting amniocentesis

Sept. 1, 1985–Aug. 31, 1989	167/196 (85.2%)
Sept. 1, 1988–Jan. 18, 1989	24/35 (68.6%)

present time. I emphasize what Dr. Nelson said; that is, these patients were somewhat unique in that each was seen by the same group of individuals each time. The ultrasonographers gained increasing experience as the study went on. I don't think this is possible in most places around the country, but our program is small. There is one disconcerting thing, as shown in Table I. The results of the study were discussed with our staff when the study was completed. During the time of the study, about 85% of the women chose amniocentesis after their counseling session. Although the numbers are obviously quite small, you can see that the number has dropped rather dramatically to 68.6% since the study was completed. I would encourage other programs not to have this kind of change in their counseling programs. We will have another session with our staff on our return because this is not what we think should be happening at the present time.

The effects of vibroacoustic stimulation on baseline heart rate, breathing activity, and body movements of normal term fetuses

Lawrence D. Devoe, MD, Nancy A. Searle, BA, David A. Ruedrich, MD, Ramon A. Castillo, MD, and William P. Metheny, PhD

Augusta, Georgia

To determine the fetal biophysical effects of vibroacoustic stimulation produced by an electronic artificial larynx we studied 20 normal term pregnancies assigned either to control (no stimulus) or experimental (stimulus) groups. Each fetus was observed for 3 hours; either no stimulus or a 3-second stimulus was delivered after the first hour. Fetal heart rate baseline and variation, breathing movement incidence, rate, and variation, and body movement incidence data were acquired concurrently and analyzed at 15-minute intervals. Intergroup comparisons showed that, after stimulation, fetal heart rate baseline and variation increased significantly, whereas breathing incidence fell during the first 15 minutes. Within-group analyses showed that poststimulus elevation of fetal heart rate baseline was the only significant time interaction over the 3 hours. Vibroacoustic stimulation appears to be primarily associated with transient alterations in fetal heart rate baseline; concomitant changes in breathing activity probably reflect normal biologic cycles. (Am J Obstet Gynecol 1989;161:524-9.)

Key words: Fetus, fetal heart rate, fetal breathing, fetal movement, acoustic stimulation

Clinical interest in vibroacoustic stimulation of the human fetus is derived from early observations[1, 2] that showed normal third-trimester fetuses had predictable tachycardic responses when brief sound or vibratory stimuli were transmitted through the material abdominal wall. More recently, artificial electronic larynges have been used in antepartum tests to stimulate fetal heart rate (FHR) "reactivity,"[3, 4] and during intrapartum monitoring to predict fetal acid-base status.[5, 6] Investigations of the possible effects of vibroacoustic stimulation on fetal activity have shown (1) gestational age–related differences in FHR, fetal breathing movement, and body movement responses and (2) possible prolongation of these effects at term for ≥1 hour.[7] Because most antepartum fetal evaluations are performed at or near term, it would be important to examine the consequences of vibroacoustic stimulation in this gestational age group with regard to the occurrence, magnitude, and duration of any observed alterations in these activity responses.

The present study was conducted to determine the

From the Department of Obstetrics and Gynecology, Medical College of Georgia.

Presented as Official Guest at the Fifty-first Annual Meeting of the South Atlantic Association of Obstetricians and Gynecologists, Hot Springs, Virginia, January 29–February 1, 1989.

Reprint requests: Lawrence D. Devoe, MD, Medical College of Georgia, Department of Obstetrics and Gynecology, Maternal-Fetal Medicine Section, Augusta, GA 30912.

6/6/12990

effects of a uniform vibroacoustic stimulus produced by an electronic artificial larynx and observed under standardized environmental conditions on baseline FHR, FHR variation, fetal breathing movement and fetal body movement, and fetal breathing movement rate and variability.

Material and methods

Study population. A total of 20 healthy women with pregnancies between 37 weeks' and 40 weeks' gestation were recruited from the general obstetric population at the Medical College of Georgia Hospital and Clinics. All patients had normal prenatal courses with gestational age confirmed by ultrasonographic measurements before week 20 of pregnancy and normal responses to glucose screening between 24 weeks' and 32 weeks' gestation. None of the patients smoked or received medications other than prenatal vitamins. All fetuses were singleton, appropriate in size for dates, and anatomically normal. Patients were entered into the study after they signed an informed consent form approved by the institutional Human Assurance Committee.

Study design. Patients were instructed to eat an approximately 400 kcal breakfast on the day of study, to refrain from eating after study began, and to avoid for the preceding 24 hours drugs with central nervous system effects or beverages that contained caffeine. Before each study, patients were assigned by random lot to either the control (unstimulated) or the experimental (stimulated) group. The principal investigator was not aware of each patient's group assignment during the study.

Studies were conducted in a quiet room with the patient in a semirecumbent position, at 2 hours after a meal. A 1-hour baseline observation period was then begun, after which the principal investigator left the study area and was unable to hear whether the stimulus was applied. An artificial electronic larynx (Western Electric Model 5C, Wallingford, Conn., courtesy of John Kasper, Corometrics Medical Systems) was placed on the maternal abdomen over the fetal vertex and, according to group assignment, either no signal was delivered or a 3-second vibroacoustic pulse was generated. The physical properties of this device have been previously described.[8] The investigator returned to the study room and observation continued for 2 additional hours.

Data collection and analysis. Concurrent observations were made of FHR baseline, acquired from an electronic fetal monitor (Hewlett Packard Model 8040A, Boblingen, West Germany) in the Doppler mode, and hardwired to the serial port of a specially programmed IBM XT microcomputer (512K random-access memory), and of real-time ultrasonographic detection of fetal breathing movement and fetal body movement with a remote-event marker, hardwired to the computer as previously described.[9] The recognition of fetal breathing movements and fetal body movements followed the criteria reported by Patrick et al.[10]

Table I. Outcome of study group patients

	Control group (No stimulus) No. = 10	*Experimental group (Stimulus) No. = 10*
Gestational age at study (wk)	38.7 ± 0.9	37.9 ± 0.3
Gestational age at delivery (wk)	40.0 ± 0.7	40.0 ± 1.3
Birth weight (gm)	3255 ± 384	3412 ± 179
5-Minute Apgar score	8.8 ± 1.1	8.8 ± 0.7

Each value expressed as mean ± SD.

At the conclusion of each study the following biophysical observations were analyzed off-line at 15-minute intervals: (1) baseline FHR; (2) long-term FHR variation (1-minute coefficient of variation of FHR baseline); (3) percentage of time spent breathing; (4) breath rate; (5) coefficient of variation of mean breath interval; and (6) percentage of time spent in body movements.

Statistical treatment of the data used a correlational analysis of variance. A p value <0.05 was considered significant.

Results

All results are presented as mean ± SD.

Obstetric outcomes. All patients were delivered of infants at term and their infants had no intrapartum or postpartum complications. Each neonate was judged to be of appropriate size for gestational age, and had a 5-minute Apgar score >7. All neonatal courses were normal. A comparison of gestational age at testing and delivery, birth weight, and mean 5-minute Apgar scores between control and experimental groups is shown in Table I. There were no significant differences between the groups for any of these variables.

FHR baseline. Mean 15-minute FHR baseline values of the control and experimental groups were 135 ± 6 beats/min and 136 ± 8 beats/min, respectively, and were not significantly different during the initial 60 minutes of observation (Fig. 1). The FHR baseline of the experimental group increased significantly (to 147 ± 9 beats/min) during the first 15 minutes after stimulation when compared with the control group ($p < 0.05$). Analysis of variance with repeated measures confirmed that the significant change in mean baseline FHR for the experimental group was confined to the first 15 minutes after stimulation ($p = 0.001$). Initial 60-minute control period values of mean 1-minute coefficients of variation of FHR baseline were similar in the experimental and control groups (0.045 ± 0.02

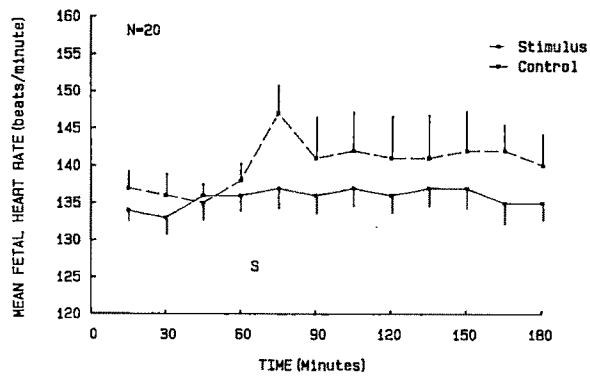


Fig. 1. Mean baseline FHR is plotted in 15-minute intervals before and after vibroacoustic stimulation(s). Experimental group is indicated by *broken line;* control group is indicated by *solid line. Bars* represent SEM.

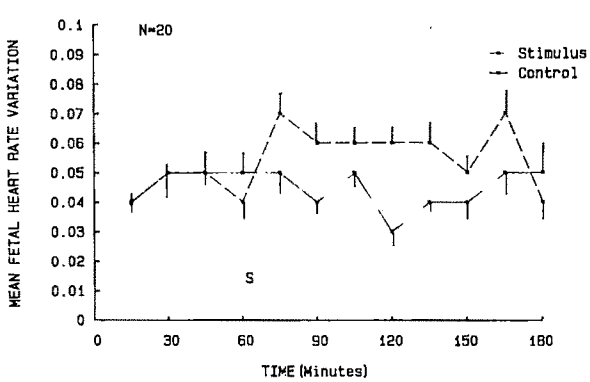


Fig. 2. Mean 1-minute coefficient of variation of baseline FHR is plotted in 15-minute intervals before and after vibroacoustic stimulation(s). Experimental group is indicated by *broken line;* control group is indicated by *solid line. Bars* represent SEM.

and 0.048 ± 0.03, respectively) (Fig. 2). Mean 1-minute coefficient of variation of baseline FHR also increased for the experimental group during the first 15 minutes after stimulation, when compared with the control group ($p < 0.05$). Analysis of variance with repeated measures showed no significant differences in either group for the remainder of the study period.

Fetal breathing activity. The mean 15-minute percentage of time spent breathing was similar for both groups (control group: 37.4% ± 27.8%; experimental group: 29.0% ± 25.3%) during the first hour of study. After stimulation there was a significant fall in breathing activity for the experimental group to 17.8% ± 15.9% ($p < 0.05$) when compared with the control group (Fig. 3). Analysis of variance with repeated measures showed that trends in breathing activity were not significantly different in either the experimental or control group. Fetal breath rates were similar in both groups during the control period and after stimulation

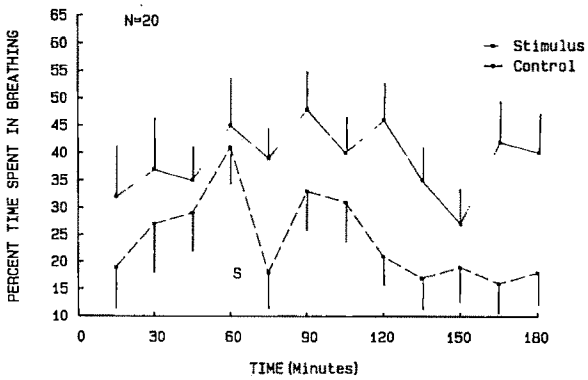


Fig. 3. Mean time spent breathing is plotted in 15-minute intervals before and after vibroacoustic stimulation(s). Experimental group is indicated by *broken line;* control group is indicated by *solid line. Bars* represent SEM.

(Fig. 4) as were mean 15-minute coefficients of variation of breath intervals (Fig. 5).

Fetal body movements. The mean 15-minute percentage of time spent in movement was 4.4% ± 6.0% and 4.9% ± 5.0% in the experimental control groups, respectively, during the control period. These values were not significantly different. After stimulation there were no immediate changes in this activity. Although a peak in fetal body movement incidence occurred at 120 minutes in the experimental group, analysis of variance failed to show significant effects with time in either group over the total study period (Fig. 6).

Individual fetal responses. Eight of the experimental group fetuses had an increase in mean baseline FHR (>10 beats/min) and six had a decline in fetal breathing movement during the first 15 minutes after stimulation (>50%). Two fetuses had no apparent biophysical response to the stimulus. When behavioral state vectors (e.g., FHR variation, fetal movement incidence, and fetal breathing patterns) were taken into consideration, there were no apparent differences in prestimulus states between fetuses with altered biophysical activities and those with unchanged poststimulation activities. Although rapid eye movements were not continuously recorded as part of the study protocol, the consistent presence of irregular breathing activity, the pattern of baseline FHR variability, and relatively low incidence of fetal body movement, suggested that all of the fetuses in both the control and the experimental group were either entering or already in an active sleep state.

Comment

This study has shown that a uniform brief vibroacoustic stimulus is associated with alterations in selected biophysical parameters of normal term human fetuses. Under standardized observation conditions with the use of a computerized data acquisition and analysis system, we have been able to describe precisely the mag-

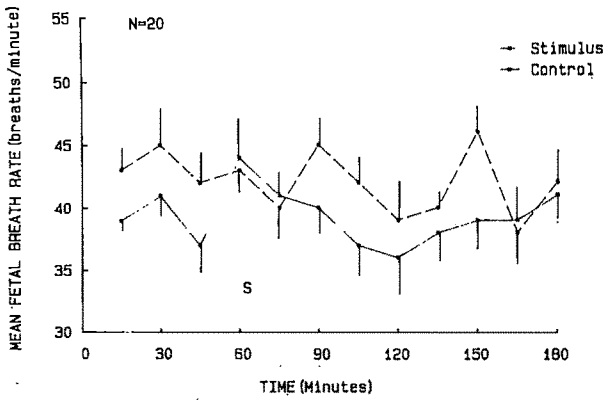


Fig. 4. Mean fetal breath rate is plotted in 15-minute intervals before and after vibroacoustic stimulation(s). Experimental group is indicated by *broken line;* control group is indicated by *solid line. Bars* represent SEM.

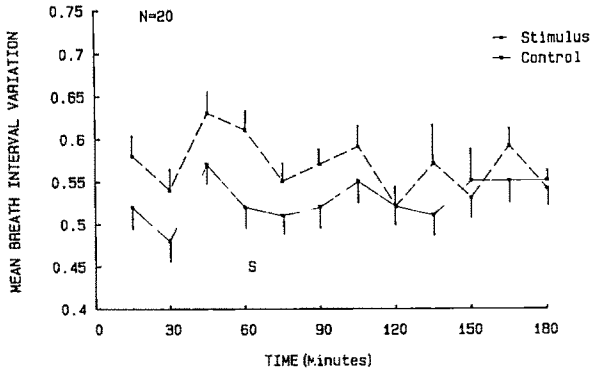


Fig. 5. Mean coefficient of variation of mean breath intervals is plotted in 15-minute intervals before and after vibroacoustic stimulation(s). Experimental group is indicated by *broken line;* control group is indicated by *solid line. Bars* represent SEM.

nitude and duration of these responses. Most healthy term fetuses appear to respond to an external vibroacoustic signal with transient increases in baseline FHR and FHR variation, largely confined to the first 15 minutes after stimulation. Concurrently, fetal breathing activity declines sharply in the stimulated group during the first 15 minutes and throughout the remainder of observation in both groups. This probably reflects naturally occurring biologic cycles[11] rather than sustained poststimulus effects. Poststimulus changes in biophysical activity appear to be selective as fetal body movement incidence and breath interval variability were not significantly changed. The presence or absence of fetal "responses" to vibroacoustic stimulation are not entirely predictable, as two fetuses did not show changes in biophysical activity. An important observation is that any alteration in biophysical response that might be attributed to vibroacoustic stimulation does not seem to be prolonged for more than 15 minutes after stimulation.

Whereas vibroacoustic stimulation of the human fetus has been studied >50 years,[1] the recent reports of Gagnon et al.[7,8] were among the first to explore possible mechanisms of its action. The present study is similar in design and intent to those of Gagnon et al. but differs in several important respects. First, the observation period was 60 minutes longer so that at least one completed biologic cycle[11] would be included during the study. The actual pulse duration was 2 seconds shorter. The statistical treatment of our study data differed from theirs in that our analysis focused on the intragroup effects of time and the possible intergroup differences that might be attributed to the experimental maneuver. These differences in study approach suggest that between groups, the most likely sources of differences in any biophysical parameter value, excluding the initial 15-minute poststimulation period, result from normal biologic cycles, as described by Campbell et al.[11]

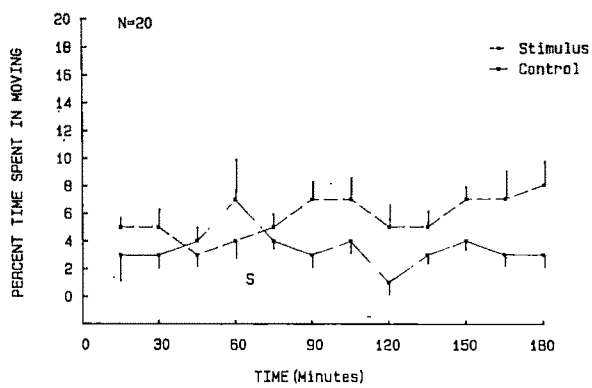


Fig. 6. Mean percentage of time spent in movement is plotted in 15-minute intervals before and after vibroacoustic stimulation(s). Experimental group is indicated by *broken line;* control groups is indicated by *solid line. Bars* represent SEM.

The mechanism of action by which vibroacoustic stimulation appears to affect the fetus is undetermined. Grimwade et al.[12] have suggested that the vibratory component is more important than the acoustic component in eliciting responses. Fetal sensitivity to this stimulus may be related to the maturation of sensory pathways that involve Meissner corpuscles, as the occurrence and magnitude of a poststimulus response are directly related to length of gestation.[7] Our data demonstrate that, even at term, not all normal fetuses respond to vibroacoustic stimulation and thus suggest that the stimulus-response characteristics of this system may continue to vary throughout pregnancy.

Our data do not support the speculation that vibroacoustic stimulation alters subsequent fetal behavioral states,[7] as characterized by coherent patterns of motor activity that persist for at least several minutes.[13] If significant or prolonged alterations in state had occurred, significant coincident differences in FHR variation, body movement incidence, breathing activity,

and FHR baseline would have been apparent, especially because the study population was at a gestational age when behavioral stages are well organized. It appears more likely that poststimulus changes involve individual state vectors for brief periods of time.

In conclusion, we have attempted to identify and clarify some of the fetal biophysical effects associated with vibroacoustic stimulation, delivered by an electronic larynx. These effects are brief and do not generally persist more than 15 minutes after the stimulus is applied. The directions in which state-related activities are changed and the lack of uniformity in poststimulus fetal responses suggest that disorganization of behavioral state may occur after vibroacoustic stimulus. It is important to obtain additional information concerning the mechanisms of vibraocoustic stimulation before it can be recommended without qualifications for assessment of fetal well-being.

REFERENCES

1. Sontag LW, Wallace RF. Changes in the rate of the human fetal heart in response to vibratory stimuli. Am J Dis Child 1936;51:583.
2. Bernard J, Sontag LW. Fetal reactivity to tonal stimulation: a preliminary report. J Genet Psychol 1947;70:205.
3. Smith CV, Phelan JP, Paul RH, Broussard P. Fetal coustic stimulation testing: a retrospective experience with fetal acoustic stimulation. AM J OBSTET GYNECOL 1985;153: 567-8.
4. Smith CV, Phelan JP, Platt LD, Broussard P, Paul RH. Fetal acoustic stimulation testing. AM J OBSTET GYNECOL 1986;155:131-4.
5. Smith CV, Nguyen HN, Phelan JP, Paul RH. Intrapartum assessment of fetal well-being: a comparison of fetal acoustic stimulation with acid-base determinations. AM J OBSTET GYNECOL 1986;155:726-8.
6. Edersheim TG, Hutson JM, Druzin ML, Kogut EA. Fetal heart rate response to vibratory acoustic stimulation predicts fetal pH in labor. AM J OBSTET GYNECOL 1987;157:1557-60.
7. Gagnon R, Hunse C, Carmichael L, Fellows F, Patrick J. Human fetal responses to vibratory acoustic stimulation from twenty-six weeks to term. AM J OBSTET GYNECOL 1987;157:1375-81.
8. Gagnon R, Hunse C, Carmichael L, Fellows F, Patrick J. External vibratory acoustic stimulation near term: fetal heart rate and heart rate variability responses. AM J OBSTET GYNECOL 1987;156:323-7.
9. Devoe LD, Searle N, Searle J, et al. Computer-assisted assessment of the fetal biophysical profile. AM J OBSTET GYNECOL 1985;153:317-21.
10. Patrick J, Campbell K, Carmichael L, Natale R, Richardson B. Patterns of human fetal breathing during the last 10 weeks of pregnancy. Obstet Gynecol 1980;56:24.
11. Campbell K. Ultradianrhythms in the human fetus during the last 10 weeks' gestation: a review. Semin Perinatol 1980;4:301.
12. Grimwade JG, Walker DW, Bartlett M, Gordon S, Wood CC. Human fetal heart rate change and movement in response to sound and vibration. AM J OBSTET GYNECOL 1971;109:86.
13. Nijhuis JG, Prechtl HFR, Martin CB Jr, Bots RSCM. Are there behavioral states in the human fetus? Early Hum Dev 1982;6:177.

Editors' note: This manuscript was revised after the discussions were presented.

Discussion

DR. GUY M. HARBERT, JR., Charlottesville, Virginia. For >50 years it has been known that the fetus responds to sound and vibration. In 1977 clinical application of these observations was stimulated by Read and Miller[1] when they published preliminary results that indicated acoustic stimulation of the fetus evoked accelerations in FHR that correlated well with fetal outcome. They proposed that a positive fetal response to auditory stimulation would obviate the need for an oxytocin challenge test. In 1984 Serafini and colleagues[2] at the University of Maryland reported that acoustic stimulation was a safe and rapid test of fetoplacental sufficiency comparable to nonstress tests in reliability. Over the next 2 years, retrospective studies championed the advantages of fetal acoustic stimulation in the decrease in the incidence of nonreactive nonstress tests and in the reduction of testing time.

With the use of the number of published manuscripts as a guide, it would appear that fetal acoustic stimulation came of age in the latter half of 1987.

In 1988 there were articles that suggested ultrasonic imaging of fetal facial reactions and response decrement patterns to acoustic stimulation may be a useful tool for in utero neurologic evaluation[3] and that differences in response may distinguish the small-for-gestational-age fetus from one with appropriate growth.[4] Investigators who performed Doppler flow velocity measurements demonstrated a significant decrease in umbilical peak-systolic/end-diastolic ratios immediately after acoustic stimulation.[5] Others have recorded the sound pressure in amniotic fluid created by application of an electronic artificial larynx to the abdominal surface of pregnant ewes.[6]

Wheras fetal acoustic stimulation is generally considered to be safe, severe fetal bradycardia caused by external vibratory acoustic stimulation has been reported.[7] With an increase in interest in and application of acoustic stimulation of the fetus, this presentation, which describes the occurrence, magnitude, and duration of some aspects of the fetal response to a vibroacoustic stimulus, is very appropriate.

The investigational protocol is well designed, the control and experimental groups are well matched and the conclusions are appropriate for the data presented. Extension of the observation period to include at least one completed biologic cycle is a significant contribution to better understanding of the fetal biophysical effects. A weakness of the experimental design is that a cross-over study was not performed. This would have doubled the data points without jeopardizing the initial randomization. Because each subject would then serve as her own control, it would have emphasized the differences or lack thereof that occurred as a result of vibroacoustic stimulation. However, failure to do this cross-over does not detract from the significance of the findings.

The statistics used are appropriate but are sometimes confusing in their presentation. For example, it is stated that fetal breathing activity in the experimental group was decreased significantly after stimulation, yet the

analysis of variance with repeated measures showed that this trend was not significantly different from that observed in the control group. The latter statement could lead one to question whether there was really a change in fetal breathing activity in response to the stimulus.

I have three other questions. First, under individual fetal responses, it is reported that eight of the experimental fetuses had an increase in mean baseline heart rate and six had a decline in fetal breathing movements. This leaves the possibility that two of the fetuses that were stimulated showed no response in either heart rate or breathing movements. Is this correct?

Second, you state that the consistent presence of irregular breathing activity, the pattern of baseline FHR variability, and the relatively low instance of fetal body movement suggests that all of the fetuses who received stimulation were either entering or already in an active sleep state. Would not this statement also apply to the control group?

And third, other investigators have reported a significant negative correlation between the maximum of the first FHR acceleration and the prestimulation basal FHR.[8] Did you note such a correlation?

I compliment Dr. Devoe and colleagues on their study of the fetal biophysical effects associated with vibroacoustic stimulation and encourage them to continue and expand their investigations. All too often in obstetrics we have seen new techniques accepted as dogma in clinical situations with only minimal understanding of the mechanisms involved. With the apparent proliferation of interest in fetal vibratory acoustic stimulation each contribution of basic information such as presented here will enable us to better interpret and understand the safety and the significance of stimulus response in clinical situations.

REFERENCES

1. Read JA, Miller FC. Fetal heart rate acceleration in response to acoustic stimulation as a measure of fetal well-being. AM J OBSTET GYNECOL 1977;129:512.
2. Serafini P, Lindsay MBJ, Nagey DA, Pupkin MJ, Tseng P, Crenshaw C. Antepartum fetal heart rate response to sound stimulation: the acoustic stimulation test. AM J OBSTET GYNECOL 1984;148:41.
3. Kuhleman KA, Bruns KA, Depp R, Sabbaga RE. Ultrasound imaging of normal fetal response to external vibratory acoustic stimulation. AM J OBSTET GYNECOL 1988; 158:47.
4. Gagnon R, Hunse C, Fellows F, Carmichael L, Patrick J. Fetal heart rate and activity patterns in growth-retarded fetuses: changes after vibratory acoustic stimulation. AM J OBSTET GYNECOL 1988;158:265.
5. Gagnon R, Morrow R, Ritchie K, Hunse C, Patrick J. Umbilical and uterine artery blood flow velocities after vibratory acoustic stimulation. AM J OBSTET GYNECOL 1988; 159:574.
6. Gerhardt KJ, Abrams RM, Kovaz BM, Gomez KJ, Conlon M. Intrauterine noise levels produced in pregnant ewes by sound applied to the abdomen. AM J OBSTET GYNECOL 1988;159:228.
7. Sherer DM, Menashe M, Sadousky E. Severe fetal bradycardia caused by external vibratory acoustic stimulation. AM J OBSTET GYNECOL 1988;159:334.
8. Gagnon R, Hunse C, Patrick J. Fetal responses to vibratory acoustic stimulation: influence of basal heart rate. AM J OBSTET GYNECOL 1988;159:835.

DR. WILLIAM F. O'BRIEN, Tampa, Florida. This is excellent and important work for a very important reason. We are getting farther along in the avoidance of contraction stress tests by doing vibroacoustic stimulation in the patient who has nonreactive nonstress test results. However, we begin to lose track of the fact that what we really are trying to do is to find that very small group of fetuses that have a high likelihood of poor outcome. Reactivity after vibroacoustic stimulation offers a degree of reassurance similar to reactivity without vibroacoustic stimulation. It is important for us to know what vibroacoustic stimulation does to other test parameters such as the biophysical profile. How long after a vibroacoustic stimulation test must we wait before performance of a biophysical profile? Dr. Devoe's study suggests to me that a biophysical profile done 30 to 45 minutes later is not affected by a preceding vibroacoustic stimulation. I want to ask Dr. Devoe whether he agrees with this conclusion.

DR. JOHN W. C. JOHNSON, Gainesville, Florida. I also congratulate Dr. Devoe on a very well-designed study and particularly on his precautionary conclusions. There are a number of people who have raised concerns about the acoustic pressure generated by this test and to date there really have not been any careful follow-up studies as to whether there might be auditory damage as a result of this study. I wonder whether Dr. Devoe has any information or concerns about this issue.

DR. DEVOE (Closing). I appreciate the constructive insights of Dr. Harbert and agree that a cross-over study would have been ideal if it would have been practical to have arranged second sessions for each patient. In response to his first question, the analysis of our data shows that extension of the study period for 2 hours after stimulation supports the conclusion that alterations in breathing activity, which occurred in both groups, are unlikely to have resulted from stimulation. As to the next question, two fetuses in the experimetnal group did not show altered FHR or breathing activity after stimulus. It is also probable that fetuses in both the control group and the experimental group were entering or already in active sleep before stimulation. Our observations with regard to prestimulus baseline and first FHR accelerations are similar to those reported by Gagnon et al.

Dr. O'Brien raises a thoughtful question (i.e., does vibroacoustic stimulation have a lingering effect on subsequent biophysical tests?). Our data suggest that such an effect would be unlikely after the first 15 minutes after stimulation.

Dr. Johnson focuses on an area of common concern: is this technology safe for the infant? Whereas no adverse effects have been reported for our study infants, I cannot offer this as a blanket endorsement as our numbers are small and the follow-up period was confined to the time spent in the normal newborn nursery.

Seminoma in pubertal patient with androgen insensitivity syndrome

W. Glenn Hurt, MD,[a] Joann N. Bodurtha, MD,[b] Janice B. McCall, MD,[c] and M. Moinuddin Ali, MD[d]

Richmond, Virginia

The case of a 14-year-old girl with complete androgen insensitivity syndrome and metastatic seminoma is reported. She was treated by bilateral adnexectomy, removal of paraaortic lymph nodes, postoperative radiation, and estrogen replacement therapy. She represents the fourth case of gonadal malignancy to be reported in a teenage patient with androgen insensitivity syndrome. (AM J OBSTET GYNECOL 1989;161: 530-1.)

Key words: Seminoma, androgen insensitivity syndrome, testicular feminization

Complete androgen insensitivity syndrome is a genetic disorder with X-linked inheritance that is associated with an XY karyotype, bilaterally maldescended testes, and androgen insensitivity caused by androgen receptor defect(s). Individuals with androgen insensitivity syndrome are prone to develop gonadal tumors but have been observed to do so at a later age and with less frequency than those with gonadal dysgenesis. We believe this is the fourth case of invasive gonadal malignancy to be reported in a teenage patient with androgen insensitivity syndrome.

Case report

A 14-year-old nulligravid patient was referred because of an abdominopelvic mass and primary amenorrhea. The patient had a female phenotype, Tanner III breast development, and no secondary sexual hair. The blind vagina was 3 cm long. A large, firm mass filled the pelvic and lower abdominal cavities and extended to within 3 cm of the lower margin of the umbilicus.

The β-subunit of human chorionic gonadotropin, α-fetoprotein, CA 125, and carcinoembryonic antigen levels were normal. Results of chest radiography and excretory urography were normal. Transvaginal fine-needle aspiration of the mass was performed; the cytopathologic diagnosis was dysgerminoma-seminoma.

Exploratory laparotomy revealed a solid left adnexal mass that filled the lower abdominal and pelvic cavities, a right adnexal mass that resembled a testicle, and two suspicious left paraaortic lymph nodes underlying the left renal vessels. There was no evidence of a uterus. Both adnexa, the two lymph nodes, and a portion of the omentum were removed.

The left adnexal mass measured 17 × 12 × 6 cm and weighed 930 gm. Histologically it was typical of a seminoma. The right adnexal mass was an immature testis that contained foci of intratubular seminoma. Metastases of 1 × 2 cm were present in each of the two left paraaortic lymph nodes.

The patient was treated with radiation therapy as for a stage IIA testicular seminoma (M. D. Anderson modification of the Dixon and Moore staging system). A 4 MeV linear accelerator was used to deliver a total dose of 2445 cGy to the pelvic and paraaortic areas with a boost of 600 cGy to the site of the positive paraaortic nodes. Three weeks later, the mediastinum and supraclavicular fossae were treated with a total dose of 2550 cGy. The patient takes conjugated estrogen as replacement therapy. She is now well and free of disease 18 months after surgery.

Cytogenetically, the patient was reported to have a 46,XY karyotype with no evidence of mosaicism. The presence of a structurally normal Y chromosome was confirmed by C-banding. Studies performed on skin from the labia majora revealed 5 α-reductase activity of 18.5 pmol/h/mg protein (normal > 1.0) and an androgen receptor level of 1.1 fmol/mg protein (normal > 15.0). The patient is reported to have had an aunt who was not able to have children because she had "the internal organs of a male."

Comment

In their study of 181 cases of androgen insensitivity syndrome, Morris and Mahesh[1] found a 22% overall incidence of malignant gonadal tumors. The majority of the malignancies were seminomas, but there was also a malignant teratoma and a Sertoli-Leydig cell tumor. Of the patients with gonadal tumors in their series, one was a teenager, two were patients in their 20s, and the remainder were 30 years of age or older. The one teen-

From the Departments of Obstetrics and Gynecology,[a] Human Genetics,[b] Pathology,[c] and Radiation Oncology,[d] Medical College of Virginia, Virginia Commonwealth University.

Presented at the Fifty-first Annual Meeting of the South Atlantic Association of Obstetricians and Gynecologists, Hot Springs, Virginia, January 29–February 1, 1989.

Reprint requests: W. Glenn Hurt, MD, Department of Obstetrics and Gynecology, Box 34, MCV Station, Richmond, VA 23298.

6/6/12994

ager, reported originally by Carmichael and Oldfield,[2] was 14 years old and had a malignant teratoma of the left gonad. This is the youngest patient with androgen insensitivity syndrome reported with an invasive gonadal malignancy.

Perez-Palacios and Jaffe[3] reported that one of their patients with androgen insensitivity syndrome had an 18-year-old sibling who died with an "abdominal malignancy" that was a gonadal stromal tumor and was associated with absence of the uterus. The fact that complete androgen insensitivity syndrome is an X-linked trait suggests that this sibling may have had the disorder.

Horcher et al.[4] reported a 15-year-old patient in whom they diagnosed androgen insensitivity syndrome. On the basis of the recommendation that patients with androgen insensitivity syndrome be allowed to complete pubertal feminization, they chose not to perform prophylactic gonadectomy at the time the diagnosis was made. When the patient was 16, she had surgery because of increasing pain in the left inguinal region. The left testis was partially within the inguinal canal, and this was thought to be the cause of the pain. The right testis was free within the abdomen; it contained a seminoma.

To calculate the potential for the development of gonadal tumors in patients with androgen insensitivity syndrome, Manuel et al.[5] combined a series of 23 cases of patients with no tumors with 82 cases from the medical literature and determined that the expectancy of tumors is 3.6% at age 25 and 33% at age 50. They conclude that "if a risk of something less than four percent is acceptable, delay in removal of the gonads may be entertained until approximately the age of thirty."

More recently Muller and Skakkebaek[6] have described carcinoma in situ in the testes of three of eight consecutive patients with incomplete androgen insensitivity syndrome at 2 months and at 13 and 14 years of age. These findings led them to recommend gonadal biopsy as soon as the syndrome is diagnosed. If the biopsy results reveal carcinoma in situ, they advocate immediate gonadectomy. If the biopsy was performed in early childhood with negative results, they recommend that it be repeated at puberty since the accuracy of a single biopsy is not known to determine whether gonadectomy can be further delayed. The recommendation that patients have serial biopsies and that gonadectomy be based on biopsy results has not gained wide acceptance.

Women with XY karyotypes are at increased risk for development of gonadal malignancies. Those with gonadal dysgenesis are more likely to develop gonadal malignancies and at a younger age than those with androgen insensitivity syndrome. It is accepted that patients with gonadal dysgenesis should have prophylactic gonadectomy as soon as their condition is diagnosed. The fact that three young patients with incomplete androgen insensitivity syndrome have been found to have carcinoma in situ of the testes and that four teenagers have been reported to have invasive gonadal malignancies suggests the need to reevaluate the recommendation that prophylactic gonadectomy in androgen insensitivity syndrome patients be postponed to permit pubertal growth and feminization. Gonadal biopsy may be a method to determine when to perform gonadectomy.

REFERENCES

1. Morris JM, Mahesh VB. Further observations on the syndrome, "testicular feminization." Am J Obstet Gynecol 1963;87:731-48.
2. Carmichael R, Oldfield C. A case of complete male pseudohermaphroditism with intra-abdominal teratoma testis. J Pathol Bacteriol 1934;39:617-22.
3. Perez-Palacios G, Jaffe RB. The syndrome of testicular feminization. Pediatr Clin North Am 1972;19:653-67.
4. Horcher E, Grunberger W, Parschalk O. Classical seminoma in a case of testicular feminization syndrome. Prog Pediatr Surg 1983;16:139-41.
5. Manuel M, Katayama KP, Jones Jr HW. The age of occurrence of gonadal tumors in intersex patients with a Y chromosome. Am J Obstet Gynecol 1976;124:293-300.
6. Muller J, Skakkebaek NE. Testicular carcinoma in-situ in children with androgen insensitivity (testicular feminization) syndrome. Br Med J1984;288:1419-20.

Successful pregnancy after a liver transplant

Cynthia J. Sims, MD, Kathy B. Porter, MD, and Robert A. Knuppel, MD, MPH
Tampa, Florida

The literature contains limited reports on successful pregnancy outcomes after a liver transplant. We report an uncomplicated pregnancy and delivery in a patient 35 months after liver transplantation because of chronic active hepatitis with resultant liver failure secondary to non-A non-B hepatitis. (AM J OBSTET GYNECOL 1989;161:532-3.)

Key words: Liver transplant, pregnancy outcome

This is a case report of a patient with a liver transplant who had a subsequent successful pregnancy outcome. Because only a few cases have been reported with varied outcomes, information gained from these reports during pregnancy and from other transplant recipients will assist in the guidance of obstetric management of these cases.

Case report

On June 11, 1987, a 26-year-old gravida 1, female was seen at an estimated gestational age of 10 weeks. Her medical history was remarkable for non-A non-B hepatitis that led to chronic active hepatitis and subsequent liver failure. In 1984 she underwent an orthotopic liver transplant with a relatively uncomplicated postoperative course. Over the subsequent year she was maintained on the immunosuppressive agent cyclosporine, 150 mg orally every 12 hours. After this regimen, results of liver function tests remained within the normal range without evidence of rejection. No serious infections were noted during the 6 months before pregnancy.

We chose to continue the patient on the immunosuppressive therapy that was established before pregnancy. Initial and subsequent laboratory evaluations of hepatic and renal function were normal. Serial sonograms revealed adequate growth of the fetus. No fetal anomalies were detected by ultrasonographic evaluation. At 32 weeks' gestation weekly nonstress tests were started and were noted to be reactive. At 38 weeks' gestation the fetus was noted to be in a breech presentation. On admission to the labor and delivery suite for an external cephalic version, the fetus spontaneously turned to a vertex presentation. The cervical examination revealed a Bishop score of 9, thus oxytocin induction of labor was begun. The patient underwent a spontaneous vaginal delivery of a 2724 gm female infant 13 hours later. Apgar scores were 9 at 1 and 5 minutes, respectively. The neonate did not have any obvious anomalies. After an uneventful 2-day nursery stay, the neonate was discharged to home with her mother. Breast-feeding was not advised while taking cyclosporine. At 3 months of age, the infant had a lower urinary tract infection that promptly responded to therapy. The mother and her infant were doing well 12 months after delivery.

Comment

The first case report of a successful pregnancy in a patient after a liver transplant was reported by Walcott et al. in 1978.[1] In 1980 Myers et al.[2] reported two different term pregnancies in the same patient. Newton et al.[3] reported the fourth third-trimester pregnancy associated with a liver transplantation patient. The pregnancy was complicated by abruptio placentae and preterm delivery at 27.5 weeks' gestation; both mother and infant survived. In each case the patient was immunosuppressed with azathioprine. In 1988 two cases were reported by Venkataramanan et al.[4] Immunosuppression was achieved with cyclosporine.

Standards of care for liver transplant recipients who conceive are not well established. When managing a liver transplant patient during pregnancy many issues still raise concern. Little is known about the effect of the pregnancy precipitating rejection of the transplanted liver. Also, the transplanted liver may affect the course of the pregnancy. These effects were not observed in our patient.

A particular concern during pregnancy in liver transplant recipients is the potential harmful effect of chronic maternal immunosuppression. The literature available on renal transplant patients served as an important guide in the management of our patient. However, the majority of the literature contains information about the immunosuppressive agent azathioprine. Hu-

From the Department of Obstetrics and Gynecology, University of South Florida College of Medicine.
Presented as Official Guest at the Fifty-first Annual Meeting of the South Atlantic Association of Obstetricians and Gynecologists, Hot Springs, Virginia, January 29–February 1, 1989.
Reprint requests: Cynthia J. Sims, MD, Medical College of Pennsylvania, Allegheny Campus, 320 E. North Ave., Pittsburgh, PA 15212.
6/6/12992

man organ transplant recipient survival rate has been improved by use of the immunosuppressive agent cyclosporine. Cyclosporine is lipid soluble, widely distributed in the body, and highly metabolized. Cyclosporine has been detected in cord blood, placenta, amniotic fluid, and breast milk. The toxicity of cyclosporine metabolites is unknown. Cyclosporine did not produce any chromosomal abnormalities in animals exposed to chronic therapy. Further follow-up is necessary to evaluate the possible effects on the fetus exposed to chronic cyclosporine immunosuppression in utero.

The care of the organ transplant recipient during pregnancy requires a team approach. Not only are the immediate concerns of the pregnancy important, but long-term follow-up of the mother and her infant will be relevant to fully evaluate the extent of possible complications.

REFERENCES

1. Walcott WO, Derick DE, Jolley JJ, Snyder DL. Successful pregnancy in a liver transplant patient. AM J OBSTET GYNECOL 1978;132:340.
2. Myers RL, Schmid R, Newton JJ. Childbirth after liver transplantation. Transplantation 1980;29:432.
3. Newton ER, Turksay N, Kaplan M, Reinhold R. Pregnancy and liver transplantation. Obstet Gynecol 1988; 71:499.
4. Venkataramanan R, Koneru B, Wang CC, Burckart GJ, Caritis S, Starzl TE. Cyclosporine and its metabolites in mother and baby. Transplantation 1988;46:468.

A clinical-pathologic study of mixed müllerian tumors of the uterus over a 16-year period—The Medical College of Georgia experience

Donald G. Gallup, MD, Donna S. Gable, MD, O. Eduardo Talledo, MD, and Luther B. Otken, Jr., MD

Augusta, Georgia

From 1972 through 1987, 40 patients at the Medical College of Georgia were assessed with a diagnosis of mixed müllerian tumors which constituted 3.5% of all female genital tract malignancies. The mean patient age was 65.3 years; 60% of the patients had stage I disease. Of those patients with clinical stage I disease, more advanced disease was found at celiotomy in 33%. Retroperitoneal nodes were positive for malignancy in 35% of 20 patients who underwent node sampling. Cell washings were positive in 12%, and 9% had omental metastases. Forty-seven percent had homologous tumors; 53% of tumors were heterologous. Relatively poor prognosis was associated with large tumor volume, vascular invasion, nodal metastases, and disease outside the uterus. The overall survival rate was 32%; 14 of 24 patients with stage I disease are dead of disease. Of patients dead of disease, 92.5% had distant metastases. Adjuvant therapy with a combination of VP-16, cisplatin, and irradiation was beneficial in four high-risk patients. (AM J OBSTET GYNECOL 1989;161:533-9.)

Key words: Mixed müllerian tumors, uterus, cancer

Mixed müllerian tumors of the uterus have been traditionally associated with a relatively poor prognosis. Controversies about some prognostic factors continue.

From the Departments of Obstetrics and Gynecology and Pathology, Medical College of Georgia.
Presented as Official Guest at the Fifty-first Annual Meeting of the South Atlantic Association of Obstetricians and Gynecologists, Hot Springs, Virginia, January 29–February 1, 1989.
Reprint requests: Donald G. Gallup, MD, Medical College of Georgia, Department of Obstetrics and Gynecology, Section of Gynecologic Oncology, Augusta, GA 30912.
6/6/12993

Mixed tumors with homologous patterns (tissue elements entirely indigenous to the uterus) have been thought to be associated with a better survival than mixed tumors with heterologous elements (tissue with cells foreign to the uterus). If the heterologous element present is other than chondrosarcoma (i.e., striated muscle), survival has been relatively poor.[1,2] The mixed müllerian tumors are highly aggressive neoplasms that spread early to regional nodes and adjacent tissues. Nodal involvement is usually associated with deep myometrial invasion. In an earlier report, DiSaia et al.[3]

Table I. Status of retroperitoneal nodes in 20 patients with stage I or II mixed müllerian tumors

Positive nodes	*No. of patients*	*%*
Pelvic	4	20
Paraaortic	1	5
Pelvic and paraaortic	2	10
Total	7	35

Table II. International Federation of Gynecology and Obstetrics clinical stage and survival

Stage	*No. of patients*	*Dead of disease*	
		n	*%*
*I	24	14†	58.3
*II	8	5	62.5
III	1	1	100
IV	7	7	100
Total	40	27	67.5

*Three in stage I and two in stage II are alive with disease.
†One patient lost to follow-up and presumed dead.

noted pelvic node metastases in one third of the patients who had operations. The single most important factor signaling poor prognosis at diagnosis is extrauterine extension of disease. Hematogenous spread of both homologous and heterologous tumors is not uncommon.[4-8] Controversy regarding the role of primary surgical staging versus preoperative pelvic irradiation continues. The role of presently available chemotherapy as an adjuvant in primary treatment is also unsettled. This article is a retrospective review of the patients assessed with a diagnosis of mixed müllerian tumor who were managed at one institution during a 16-year period.

Material and methods

The clinical records of all patients assessed with a diagnosis of mixed müllerian tumor of the uterus at the Medical College of Georgia from Jan. 1, 1972, to Dec. 31, 1987, were reviewed. Follow-up information was obtained from tumor records, and when necessary by telephone and correspondence. Histopathologic material was reviewed with two pathologists (D. S. G. and L. B. O.). Re-cuts and special stains were obtained and reviewed when necessary.

The patients were retrospectively staged based on the 1971 International Federal of Gynecology and Obstetrics criteria for adenocarcinoma of the uterine corpus. In addition to the clinical stage, patients who underwent celiotomy were also staged on a surgical-pathologic basis.

Results

During the study, 49 patients were assessed with a diagnosis of mixed müllerian tumor of the uterus. Nine patients were removed from the study group because of inadequate material to make the diagnosis (5 patients), or because of misdiagnosis after the slides were reviewed; thus 40 patients qualified for the study group. These cases constituted approximately 3.5% of all female genital tract malignancies managed at the Medical College of Georgia during the same period.

The mean age was 65.3 years, with a range from 47 to 89 years. Only one patient was <50 years old, and 26.2% were <60. All but three women were postmenopausal. The mean parity was 2.9, but 14.3% of the patients were nulliparous. One third of the patients were white; 67% were black. Thirty-three percent had diabetes mellitus. The most frequent initial primary symptom was abnormal vaginal bleeding, which occurred in 95% of patients. Of the two patients who had no bleeding, one had passage of tissue, and the other, who had prior irradiation treatment, had ascites. Other symptoms noted in addition to bleeding were pain (17%), increased girth (11%), and weight loss (5%). Exogenous estrogen was used from 5 to 25 years by 10% of the patients. Of those with symptoms, 9.5% of patients gave a symptom history of <1 month; 48%, <3 months, and 90%, <6 months. Only 7.5% had a history of prior pelvic irradiation treatment for cervical cancer. Additionally, one patient had prior breast cancer and one had chronic lymphocytic leukemia. Twenty-five percent of the patients weighed >20% of their ideal body weight. Results of cervical cytologic testing, available in 25 patients, were suspicious for malignancy in 44% and inflammatory in 12%.

When International Federal of Gynecology and Obstetrics criteria for staging were applied to the study cases, 60% had clinical stage I disease; 20% had clinical stage II disease; 2.5% had clinical stage III tumors, and 17.5% had clinical stage IV disease. Six women with clinical stage I and II disease did not undergo hysterectomy. Thirty-three percent of women undergoing surgery were restaged from clinical stage I to more advanced disease. Four of five patients with clinical stage II disease had more advanced disease at celiotomy. Retroperitoneal nodes were positive for tumor in 35% of 20 patients who underwent node sampling (Table I). Nine percent of patients undergoing celiotomy with hysterectomy and removal of the ovaries had omental metastases, regardless of whether nodes were sampled. Cul-de-sac washings were positive in 12%. All living patients have been followed up at least 9 months. When survival was correlated with clinical

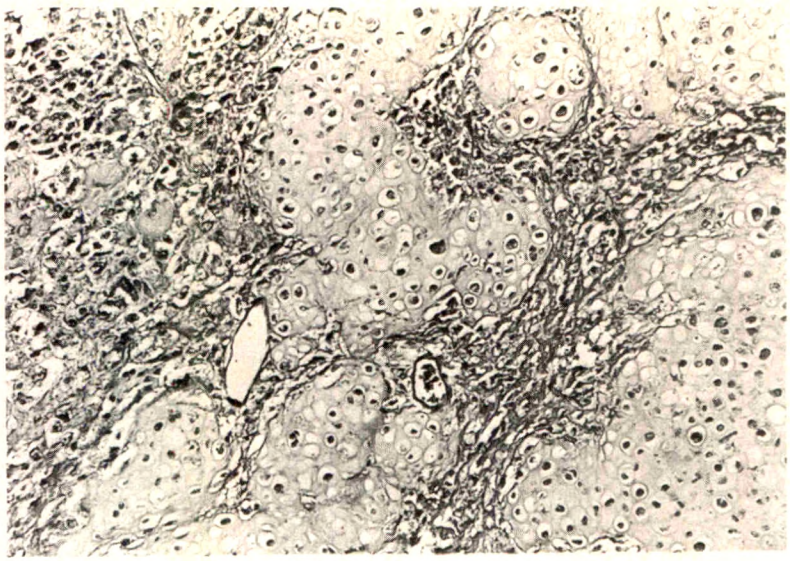


Fig. 1. Chondrosarcoma. Elements of cartilage are noted with a heterologous tumor in a 72-year-old woman who died within 6 months of surgery.

International Federal of Gynecology and Obstetrics stage, 58.3% with stage I were dead of disease (Table II). In one of the patients with stage I who was considered dead of disease, death was treatment-related. She developed pelvic recurrence with an obstructed ureter and was treated with methotrexate, actinomycin D, and cyclophosphamide. After the second chemotherapy course she died of toxicity manifested by thrombocytopenia, leukopenia, and eventual disseminated intravascular coagulation. Three patients with clinical stage I are living with disease at 9, 13, and 29 months. Two patients with clinical stage II disease are living with disease at 12 and 13 months. Of patients dead of disease, 39% died within a 6-month period; 50% died in a period of 7 to 12 months; and 11% died after a period of 13 months. One of these latter three died 36 months after diagnosis. Seventy-four percent of the 27 black patients are dead of disease, compared with 46% of the 13 white patients.

Of 11 patients with clinical stage I or II disease who underwent surgery and were found to have extrauterine spread, 6 are dead and 5 are living and are presumed to have disease. Of the 15 patients with disease confined to the uterus who underwent surgery, 46.7% are dead of disease. If patients with stage III and IV are added to the group with extrauterine spread, there are no survivors without evidence of disease. Nineteen patients (47.5%) had homologous mixed müllerian tumors, and 63% are dead of disease, whereas 67% of the 21 patients with heterologous mixed müllerian tumors are dead of disease. The various elements of the sarcoma (i.e., chondrosarcoma) did not appear to affect survival (Fig. 1). Tumor volume was obtained from pathology reports in 26 patients. Of 18 patients with tumor size ≥5 cm, 55% are dead of disease. Eight patients had tumors <5 cm in size, and only 25% are dead from disease. Tumor volume was not always related to depth of myometrial invasion. Five patients with tumor >5 cm who are dead of disease had only superficial invasion. Vascular invasion was identified in 14 patients; 64.2% are dead of disease, compared with 12 patients without identifiable vascular invasion, only 25% of whom are dead of disease. In patients with vascular invasion, 43% had sarcoma in the vessels and 28% had carcinoma (Fig. 2). The remaining patients had a mixture of the elements in vascular spaces.

The presence of vascular invasion as a poor prognostic factor is illustrated by the case of a 67-year-old woman with a stage IB heterologous mixed müllerian tumor. She had only superficial myometrial invasion of a polypoid lesion that filled the cavity. Paraaortic and pelvic nodes removed contained no tumor. Cell washings were negative, and an omental biopsy revealed no tumor. Because of tumor in vascular spaces, she was offered adjuvant chemotherapy but refused. Four months after surgery, she died of massive liver and lung metastases.

Mitotic activity did not play a significant role in survival. Regardless of outcome, almost all patients had high counts (>10 mitoses per 10 high-power fields in the sarcomatous element. The carcinoma/sarcoma ratio also did not affect survival. The overall mortality in this series was 67.5%.

In patients dying of disease, the location of tumor was assessed by physical examination, x-ray studies, or postmortem examination. Two patients died with local recurrence only, whereas 92.5% had distant metastases, either alone or combined with local recurrence (Fig.

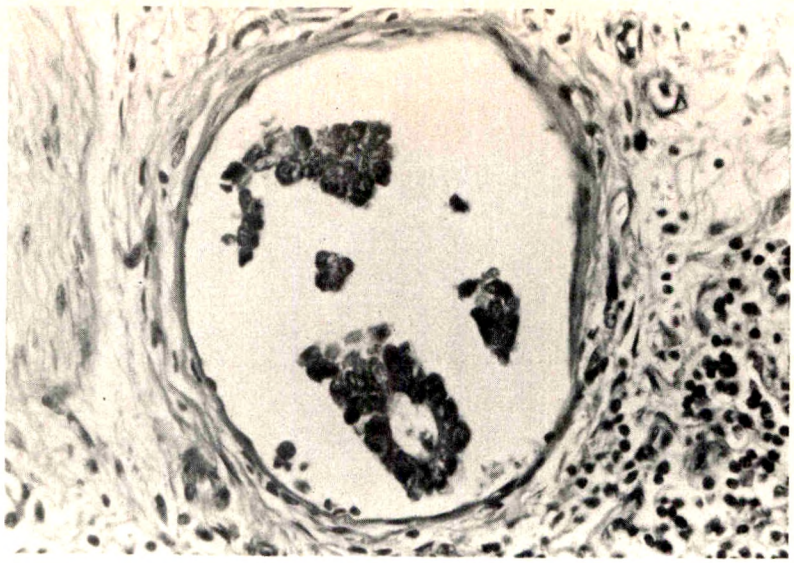


Fig. 2. Metastatic carcinoma in a vascular-lymphatic space in the uterus of a patient who died 9 months after treatment.

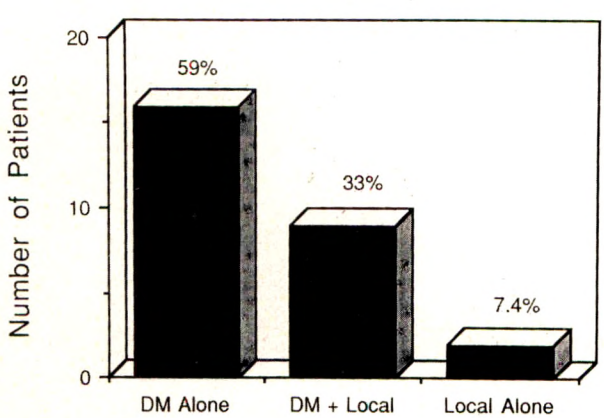


Fig. 3. Location of disease at time of death. *DM*, Distant metastases.

3). The most common sites of metastatic disease were lung (17 cases) and liver (7 cases). Other extrapelvic sites of disease included the upper abdomen, supraclavicular nodes, mediastinum, and bone.

Treatment for patients with operable disease was variable. The addition of radiation therapy did not significantly contribute to survival (Table III). Only two patients had postoperative external beam irradiation without chemotherapy; both are long-term survivors. Although nodes were not sampled in these two patients, these nodes were not suspicious at the time of operation. Four patients were treated with two courses of VP-16 and cisplatin followed by whole pelvis irradiation. Two of these also had 4500 cGy radiation treatment to the paraaortic nodes. None of the four patients has clinical evidence of disease, but all are considered to be

Table III. Role of radiation therapy in stage I and II disease*

Treatment	*No. of patients*	*Dead of disease (%)*
Preoperative irradiation	7	57.1
No irradiation	15	60
Postoperative irradiation†	2	0

*Doses = 4000 to 4500 cGy external beam with average 5000 mg hr of cesium.

†Doses = 4500 to 5000 cGy; does not include patients treated with cisplatin and VP-16.

alive with disease because of the short follow-up period. One additional patient was treated with six courses of VP-16 and cisplatin (Table IV).

Comment

Although this study population had a relatively high incidence (3.5%) of mixed müllerian tumor in all female genital tract tumors, the past reported incidence is relatively rare.[4, 6, 9, 10] Any increase in incidence of this tumor is probably associated with increased recognition of the neoplasm. These tumors most often occur in women in the last menopausal years. The mean age of our patients (65.3 years) is consistent with other reports, with a mean age of 63 to 70 years in studies reported from European countries and the United States.[4, 6, 10-12] Of 45,342 female patients admitted to the Medical College of Georgia Hospital in a recent 4-year period, 49.6% were black. Two third of the patients in this

Table IV. Role of VP-16 and cisplatin

Original clinical stage	*Adjuvant radiation therapy*	*Reason for treatment*	*State of disease*	*Months after treatment*
IB	Paraaortic whole pelvis	Positive pelvic and paraaortic nodes	Alive with disease	15
II	Whole pelvis	Positive pelvic nodes	Alive with disease	12
II	Paraaortic whole pelvis	Positive pelvic and paraaortic nodes	Alive with disease	12
IB	Whole pelvis	Positive pelvic nodes	Alive with disease	9
III	No	Tumor of ovaries and in vessels	Dead of disease	18

series with mixed müllerian tumor were black. More black patients died from disease in this report than whites. Macasaet et al.[7] have suggested that mixed müllerian tumors in black women may have a different biologic origin since black women in their series tended to have more advanced disease at diagnosis. Seven percent of the patients reported herein had a history of prior pelvic irradiation. Most series report that approximately 15% of patients have a history of prior pelvic irradiation, but individual series may vary from none to 29%.[3, 4, 6-9, 11, 13] Others have also reported nulliparity to occur in from none of the patients to 32%.[4, 7-9, 12] Obesity has been noted in from 20% to 31% to two other reports.[7, 9] Patients who have appropriate staging laparotomies have almost a 35% chance of having positive retroperitoneal nodes, as reported in our series and other current reports[3, 14] (Table V).

Earlier reports on mixed müllerian tumors suggested that there may be a somewhat better outcome in patients with homologous elements only.[1, 2] However, we and other recent investigators[4, 7-9, 12, 14] found no difference in outcome between homologous and heterologous tumors. The survival (32% reported herein) continues to be dismal. Survival rates reported by others, when all stages of mixed müllerian tumor are evaluated, range from 14% to 41%.* A group of patients at high risk for failure was identified in our study and included those with vascular or lymphatic space involvement, extrauterine spread noted at the time of operative procedure, large (>5 cm) tumor bulk, and nodal metastases.

How might survival be improved? Although the number of patients in our study is small, we found no significant impact on survival when perioperative irradiation was added to surgery. Vongtama et al.[17] noted that radiation therapy was of adjunctive value to surgery in terms of decreasing pelvic recurrences and increasing survival. Salazar et al.[18] and Spanos et al.[19] also noted that adjuvant irradiation significantly improved disease controlability in the pelvis. However, other recent studies indicate no significant added benefit of adjuvant pelvic irradiation.[2, 11, 14, 15]

*References 2, 4, 5, 8, 9, 11, 13, 15, 16.

Table V. Incidence of positive retroperitoneal nodes in patients with mixed müllerian tumors

Authors	*Total No. of patients*	*Positive nodes*	
		n	%
DiSaia et al.[3]	28	10	35.7
Peters et al.[14]	13	4	31
Present series	20	7	35
Total	61	21	34.4

Preoperative irradiation could be detrimental to survival. These tumors tend to be blood-borne neoplasms. Macasaet et al.[7] strongly advocated a primary surgical approach because 55% of women with clinical stage I were found to have widespread disease at surgery; this incidence is more common than the 33% of patients in our series with clinical stage I who had extrauterine spread. Other investigators have also noted an upgrading of clinical stage I or II to more advanced disease in from 23% to 29% of patients.[13, 14] As in our series, others have reported that extrauterine spread was associated with a poor prognosis with survival rates from 0 to 28%.[5, 8, 11, 13, 19] Eighty-nine percent of our patients dead of mixed müllerian tumor died within 12 months. Spanos et al.[19] and Salazar et al.[18] noted that 90% of local and distant failures were apparent within 2 years. Shaw[11] reported that 53% of his patients died within 12 months of diagnosis. Others have also reported that when distant metastases occurred, the lungs were often involved.[6, 9, 15]

Response rates, partial or complete, are below 15% in patients with advanced or recurrent mixed müllerian tumor treated with Adriamycin alone or Adriamycin plus cyclophosphamide or dimethyl triazeno imidazole carboxamide.[20, 21] Recently Seltzer et al.[22] reported a 50% response rate in six patients treated with a combination of cisplatin and Adriamycin, with duration of response from 9 to 11 months. Gershenson et al.[23] reported a 42% response rate in patients with disseminated mixed müllerian tumor treated with cisplatin alone. Cisplatin seems to have moderate activity in this neoplasm. Kohorn et al.[13] reported an 80% survival rate

in patients who received adjuvant radiation therapy and combination chemotherapy. All of our high-risk patients treated with irradiation plus VP-16 and cisplatin are alive from 9 to 18 months after diagnosis and surgery. These patients have no apparent progression of tumor. However, the follow-up period is too short and the number of patients too small to draw any conclusions about these adjuvant modalities.

In summary, the survival in our series and others has not been improved by use of adjunctive radiation alone. High-risk factors can be identified, but only if a primary surgical approach is used to delineate the extent of disease and these risk factors. A pilot study is warranted with cisplatin-based combination chemotherapy, with or without irradiation, in these high-risk patients. All treatment modalities should be considered in women with mixed müllerian tumors of the uterus.

REFERENCES

1. Kempson RL, Bari W. Uterine sarcomas: classification, diagnosis, and prognosis. Hum Pathol 1970;1:331-49.
2. Norris HJ, Roth E, Taylor HB. Mesenchymal tumors of the uterus II. A clinical and pathologic study of 31 mixed mesodermal tumors. Obstet Gynecol 1966;28:57-63.
3. DiSaia PJ, Morrow CP, Boronow R, Creasman W, Mittelstaedt L. Endometrial sarcoma: lymphatic spread pattern. Am J Obstet Gynecol 1978;103:104-5.
4. Chaung JT, Velden V, Graham JB. Carcinosarcoma and mixed mesodermal tumor of the uterine corpus. Obstet Gynecol 1970;35:769-80.
5. Mortel R, Koss LG, Lewis JL, D'Urso JR. Mesodermal mixed tumors of the uterine corpus. Obstet Gynecol 1974;43:248-52.
6. Marchese MJ, Liskow AS, Crum CP, McCaffrey RM, Frick HC. Uterine sarcomas: a clinicopathologic study, 1965-1981. Gynecol Oncol 1984;18:229-312.
7. Macasaet MA, Waxman M, Fruchter RG, et al. Prognostic factors in malignant mesodermal (Mullerian) mixed tumors of the uterus. Gynecol Oncol 1985;20:32-42.
8. Lotocki R, Rosenshein NB, Grumbine F, Dillon M, Parmley T, Woodruff JD. Mixed Mullerian tumors of the uterus: clinical and pathologic correlations. Int J Gynaecol Obstet 1982;20:237-43.
9. Williamson EO, Christopherson WM. Malignant mixed Mullerian tumors of the uterus. Cancer 1972;29:585-92.
10. George M, Pejovic MH, Kramar M. Uterine sarcomas: prognostic factors and treatment modalities—study on 209 patients. Gynecol Oncol 1986;24:58-67.
11. Shaw RW. Mullerian mixed tumour of the uterine corpus: a clinical histopathological review of 28 patients. Br J Obstet Gynaecol 1983;90:562-9.
12. Barwick KW, LiVolsi VA. Malignant mixed Mullerian tumors of the uterus. A clinicopathologic assessment of 39 cases. Am J Surg Pathol 1979;3:125-35.
13. Kohorn EI, Schwartz PE, Chambers JT, Peschel FE, Kapp DS, Merino M. Adjuvant therapy in mixed Müllerian tumors of the uterus. Gynecol Oncol 1986;23:212-21.
14. Peters WA III, Kumar NB, Fleming WP, Morley GW. Progostic features of sarcomas and mixed tumors of the endometrium. Obstet Gynecol 1984;63:550-6.
15. Rachmaninoff N, Climie ARW. Mixed mesodermal tumors of the uterus. Cancer 1966;19:1705-10.
16. Tanga R, Delgado G. Uterine sarcoma. Int Surg 1982; 67:339-44.
17. Vongtama V, Karlen JR, Piver MS, Tsukaoa Y, Moore RH. Treatment results and prognostic factors in stage I and II sarcomas of the corpus uteri. Am J Roentgenol Rad Ther Nucl Med 1976;126:139-47.
18. Salazar OM, Bonfiglio TA, Patten SF, et al. Uterine sarcomas. Natural history, treatment and prognosis. Cancer 1978;42:1152-60.
19. Spanos WJ, Wharton JT, Gomez L, Fletcher GH, Oswald MJ. Malignant mixed mullerian tumors of the uterus. Cancer 1984;53:311-6.
20. Omura GA, Major RJ, Blessing JA, et al. A randomized trial of Adriamycin with and without dimethyl triazeno imidazole carboxamide in advanced uterine sarcomas. Cancer 1983;52:40-6.
21. Muss HB, Bundy B, DiSaia PJ, et al. Treatment of recurrent or advanced uterine sarcoma: a randomized trial of doxorubicin versus doxorubicin and cyclophosphamide (a phase III trial of the Gynecologic Oncology Group). Cancer 1985;55:1648-53.
22. Seltzer V, Kaplan B, Vogl S, Spitzer M. Doxorubicin and cisplatin in the treatment of advanced mixed mesodermal uterine sarcoma. Cancer Treat Rep 1984;68:1389-90.
23. Gershenson DM, Kavanagh JJ, Copeland JT, Edwards CL, Shinger CA, Wharton JT. Cisplatin therapy for disseminated mixed mesodermal sarcoma of the uterus. J Clin Oncol 1987;5:618-21.

Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. R. Henry Temple, Wilmington, North Carolina, (Official Guest). In the 16-year time span of this study, the records of 41 patients with mixed müllerian tumors of the uterus were analyzed. This is a very large series of these rare and deadly tumors.

They represented 3.5% of the female genital tract tumors at the Medical College of Georgia. This is an unusually high incidence of these unusual tumors and represents a fourfold increase over what one would normally expect. Other views of the subject quote mixed müllerian tumors as representing 3% to 6% of uterine cancers.

When International Federal of Gynecology and Obstetrics criteria were applied, 61% had stage I disease, 19.5% had stage II, 2.5% had stage III, and 17% had stage IV disease. The deadly nature and dismal prognosis for this disease is reflected in the fact that there were no cures in patients with stage III or IV disease and only 12.5% of patients with stage II and 28% of patients with stage I disease were free of disease after treatment.

The demographic data presented by Dr. Gallup were generally in agreement with other large series. The mean patient age was 65.3 years, and the mean parity was 2.9. Two thirds of the patients were black; one third had diabetes. Seven percent had previous pelvic irradiation, and 9.5% had a history of previous use of exogenous estrogen. One of the few criticisms I have of this review and, indeed, of the oncologic literature in general is the failure to define the population from which the study patients are drawn. Figures that show a third of patients with diabetes mellitus or two third being black have little meaning if the prevalence of diabetes or the racial makeup of the community at large from which these patients are drawn is also unknown.

From his data, Dr. Gallup clearly delineates some very important truisms regarding mixed mülerian tumors of the uterus. They are:

1. The single most important factor signaling poor prognosis is extra uterine extension of disease. There were no survivors free of disease in the group with cancer outside the uterus.
2. In patients with disease confined to the uterus, poor prognostic factors were increasing depth of myometrial invasion, vascular or lymphatic space involvement, and large tumor bulk (>5 cm) even without myometrial invasion. The correlation between bulk of tumor and prognosis is one not appreciated in most other reviews of the subject.
3. Finally, surgery is the mainstay of treatment. Only surgery seems to make any difference incure rates. Neither adjuvant radiation nor chemotherapy significantly increased cure rates. Furthermore, their effect on length of survival is uncertain.

Somewhat as an addendum, the paper describes five patients treated with cisplatin and VP-16. One patients with stage III disease was dead of disease 18 months after treatment. The other four (two patients with stage IB and two with stage II disease) were described as alive with disease 9 to 15 months after treatment. On the basis of these results, the paper calls for a pilot study using cisplatin-based combination chemotherapy for high-risk patients.

In closing, I would like to pose some questions. Although there have been several previous studies pointing out cisplatin's possible activity in mixed müllerian tumors, the role of VP-16 has not been reported. How was this agent chosen?

Was there a control group for the group treated with cisplatin and VP-16? What might be the expected natural course of the disease in similarly matched stage IB and II patients not treated with chemotherapy?

Dr. Gallup (Closing). I appreciate Dr. Temple's kind comments. I again apologize for the slight change in the numbers. After review of re-cut slides on one patient, she was dropped from the study group; this left 40 patients for analysis. He is correct in his criticism about our failure to more clearly define the overall population makeup of our study group. We presented a group of patients who underwent running midline incision closures at the annual clinical meeting in May last year. Of those Medical College of Georgia patients, 54% were black and one third had diabetes mellitus.

Why did we choose VP-16 and cisplatin as chemotherapeutic agents? We have treated a group of about 15 patients with ovarian carcinoma and failed treatment with cisplatin-based chemotherapy, with second-line VP-16 and cisplatin. We have had significant long-term responses, some of which were complete, in this group. One of these long-term responders had a primary mixed mesodermal sarcoma of the ovary. We did not have a matched control group, but no patients with clinical stage I or II disease who had positive nodes and did not receive VP-16 and platinum are survivors.

I agree with Dr. Underwood that we should try to give chemotherapy as soon after surgery as possible.

Evaluation of surgical staples in cesarean section

Gene Burkett, MD, L. P. Jensen, MD, A. Lai, MD, M. J. O'Sullivan, MD, S. Yasin, MD, S. Beydoun, MD, and A. G. W. McLeod, MD

Miami, Florida

Experience with Lactomer absorbable surgical staples (Auto Suture Poly CS 57 disposable surgical stapler) in 62 cesarean sections was compared with an equal number of operations performed with standard techniques. The technique of appliciation of the stapler evolved with experience. Countertraction to staplers with Allis' clamps prevented uterine V incisions. Operative time was longer for the stapled suture group for primary cesarean sections, but was unchanged for repeat procedures. Excellent hemostasis of the uterine incision was achieved even when varicose sinusoidal veins were present. Significant reductions of hemoglobin deficits from 2.16 to 1.3[illegible] gm/dl, postoperative anemia from 29% to 6.4%, and endometritis from 22.5% to 6.4% occurred respectively in nonstapled and stapled groups. A reduced hospital stay, shortened by 2.1 days, saved each patient $2950. Blood loss estimates by surgeons or anesthetists were often at variance. Surgeons' acceptance of the procedure grew with experience. On the basis of these results, use of absorbable staples offers certain advantages in reducing blood loss, infection, and hospital stay in cesarean deliveries. (Am J Obstet Gynecol 1989;161:540-7.)

Key words: Absorbable staples, cesarean section

Surgical stapling has undergone major modifications since it was first introduced in the United States in the early 1960s. Early staples were made from stainless steel, and complications such as infection and dyspareunia were frequently reported after hysterectomy and vaginal vault stapling.[1-3] Removal of the staples was almost mandatory. Major modifications to the material of the staples has led to widespread acceptance of stapling in many surgical specialties, especially in vascular, gastrointestinal, and genitourinary surgery, and gynecologically, especially in abdominal and vaginal hysterectomy.[4-9] In cases of malignant disease of female genital organs, several reports have related reductions in blood loss and operating times to the use of absorbable staples.

The most significant modification was the change to absorbable suture material. Currently glycolide-lactide copolymer (Lactomer* absorbable surgical staples) is used, which in gynecologic patients has resulted not only in reduction in operating time but also in lower postoperative morbidity as infections, granulomas, and in chronic dyspareunia.[9-11] Postoperative stay and hospital costs have decreased.[10] Removal of visible staples in the vaginal vault is now rarely necessary and a resurgence of interest in their use has surfaced.

From the Department of Obstetrics and Gynecology, University of Miami School of Medicine.

Presented as Official Guest at the Fifty-first Annual Meeting of the South Atlantic Association of Obstetricians and Gynecologists, Hot Springs, Virginia, January 29–February 1, 1989.

Reprint requests: Gene Burkett, MD, Department of Obstetrics and Gynecology, University of Miami School of Medicine (R-136), P.O. Box 016960, Miami, FL 33101.

**Reg. U.S. Patent and Trademark Office. Auto Suture Company, Div. of United States Surgical Corporation, Norwalk, Conn.*

6/6/13909

The progression to obstetric use of surgical staples for abdominal delivery of the infant seemed reasonable since bleeding from the incision of the highly vascular gravid uterus could be easily curtailed. The speed, safety, and convenience of the stapling device to open the uterus were also attractive incentives.[12] In experiments with nonhuman primates, problems of infertility caused by migration of metal staples were no longer a risk with the modern copolymer counterparts.[12, 13]

Retrospective analysis of our experience developed out of need to assess the efficacy and cost of the absorbable staple (Auto Suture Poly CS* 57 disposable surgical stapler) in cesarean section, especially because the cost of sutures in nonstapling techniques seemed so much less. The findings of this audit are reported in this article.

Material and methods

The hospital records of 62 patients who were delivered by cesarean section in which absorbable staples (Auto Suture Poly CS 57 disposable surgical stapler) were used to open the uterine incision were compared with 62 patients whose cesarean sections were performed by standard nonstaple techniques with scissor and sutures. The comparison group was chosen by reviewing the records of the next cesarean section following the case done by surgical stapler.

All patients were delivered at the University of Miami/Jackson Memorial Medical Center between June 1986 and April 1987. Demographic data were collected

*Reg. U.S. Patent and Trademark Office. Auto Suture Company, Div. of United States Surgical Corporation, Norwalk, Conn.

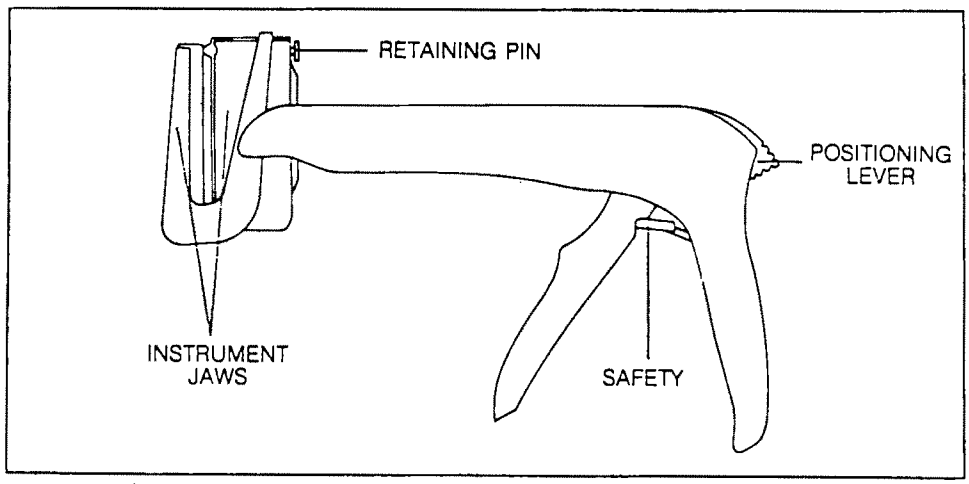


Fig. 1. Schematic view and nomenclature of Auto Suture Poly CS 57 disposable surgical stapler.

from each group on age, parity, race, gestational age, indication for cesarean section, labor or nonlabor, number of vaginal examinations in labor, rupture of membranes (length of time), and type of uterine incision.

Outcome variables were related to blood loss as estimated by surgeons, hemoglobin deficits (calculated from differences of hemoglobin at admission and discharge), and anemia (hemoglobin <10 gm/dl at discharge). Postoperative infection (febrile morbidity, temperature ≥100.4° F) and length of hospital stay were also compared. The acceptance of the procedure was gauged by comments of each surgeon after the first use of staples and later after 62 cases had been completed.

Method of application of the staples initially was that recommended by the manufacturers but quickly evolved with modification to avoid V-shaped incisions in the uterus. To easily accommodate the instrument under the abdominal wall, a small Richardson's retractor was used to elevate (rather than a lateral pull) the abdominal wall. A transverse incision of 1 to 2 cm is made with a knife in the central part of the uterus in the usual way for any cesarean section.

The Auto Suture Poly CS 57 disposable surgical stapler was applied to extend the incision laterally on both sides of the uterus. The following steps were taken (Fig. 1).

1. The jaws of the instrument are parted by opening the positioning lever.
2. The lower jaw of the stapler is passed through the initial incision in the uterus with fingers between the fetus and instrument. Use of Allis' clamps on the initial uterine incision provides countertraction and rotates the uterus so that the stapler can be applied straight or only very slightly inclined upward. In this way V-shaped uterine incisions are avoided. The technique of protecting the fetus is the same as that when the uterine incision is extended with the bandage scissors.
3. The positioning lever of the instrument is closed so that the instrument is locked in place and the position is rechecked. It is important to check that no inappropriate structures, such as fetal parts, are within the jaws of the instrument. Tissue is only incised if the stapler is fired.
4. Release of the safety guard allows the trigger to be pressed firmly toward the handle (as far back as possible). This will fire two rows of eight staples per row from each jaw, so that the upper staples lock into the lower staples, compressing the tissue between the staples. At the same time that the staples are fired, a knife blade cuts the uterine segment between the two rows of staples. The uterine incision now has a row of staples on the upper and lower edges and hemorrhage is controlled. Actual measurements of the two sizes of staples available are a height of 0.140 inches (3.6 mm) or 0.170 inches (4.3 mm) when closed, and the width (6.2 mm at the top and 6.6 mm at the bottom) is the same for each size.

Closure of the uterine incision is accomplished with a single-layer continuous suture of chromic catgut 0. As the method evolved, polyglycolic suture 0 was substituted for the catgut. At first the incision was closed by suturing completely around the staples, but apposition of the staples on each side of the incision was better achieved by passing the suture through the middle of each locked staple pair. Finally, the complete evolution resulted in alternating one stitch through the staples for proper apposition with the next stitch around the following staple pair to suture full thickness of the uterine musculature.

In the nonstapled cesarean sections, closure of the uterine incision was done by two continuous layers of either chromic catgut 0 or polyglycolic 0; the first layer was hemostatic and full thickness, while the second was an invaginating watertight seal. Suture material was chosen by the surgeon.

Table I. Demographic data

	Nonstapled (n = 62)	*Stapled group (n = 62)*	*p value*
Age (yr)			
Mean	26.4 ± 5.4	28.5 ± 6.8	0.05
Range	14-35	15-38	
Gravidity			
Mean	3.0 ± 2.1	2.7 ± 1.7	
Range	1-8	1-8	
Race*			
White	10	16	
Black	26	24	
Hispanic	26	22	
Pregnancies†			
Single	60	61	
Multiple	2	1	
Cesarean section			
No labor	30	28	NS
After labor	32	34	
Primary	43	30	0.02
Repeat	19	32	(χ^2 = 5.629)
Type of uterine incision			
Low-segment transverse	60	60	
Low-segment vertical	2	1	
Classic	0	1	
No. of vaginal examinations	4.2 ± 1.4	3.9 ± 1.9	

*χ^2 = 1.80; *df* 2; p = 0.4.
†Fisher's exact test, p = NS.

Table II. Indications for cesarean section*

	Nonstapled group	*Stapled group*
Repeat cesarean section	32	30
Cephalopelvic disproportion	24	16
Failure to progress	4	7
Fetal distress	6	8
Breech	4	3
Others	13	17

*More than one indication in some patients.

Statistical analysis was done by Student's t test for the nonstapled pairs versus stapled groups. Evaluation of cesarean section by labor/nonlabor, primary/repeat operation was by χ^2 analysis. The χ^2 analysis was used to compare postoperative anemia and endometritis. Fisher's exact test was used in analysis of single and multiple pregnancies.

Results

Records of 62 patients who had cesarean section with the use of absorbable stapler-knife hysterotomy were compared with an equal number of patients whose operations were done by standard nonstaple techniques.

Demographic data in Table I indicate that age, parity, and race of the two cesarean groups (stapled and nonstapled) were approximately the same. There were no significant differences in the number of elective cesarean sections or cesarean sections after labor. However, the number of primary cesarean sections was significantly more in the nonstapled group (χ^2, 5.629; *df*, 1; p = 0.02). The mean number of vaginal examinations during labor was the same in each group. The range of vaginal examinations was zero to nine in the nonstapled group and zero to eight in the stapled group. Uterine incisions were predominantly transverse lower uterine incisions, and the gestations were primarily singleton pregnancies.

Indications for cesarean sections are listed in Table II and did not differ between the two groups. Previous cesarean sections and cephalopelvic disproportion predominate as the usual indications for cesarean section. Among the indications listed as "other" are previous myomectomy (2 cases), active lupus (1), severe intrauterine growth retardation (3), severe preeclampsia with deteriorating HELLP syndrome (hemolysis, elevated liver enzymes, low platelets) (3), placenta previa (1), rhesus isoimmunization (2), diabetes mellitus (3), and twins in breech-vertex presentation (2). More than one indication occurred in many patients.

Table III shows the outcomes of the cesarean sections of each group.

The operating time from first incision to placement of the last skin staple or suture was significantly different between the stapled and nonstapled group in primary but not in repeat cesarean sections. However, the mean surgical operating time when *all* cesarean sections were considered, irrespective of the group, was 41.4 minutes (n = 73) for primary and 45.2 minutes

Table III. Outcome of cesarean section

	Nonstapled group (N = 62)	*Stapled group (N = 62)*	*p value*
Operating time (min)			
Primary	39.1 ± 9.7* (*n* = 43)	43.8 ± 9.2 (*n* = 30)	= 0.05
Repeat	46.0 ± 12.2* (*n* = 19)	44.7 ± 7.5 (*n* = 32)	NS
All cesarean sections			
Primary	41.44 ± 9.64 (*n* = 73)		
Repeat	45.20 ± 10.2 (*n* = 51)		
	(*t* = 2.09; *df* 0.172; *p* = 0.04)		
Blood loss (ml)			
Estimated by surgeon	760 ± 150	680 ± 150	⩾ 0.01
Estimated by anesthetist	1010 ± 120	1120 ± 140	< 0.0001
Hemoglobin deficit (gm/dl)	2.16 ± 1.01	1.13 ± 0.92	< 0.00001
Postoperative anemia† (10 gm/dl)	29.0% (*n* = 18)	6.4% (*n* = 4)	< 0.001
Endometritis‡	22.5% (*n* = 14)	6.4% (*n* = 4)	< 0.01
Length of postoperative hospitalization (days)	6.9 ± 2.4	4.8 ± 1.2	< 0.00001
Complications			
Uterine incision—tears	6	4	NS
Uterine incision—bleeding angles	3	6	= 0.04
Instrument failure	N/A	2	—
Expulsion of staples	N/A	4	—

*For column, $t = 2.38$; df 60; $p < 0.02$.
†$\chi^2 = 10.83$; df 1; $p < 0.001$.
‡$\chi^2 = 6.50$; df 1; $p < 0.01$.

(*n* = 51) for repeat cesarean sections, indicating statistically longer intervals for repeat cesarean sections ($p = 0.04$).

Estimated blood loss by surgeons and anesthetists was significantly different; larger volumes were reported by anesthetists (*t* test, 29.2; *df*, 0.61, $p = 0.0001$). Between nonstapled and stapled groups, the estimated blood loss by the surgeons was statistically higher in the nonstapled group, whereas estimation by the anesthesiologists (also significant), was in the opposite direction, that is, higher in the stapled group.

Conversely, hemoglobin deficits calculated from hemoglobin at admission and hemoglobin on the third postoperative day showed that the mean hemoglobin deficit was significantly lower in the stapled group than in the nonstapled group (2.16 vs. 1.31 gm/dl). By comparison, the mean deficit in 40 vaginal deliveries was 0.9 gm/dl (vaginal delivery vs. cesarean section in both groups, $p < 0.001$ χ^2, stapled vs. nonstapled, $p < 0.00001$, *t* test). Postoperative hemoglobin of ≤10 gm/dl was present in 18 (29.0%) patients in the nonstapled group and only in four (6.4%) patients in the stapled group. Preoperatively six patients of the nonstapled group and three from the stapled group had admission hemoglobin levels of ≤10 gm/dl. Therefore blood loss at operation would account for postoperative anemia in 12 nonstapled and one stapled patient and is statistically significant ($p < 0.001$).

Postpartum infection included endometritis in 14 (22.5%) patients in the nonstapled group versus 4 (6.4%) in the stapled cesarean section patients; urinary tract infections occurred in two patients in the nonstapled group.

As a result of the increase in endometritis, the mean postoperative hospital stay was significantly higher in the nonstapled group (6.9 vs 4.8 days); thus patients in the stapled group spent 2.1 days less in the hospital. The range of stay for the nonstapled group was 4 to 15 days, whereas for the stapled group it was 3 to 8 days. This reduction in postoperative stay resulted in a savings of $2950 (daily hospital cost, excluding cost of staples $1500/day times 2.1 days minus extra cost of staples, $200) per patient.

Angular tears were similar in each group. In two cases the staples did not close properly and compression of the uterine incision was not accomplished. Four patients reported passing staples between 3 and 6 weeks post partum, and one patient passed staples on several occasions over a 2-week interval.

Opinions of the surgeons using the Auto Suture Poly CS 57 disposable surgical stapler were recorded after the first use of the instrument and after completion of the 62 stapled cesarean sections. All evaluations were sought before analysis of all data. Initial comments were approval by two surgeons, disapproval by three, and three were unsure. Six surgeons approved the use of the stapler in selected cesarean sections after 62 cases with stapled sutures were completed. These indications included lower uterine segments with multiple large veins, thick lower uterine segments, possibly in anterior placenta previa. Two surgeons continued to have doubts about the usefulness of the stapler; one surgeon

believed that this was particularly disappointing because the staples were not thick enough to encompass and compress the thick uterine muscle of the classical incision. In his case, staples tore off the edge of the incision as soon as applied.

Female surgeons also complained that pressure necessary to squeeze the trigger to fire the staples required both hands and increased the degree of difficulty in application, whereas residents complained that stapling "slowed them down."

All agreed that hemostasis, and consequently visibility, of uterine incisions was excellent and better than in the nonstapled group.

Comment

The Auto Suture Poly CS 57 disposable surgical stapler was approved by the Food and Drug Administration for use in hysterectomy and cesarean section on the basis of animal studies and the experience of the use of absorbable staples in other specialties. It was expected that these staples would reduce blood loss and operative and postoperative morbidity, as had occurred when they were used in other specialties. However, the cost was far in excess of the suture material required for standard techniques of cesarean section, and therefore an audit to determine the efficacy of the knife-stapler was undertaken. The trends suggested from this data strongly support a reduction in blood loss and postoperative morbidity. No apparent gain in operating time was achieved. On reflection, the technique varies little from that of the standard method for opening the uterine incision.

Blood loss calculations in cesarean sections provide many difficulties. Significant variations of the estimates for blood loss by the anesthetist and surgeon emphasize the unreliability of that method. Differences varied statistically, and the trends were in opposite directions between the two groups of patients. However, in combination with the vital signs of the patients, such clinical estimates continue to be important in intraoperative assessment of blood loss. The accuracy of such estimates is amplified by contamination with amniotic fluid, blood soaked into operating drapes, sponges, and perhaps even on or under the operating table. However, a most important consideration is that blood lost in this operation includes not only that from the uterine incision but also a large amount from the placental site and a small amount from the abdominal incision. The knife-stapler can only affect blood loss associated with the uterine incision.

To circumvent some of these difficulties, we decided to compare the hemoglobin at admission with that of the third postoperative day. Previous analysis had indicated that hemoglobin concentrations and fluid shifts associated with delivery were usually stable by the third day. Since expected blood loss at cesarean section does not usually require replacement by transfusion, the third-day estimation of hemoglobin concentration allows the surgeon to evaluate surgical morbidity and decide whether or not blood loss at surgery necessitates any further treatment. The data show that autosuture stapling plays a role in the reduction of blood (on the basis of lower mean hemoglobin deficit of 2.16 gm/dl for the nonstapled group and 1.31 gm/dl for the stapled group). The mean deficit for vaginal deliveries of 0.9 gm/dl probably indicates the loss of blood from the placental site and brings into perspective the values obtained from patients who were delivered surgically.

The larger number of patients with postoperative anemia in the nonstapled group (29% vs. 6%) follows the trend seen with the hemoglobin deficit analysis and strengthens the concept that stapling reduces blood loss.

The postoperative incidence of endometritis and urinary infections was less common in the women with stapled cesarean sections (6.4%), than in the nonstapled group (22.5%). There is no immediate explanation for this wide variation since the patients were from the same pool of a lower socioeconomic group in a multiethnic, inner-city hospital. In a separate additional audit of 35 consecutive cesarean sections, seven cases (20%) with perioperative mild to severe endometritis indicated that the findings in our study group were not a chance cluster, which would falsely elevate the incidence in our comparison nonstapled group. The difference in the incidence of endometritis in the two study groups may be partially a result of the material used for the staples, a glycolide-lactide copolymer, which is more inert than other suture material used in the nonstapled group and therefore may be less enhancing to infection.

However, this incidence of postoperative endometritis is unexpectedly high, even for our hospital. This is particularly so because of the use of prophylactic antibiotics for all patients who labor before cesarean section. Mefoxin irrigation of the uterine cavity, uterine incision, and the abdominal incison has proved efficacious in our institution for the past 5 years, and the incidence of postoperative endometritis has been reduced from 33% to 6%.[14] An incidence of 22.5% on the nonstapled group, or 20% in the group of consecutive cesarean section which was audited separately, is therefore surprising and unexplained.

Complications were few, but the most disconcerting to the patient was the passing of the staples vaginally. It is therefore important that the patients be alerted that as the staples dissolve and loosen, particles may be extruded usually at 3 to 6 weeks after operation, as occurred in four patients. Bleeding from the angles of

the uterine incision occurs with the stapler because of a pin in the instrument that fixes it in place once the stapler is closed. After the stapler is removed, bleeding often occurs from the site of the pin. In the nonstapled cesarean sections, incursions into the uterine vessels when the uterine incision was extended too far laterally resulted in bleeding at the angles of uterine incision. Extension tears of the uterine incisions occurred evenly in both groups and can be attributed to technical errors.

No fetal injury was encountered in our study. We believe that this is largely a result of our insistence that a protective finger must be inserted between the fetus and the undersurface of the uterus whenever any instrument, scissors or stapler, is used to extend the initial uterine incision laterally. This is also done in performing an episiotomy. There is no room for compromise on this maneuver, and anecdotal reports of severed fetal digits can only be attributed to poor surgical techniques rather than instrument failure. Beresford[9] emphasizes that tissue damage will not occur until the stapler is fired; therefore a recheck on position and the tissue that is gripped can prevent fetal injury. It is possible that a short period of training may be necessary for each obstetrician to become familiar with use of the instrument. This will enhance versatility in usage and will certainly minimize the risk of serious complications. As the surgeons in our group gained experience with the stapler, their confidence grew and led to acceptance of the instrument. The opinions of two surgeons from another hospital changed from skepticism to resounding approval.

Finally, the savings of hospital costs of $2950 per patient (the reduced hospital stay of 2 days seemingly related to the lower incidence of endometritis) should encourage acceptance of surgical staples for use in cesarean sections. Our observations lead us to believe that routine stapling may not be practical, but candidates selected for application would include those cases in which multiple uterine sinusoid-like vessels occupy the desired incision site or where the lower uterine segments are thick, or some cases of placenta previa in which lower segment incisions are possible, and in patients in whom long labors have been unsuccessful for a vaginal delivery.

Currently a randomized trial is in progress to verify these findings and to carefully determine by protocol the role of staples in cesarean section, but this audit of a relatively costly instrument even retrospectively suggests trends to reduction of blood loss, lowered postoperative morbidity, and decreased hospital stay. Concurrently it also raises questions with regard to future pregnancies and vaginal delivery after cesarean section performed with the absorbable staple–knife instruments. Long-term follow-up of such patients should be performed.

REFERENCES

1. Kahn E. Autosuture in abdominal hysterectomy. Obstet Gynecol 1968;31:852.
2. Albert RO. Uses of automatic staplers in vaginal vault hysterectomy, anterior colpoplasty and posterior colpoperineoplasty. Tex Med 1974;70:66.
3. Messitt JJ. Dyspareunia from autosuture staples: a report of two cases. Obstet Gynecol 1977;49:369.
4. Ravitch MM, Steichen FN. Technics of staple suturing in gastrointestinal tract. Ann Surg 1972;175:815.
5. Heney MM, Dretler SP, Hensle TW. Autosuturing device in intestinal urinary conduits. Urology 1978;12:650.
6. Delgado G. Use of the automatic stapler in urinary conduit diversions and pelvic exenterations. Gynecol Oncol 1980;10:1.
7. Beresford JM. Automatic stapling techniques in abdominal hysterectomy. Surg Clin North Am 1984;64:609.
8. Penalver MA, Girtanner RE Jr, Averette HE. Benefits of stapling in abdominal hysterectomy. Contemp Ob Gyn 1985;26:189.
9. Beresford JM. Using automatic staplers in gyn surgery. Contemp Ob/Gyn 1987;30(June, special issue):147.
10. Penalver MA, Averette HE, Sevin BU, Lichtinger M, Girtanner R. Gastrointestinal surgery in gynecological oncology: evaluation and surgical techniques. Gynecol Oncol 1987;28:74.
11. Wheeless CR. Stapling techniques in operations for malignant disease of the female genital tract. Surg Clin North Am 1984;64:591.
12. Bond SJ, Harrison MR, Slotnick RN, et al. Cesarean delivery and hysterotomy using an absorbable stapling device. Obstet Gynecol 1989;74:25.
13. Adzick NS, Harrison MR, Flake AW, Glick PL, Bottles K. Fetal surgery in primate III: maternal outcome after fetal surgery. J Pediatr Surg 1986;21:477.
14. Jensen LP, Dobin AJ, O'Sullivan MJ, Hilsenbeck CE. Prevention of endomyometritis by local application of antibiotic solution during cesarean section. Am J Obstet Gynecol 1985;152:565.

Discussion

Dr. J. Terrell Pope, Macon, Georgia (Official Guest). Dr. Burkett has presented data from a retrospective audit of 62 cesarean sections using copolymer surgical staples. This surgical instrument was introduced in 1987. We are indebted to Dr. Burkett and his colleagues for providing the first clinical data on the use of staples in cesarean section. Since absorbable staples cost $200 versus $2 to $4 for conventional sutures, their efficacy needed to be established.

In this study use of the staples suggested two trends: first, a significant reduction in blood loss and second, a significant decrease in the incidence of postoperative endometritis, thereby reducing hospital stay. This resulted in substantial cost savings. The study did not support the finding of a shortened operative time using absorbable staples, as has been shown in other operations in gynecology and other specialties.

Difference in blood loss between the two groups was significant. The hemoglobin deficit was used by Dr. Burkett to eliminate the subjective estimates by surgeons and anesthesiologists. Brant[1] and Wilcox et al.[2] found average blood loss at cesarean section to be 1000 ml. This study supports their findings since the nonstapled group had a hemoglobin deficit of 2.16 gm/dl, which represents approximately 1080 ml blood loss

(1 gm of hemoglobin deficit = 500 ml of blood loss). Blood loss in the stapled group was 565 ml, compared with 450 ml blood loss at vaginal delivery. These findings suggest that blood loss from the hysterotomy incision alone with conventional technique is approximately 500 ml. From a cost-saving standpoint, it would be interesting to know if any of the 22 patients (18 nonstapled and four stapled) with postoperative anemia required transfusion.

Postoperative endometritis was significantly lower in the stapled group (6.4% vs. 22.5% in the nonstapled group). Assuming that the same criteria were applied to both groups to make the diagnosis and that essentially the same percentage of patients received prophylactic antibiotics, what accounted for this significant discrepancy between the two groups? Can difference in surgical technique alone explain this discrepancy? Although socioeconomic status, number of vaginal examinations, internal fetal monitoring, anemia, obesity, prolonged operating time, and type of anesthesia have all been identified as risk factors for postoperative endometritis, prolonged labor and prolonged rupture of membranes are generally considered to be the most important factors.[3] Certainly, it would be relevant to compare both groups using these two variables. Moreover, Green and Sarubbi[4] and Kreutner et al.[5] found no increase in febrile morbidity in elective repeat cesarean sections. Fifty-one percent of the stapled group had repeat cesarean sections. If most were done electively, one would predict a lower postoperative infection rate in this group. Eight different surgeons used the staples. Did the same surgeons or colleagues at their same level of training perform the 62 sections using conventional technique? Miller et al.[6] recently reported that postoperative endometritis is significantly increased when surgery is performed by residents below the third-year level. If postoperative endometritis remains significantly lower with staples after the two groups are compared for duration of labor, duration of ruptured membranes, number of elective repeat cesarean sections, and the level of surgical training, then their use would seem efficacious.

Finally, will the myometrial scars produced by use of copolymer staples withstand the rigors of labor as vaginal birth after cesarean section becomes common? Will dissection of the bladder flap be more difficult? McNeeley et al.[7] compared copolymer staples with chromic suture during hysterectomy in the animal model and found more extensive necrosis and exudate with increased adhesion formation using the staples.

In summary, despite my questions arising from the retrospective nature of this study, Dr. Burkett and his colleagues have provided us with important clinical information on the use of a new surgical instrument that decreased blood loss from the hysterotomy incision in cesarean sections. Postoperative endometritis appears to be lowered with use of staples. I look forward to learning the results of his randomized study and to the reports on long-term follow-up of these patients.

REFERENCES

1. Brant HA. Blood loss at cesarean section. J Obstet Gynaecol Br Commonw 1966;73:456.
2. Wilcox CF, Hunt AB, Owen CA. The measurement of blood lost during cesarean section. AM J OBSTET GYNECOL 1959;77:772.
3. Gibbs RS. Clinical risk factors for puerperal infection. Obstet Gynecol 1980;55:1785-845.
4. Green SL, Sarubbi FA. Risk factors associated with postcesarean section febrile morbidity. Obstet Gynecol 1977; 49:686-90.
5. Kreutner AK, Del Bene VE, Delamar D, et al. Perioperative cephalosporin prophylaxis in cesarean section: effect on endometritis in the high-risk patient. AM J OBSTET GYNECOL 1979;134:925-35.
6. Miller PJ, Searcy M, Kaiser L, Wenzel RP. The relationship between surgeon experience and endometritis after cesarean section. Surg Gynecol Obstet 1987;165:535-9.
7. McNeeley SC Jr, Elkins TE, Portz DM, Warren J, DeLancey OL. Comparison of copolymer staples versus chromic suture during hysterectomy: gross, histologic, and microbiologic findings. Obstet Gynecol 1988;72:862.

DR. JOHN W. C. JOHNSON, Gainesville, Florida. I enjoyed the details of Dr. Burkett's study. He omitted the outcome for one of the patients, the fetus. There have been reports in the lay literature of a fetal finger being amputated with the use of this clamp. I am sure this did not occur in Miami, but I would like Dr. Burkett to reassure us that all the infants did well and there were no morbidity problems with the infants.

DR. BURKETT (Closing). First I'll answer some of Dr. Pope's questions. We do not routinely use transfusions on cesarean sections. Generally, although we reported anemia under 10 gm in up to 29%, we do not use transfusions unless the patient is very anemic and symptomatic. In the prospective series, we have at least one patient who developed disseminated intravascular coagulation from obstetric complications and who was transfused, but we will report on that later.

The more important question posed by Dr. Pope was the etiology of postoperative endometritis. We tried to evaluate this, but with only 18 patients the numbers are so very small when broken down by variables that we could not reach a conclusion as to whether any of the factors he mentioned were in fact causal. We really do not know why there is a higher incidence of endometritis in the nonstapled group. To ensure that this was not a chance cluster, we studied 35 consecutive cesarean sections about the same period and found that the incidence of endometritis was almost as high, 22.5%. We may have been going through a period when we were not attentive enough to prevention of infection. We routinely use prophylactic antibiotic irrigation in the uterus with 2 gm of Mefoxin (1 gm injected into the cavity, 0.5 gm on the uterine incision, and 0.5 gm in the abdominal incision). This study was reported by Jensen (one of the co-authors) et al. (see reference 14 of article), and postoperative infection rates were lowered to acceptable levels of approximately 8% to 10%.

With regard to the experience of the surgeons, certainly the use of the staples was not entirely confined to our authors. That may be one factor in cause of

infection that we cannot adequately control because we must expose our residents to this experience. The residents usually were third-year or chief residents, and usually there was an attending physician present at the cesarean section. However, one aspect we have not mentioned here is that the negative or positive attitudes of surgeons to the new instrument varied considerably and influenced the decision for use of the staples and possibly its acceptance.

In terms of vaginal birth after cesarean section, currently we don't have the answer. We will obviously have to do follow-up and carefully evaluate these patients. Certainly because glycolide-lactide copolymer material causes less tissue reaction, then there should be less fibrous tissue in the uterine incision and less weakness.

Dr. Johnson, there were no fetal injuries. We insisted that in any instrumentation like an episiotomy or uterine incision with bandage scissors or the stapler that a protective finger be inserted between the instrument and the fetus. In the anecdotal report from Minnesota, this surgical axiom was not practiced. There is one other case that I know occurred in Houston; again the same circumstances occurred: care was not taken to protect the fetus.

Finally, concerning the clumsiness of the instrument, we have reported to the manufacturers that redesign is necessary and they are responding to that situation.

Outpatient triplet management: A contemporary review

Roger B. Newman, MD, Cheryl Hamer, RN, BS, and M. Clinton Miller, PhD
Charleston, South Carolina

The antepartum management of 198 women who were delivered of triplets between 1985 and 1988 is reviewed. Women were managed with the assistance of ambulatory perinatal nursing to provide outpatient surveillance. Modified bed rest, prophylactic tocolysis, and betamethasone were liberally used and patients were hospitalized only when obstetrically indicated. The most common antepartum complication was preterm labor (66.2%) and the success of therapy with tocolytic agents is described. The mean gestational age and birth weight at delivery were 33.6 ± 3 weeks (mean ± SD) and 1871 ± 555 gm, respectively. Comparison of the gestational age distribution at delivery with previous reviews demonstrates fewer deliveries <29 weeks' gestation and significantly more deliveries between 32 and 37 weeks' gestation. Cesarean delivery occurred in 94% of the triplets, which eliminated birth order as a factor that affects survival. The corrected perinatal survival rate was 95% in this contemporary review of outpatient triplet management and represents a major improvement in the expected outcome for triplets. (Am J Obstet Gynecol 1989;161:547-55.)

Key words: Triplets, outpatient management, ambulatory nursing, perinatal survival

In 1958 Geza Kurtz[1] prefaced his excellent review of the factors that influence triplet survival with the following, "Because of the rarity of triplet birth the human interest appeal of such an event is greater than its practical significance." In 1989, however, advances in reproductive biology necessitate a reevaluation of this statement. Ovulation induction agents have dramatically increased the observed frequency of triplet birth in the most recent reviews.[2-6] Unfortunately, these reviews are limited by either their small size or the prolonged period of time required to achieve a reportable number of cases. Obstetricians are challenged by an increasing incidence of triplet pregnancies without adequate information on which to base management decisions and to evaluate outcome. The following large series of women who were delivered of triplets between 1985 and 1988 is presented to describe changing trends in the management of triplets and the dramatically improved prognosis of these high-risk pregnancies.

From the Department of Obstetrics and Gynecology, Medical University of South Carolina.
Presented by invitation at the Fifty-first Annual Meeting of the South Atlantic Association of Obstetricians and Gynecologists, Hot Springs, Virginia, January 29–February 1, 1989.
Reprint requests: Roger B. Newman, MD, Department of Obstetrics and Gynecology, Medical University of South Carolina, 171 Ashley Ave., Charleston, SC 29425.
6/6/12996

Material and methods

This study reviews the antepartum management and pregnancy outcome of 198 women who were delivered of triplets between 1985 and 1988. These women were

Table I. Demographic and causal characteristics of the 198 triplet pregnancies

Characteristic	*No.*	*%*
Age (yr)	29.5 + 4.4	
Race		
White	160	80.8
Black	30	15.2
Other	8	4.0
Parity		
Nulliparous	104	52.5
Parous	94	47.5
Causes		
Spontaneous	102	51.5
Induced	82	41.4
In vitro fertilization	14	7.1

Table II. The duration of therapy with tocolytic agents in 131 triplet pregnancies with preterm labor

Duration of therapy	*No.*	*%*
Noncandidate for therapy*	38	29.0
Tocolytic therapy < 48 hours	13	9.9
Tocolytic therapy 2 to 7 days	12	9.2
Tocolytic therapy < 7 days	68	51.9

*Preterm premature rupture of the membranes, advanced cervical dilation with intact membranes, and elective preterm delivery.

all referred for ambulatory perinatal nursing provided by the Tokos Medical Corporation at 24 regional centers across the country. The busiest regional center was in Orange, Calif., which managed 23 patients during the study period, whereas centers in Detroit, Charlotte, N.C., and Minneapolis managed the fewest at two each. Only four of the regional centers (Orange, San Francisco, Philadelphia, and Tampa) followed-up more than 15 patients during the study period. Individual patient management was at the discretion of a large number of attending obstetricians in both private and academic practices.

Through the involvement of ambulatory nursing personnel, each patient was contacted daily by telephone to (1) assess maternal symptomatology as it may relate to preterm labor; (2) provide education and reinforcement of preterm birth prevention principles; (3) assess patient compliance with physician-directed treatment plans; (4) perform uterine activity monitoring 2 hours daily with a portable tocodynamometer designed for outpatient use[7]; (5) provide a liaison between the patient and physician available 24 hours a day, 7 days a week; and (6) provide emotional support and patient advocacy. In addition, a daily patient-care log was maintained in which alterations in antepartum management, hospitalizations, secondary diagnoses, delivery, and neonatal outcome data were carefully recorded. Retrospective analysis of these patient-care records provided the management and outcome data reported in this review. Patient data sheets were coded to ensure patient confidentiality.

Daily ambulatory nursing contact and uterine activity monitoring began at an average gestational age of 23.8 ± 3.3 weeks (mean ± SD) and continued until 32.6 ± 3.2 weeks. When patient symptomatology or objectively recorded uterine activity exceeded physician-prescribed criteria, the perinatal nursing service would immediately contact the referring physician. In the event of hospitalization, the ambulatory nursing service was kept advised of the patient's progress and resumed daily contact if the patient was discharged undelivered.

The length of gestation was established based on the date of in vitro fertilization or medically induced ovulation when known. In spontaneous triplets, the gestational age was determined based on the date of confinement estimated by obstetric and ultrasonographic criteria. All triplet gestations were known at the time of their referral for ambulatory nursing surveillance.

Preterm labor was diagnosed when excessive uterine activity was accompanied by progressive cervical dilation or effacement at <37 completed weeks' gestation. Premature rupture of the membranes at <37 completed weeks' gestation was also considered preterm labor. Hospitalization because of increased uterine activity without associated cervical change was considered threatened preterm labor. Hospitalization without either increased uterine activity or cervical change was considered observatory unless other specific maternal or fetal complications were identified.

A variety of tocolytic agents were used both therapeutically and prophylactically at the discretion of the referring obstetrician. Intravenous magnesium sulfate was the most common agent administered for treatment of preterm labor. Oral β-adrenergic agents (terbutaline sulfate and ritodrine hydrochloride) were commonly given for both prophylaxis and maintenance therapy to prevent recurrent preterm labor. The duration of successful therapy with tocolytic agents was calculated on the basis of the number of days between the first episode of documented preterm labor and the date of delivery.

The primary indication for delivery was carefully sought in each case. Deliveries were considered to have been elective if they occurred without benefit of labor or other maternal-fetal complications that required delivery. Cases of early preterm labor (cervical dilation <4 cm) with intact membranes in which tocolysis was not used but would have been a reasonable alternative were also considered to be elective deliveries. Indications for preterm delivery included advanced preterm

labor (cervical dilation ≥4 cm and tocolysis was not considered a reasonable alternative), preterm labor with failed tocolysis, and preterm premature rupture of the membranes. Rupture of the membranes or the onset of regular uterine contractions after 37 completed weeks' gestation was considered term labor. Maternal or fetal indications for delivery were based on the clinical impression of the referring obstetrician.

The stillbirth rate was defined as a fetal loss at ≥20 weeks' gestation or weight ≥500 gm. The neonatal death rate was defined as the death of a liveborn infant within the first 28 days after birth. The perinatal mortality rate was the summation of the stillbirth and neonatal death rates. The corrected perinatal mortality rate included those stillbirths and neonatal deaths beyond 24 weeks' gestation and excluded those infants with lethal congenital anomalies.

Data are presented as means ± SD. Statistical comparisons were performed with the unpaired Student's t test and the χ^2 test where appropriate.

Results

The demographic and causal characteristics of the 198 triplet gestations in this review are presented in Table I. Almost half of the triplets in this review were a result of either in vitro fertilization or medical induction of ovulation.

The goal of antepartum management in this series was to provide obstetric surveillance on an outpatient basis. Almost all patients were prescribed some degree of bed rest or activity restriction in addition to ambulatory perinatal nursing. A total of 115 patients (58.1%) were placed on prophylactic tocolytic therapy by their referring obstetrician, and seven women (3.5%) were managed with the aid of a cervical cerclage. Therapy with corticosteroids for the enhancement of fetal lung maturation was used in most cases of preterm labor <34 weeks' gestation.

Despite a goal of outpatient management, 88 women (44.4%) required antepartum hospitalization on 110 occasions for reasons other than preterm labor. The most common indications were threatened preterm labor (51%), observation (20%), and pregnancy-induced hypertension (15.5%). The average duration of stay for each antepartum hospitalization was 15.2 ± 15 days.

In addition, 131 of the triplet pregnancies (66.2%) had preterm labor at an average gestational age of 29.9 ± 3.5 weeks. The success of therapy with tocolytic agents in the inhibition of preterm labor is described in Table II. The mean time gained in utero for those women who received therapy with tocolytic agents was 25.6 ± 22 days. However, 174 women (87.9%) still were delivered of their triplets at <37 completed weeks' gestation.

The indications for delivery are identified in Fig. 1.

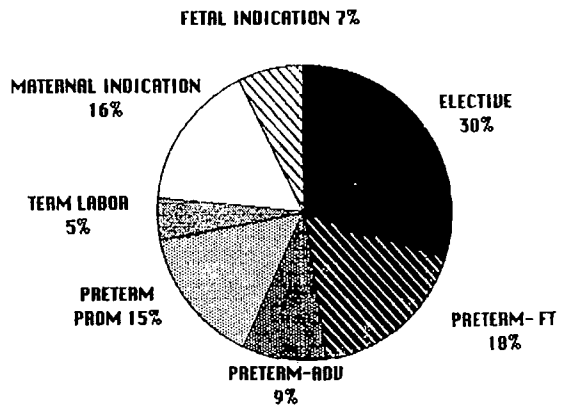


Fig. 1. Distribution of indications for delivery among 198 triplet gestations. Fetal indications include intrauterine fetal demise (4), intrauterine growth retardation (3), placental abruption (3), and fetal distress (4). Maternal indications include pregnancy-induced hypertension (28), pulmonary edema (2), abdominal pain (1), and chorioamnionitis (1). (*FT* = failed tocolysis; *ADV* = advanced cervical dilation and effacement; *PROM* = premature rupture of membranes.)

Premature rupture of the membranes is not always an indication for delivery; however, of the 30 women with preterm premature rupture of the membranes, 25 were delivered of infants on the same day that rupture of the membranes occurred. Three more women were delivered within 48 hours and the remaining two were delivered within 72 hours. Fifty-nine women (29.8%) were delivered electively by their obstetricians; 35 of those were premature (<37 weeks' gestation) and the remaining 24 were at term. Fig. 2 presents the gestational age distribution at which these elective deliveries occurred. Fig. 2 also indicates which of these deliveries were preceded by preterm labor at an earlier point in gestation (19) and those uncomplicated by prior preterm labor (40). In only five of the 19 elective deliveries complicated by prior preterm labor did this labor immediately precede delivery. The preterm labor in these five cases was considered early enough that therapy with tocolytic agents would have been considered a reasonable treatment alternative.

Cesarean section was the mode of delivery in 186 cases (93.9%). Nine women (4.6%) were delivered vaginally whereas another three (1.5%) were delivered of the first infant vaginally but required emergent cesarean delivery because of intrapartum complications.

The average gestational age at delivery was 33.6 ± 3 weeks and the mean birth weight was 1871 ± 555 gm. Only 13 women (6.6%) were delivered at <29 weeks' gestation and only nine (4.5%) were delivered at >37 weeks' gestation. A total of 149 women (75.3%) were delivered between 32 and 37 weeks' gestation. Comparison of the gestational age distribution at delivery with several previously reported reviews[1-3, 8-10] reveals

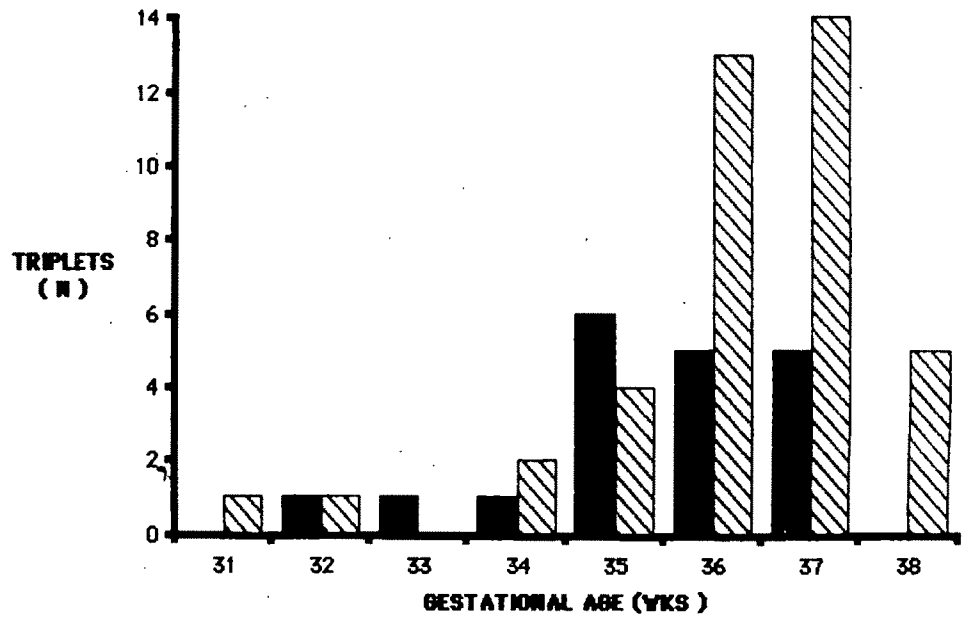


Fig. 2. Number of triplet gestations (n = 59) delivered electively between 31 and 38 weeks' gestation. Pregnancies (n = 19) previously complicated by premature labor *(black bars)* are differentiated from gestations (n = 40) uncomplicated by preterm labor *(striped bars)*.

significantly fewer deliveries at the extremes of gestational age in the current report and a greater number of deliveries clustered between 32 and 37 weeks' gestation (Fig. 3). There was no improvement in the gestational age at delivery (33.8 ± 2.8 weeks) or birth weight (1894 ± 486 gm) for the 115 women who received prophylactic tocolysis, as compared with the 83 women who did not receive these medications (33.2 ± 3.5 weeks and 1869 ± 518 gm). Of the seven women who received a cerclage, most were placed after the discovery of very early cervical change. The outcome for these women was generally poor with an average gestational age at delivery of 29.2 ± 4.1 weeks and a birth weight of 1271 ± 489 gm.

There were 19 stillbirths (3.2%) among the 594 triplets in this series. Among the 575 liveborn infants, there were 421 (73.2%) admissions to neonatal intensive care units and respiratory distress syndrome was diagnosed in 131 (22.8%) infants. There were 20 neonatal deaths (3.5%) in this review. Correction of these mortality rates to exclude nine triplets delivered <24 weeks' gestation (all delivered at 22 weeks' gestation; six stillbirths, three neonatal deaths, and a birth weight range of 430 to 510 gm) and one triplet with a lethal congenital anomaly (hypoplastic left ventricle), these triplet pregnancies resulted in a stillbirth rate of 2.2% (13:584), a neonatal death rate of 2.8% (16:571), and a corrected perinatal mortality rate of 5% (29:584). There was no significant difference in the neonatal death rate when birth order was considered [Triplet A, 5:192 (2.6%); Triplet B, 6:192 (3.1%); Triplet C, 8:191 (4.2%)].

Comment

The recent suggestion that triplet gestations may be candidates for selective first-trimester reduction crystalizes the importance of reassessment of the perinatal risk associated with these gestations.[11] The ethical justification for this recommendation is based on an assessment of the proportional risks encountered by either continuation of the triplet gestation or by selective reduction to a singleton or twin gestation. It is imperative that up-to-date information be available on which to base such risk versus benefit analyses.

The available recent literature on the outcome of triplet gestations indicates a perintal mortality rate that ranges from 13.3% to 33%.[1-4, 6, 8-10] The applicability of these reviews is limited either by their small size or by the prolonged period of time required to accumulate sufficient cases. The current review is unique not only because of its large size but also because these triplets were managed during a contemporary time frame. The corrected perintal mortality rate of 5% in this review represents a threefold to sevenfold improvement on previously reported series.

It is important to realize that this review does not represent an intensive management protocol at a single university center, but rather the accumulated experience of literally hundreds of obstetricians in the United States. This excellent perinatal survival rate (95%) reflects the general improvement in obstetric and neonatal care that is available throughout the country to women with all manner of obstetric complications.

Another unique aspect of this review was the goal of

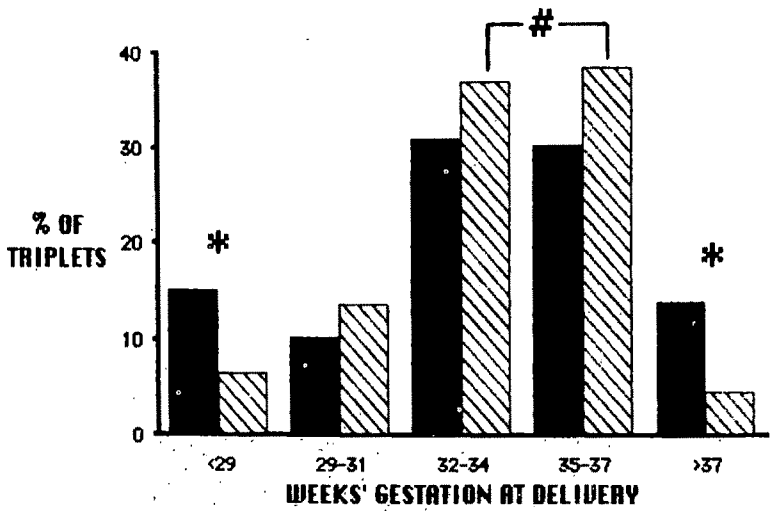


Fig. 3. Gestational age distribution at delivery of 198 triplets in current review *(striped bars)* and compilation of 159 triplets reported in previous reviews[1-3, 8-10] *(black bars)*. *Represents significant increase in deliveries at these gestational ages compared with current series ($p \leq 0.01$). #Represents significant increase in deliveries between 32 and 37 weeks' gestation compared with previous reviews ($p \leq 0.01$).

provision of antepartum care for the triplet pregnancy on an outpatient basis. Hospitalized bed rest was ineffective in the prolongation of gestation or the improvement of outcome for twin pregnancies in two prospective and randomized trials.[12, 13] However, the frequently stated recommendation for triplet pregnancies remains hospitalized bed rest in the third trimester.[1-5] In the triplet series reported by Ron-el et al.[2] and Berg et al.,[5] the average duration of hospitalization for each triplet pregnancy was approximately 40 days.

Despite a desire to provide outpatient triplet management, 44% of the triplets required admission on one or more occasions not including admissions because of preterm labor. Each of these 88 women was hospitalized for an average of 15 days. Despite this very high antepartum admission rate, the overall savings in terms of hospital days by the avoidance of routine hospitalization is remarkable. If all 198 women in this series had been admitted for an average of 40 days each, the hospital days consumed would have totaled 7920 rather than the 1338 actually required. This represents a savings of 18 years of hospitalization for this one clinical series. Given the excellent perintal survival achieved in this series, it is clear that outpatient management of the triplet gestation is justifiable.

It is tempting to speculate as to what specific factors contributed to the improved outcome reported in this series. Certainly the contemporary benefits of early diagnosis, improved ultrasonic visualization, antepartum surveillance, therapy with tocolytic agents, in utero fetal therapy, and dramatic improvements in the neonatal care of the very-low-birth-weight infant have all contributed independently. However, the particulars of this review do raise some interesting points for discussion.

This review demonstrates that the use of prophylactic tocolysis with triplet gestations is very popular among obstetricians in the United States. However, the effectiveness of prophylactic tocolysis remains a very controversial issue, at least as it pertains to the management of the twin gestation.[14-16] Ron-el reported no differences in the average length of triplet gestation between six women without prophylactic tocolysis and eight women who received β-adrenergic agents prophylactically. Eleven other triplets who received a combination of oral β-adrenergic agents and intramuscular progesterone prophylactically actually had gestation shortened by almost 2 weeks.[2] In this particular series, we also found that use of prophylactic tocolysis was not associated with any obvious improvement in either gestational age or birth weight at the time of delivery. These findings may be biased if practitioners were more likely to use prophylactic tocolysis in cases they perceived to be at the greatest risk. This selection bias probably explains the poorer obstetric performance of women who underwent cervical cerclage.

The involvement of ambulatory perinatal nursing in the outpatient surveillance of the triplet gestation represents a significant new trend in the management of these pregnancies. Daily ambulatory nursing contact and uterine activity monitoring represents an intensive antepartum surveillance effort. This effort is directed at making the earliest possible diagnosis of preterm labor, thereby allowing the more effective use of tocolysis.[17, 18] It is controversial as to whether daily uterine activity monitoring is additive to the value of frequent

personal contact and reinforcement of the principles of good prenatal care offered by the perinatal nursing team.[19] That question cannot be addressed by the current review as both elements were emphasized in the antepartum surveillance of these women.

Despite the very high incidence of preterm labor in all reported series of triplet gestations, the use of tocolytic therapy is only rarely described. Itzkowic[9] attempted tocolysis in seven of 46 women and was successful in delaying delivery more than 48 hours in three women. Holcberg[3] used tocolysis in five of 23 patients with a >48-hour success in four women. Certainly others have used tocolysis successfully with triplet gestations but no one has reported the frequency of use or the degree of success currently reviewed. Only 29% of the triplets with preterm labor were not candidates for tocolysis and 52% were tocolyzed for >7 days. The mean time gained in utero for those women who received tocolysis was almost 4 weeks.

It was surprising that the mean age and birth weights at delivery in this series were not significantly different from many previous reports without such extensive use of tocolytic agents.[1-3, 8-10] However, comparison of these previous reports with the current review made it apparent that the gestational age distribution at delivery was altered. It is possible that the effect of aggressive surveillance and therapy with tocolytic agents was to prevent very early preterm delivery (<29 weeks), which is associated with the highest rate of perinatal mortality. The reduced frequency of very early deliveries is counterbalanced by fewer deliveries that occurred >37 weeks' gestation and therefore more deliveries that clustered between 32 and 37 weeks' gestation. The reduced number of triplet gestations delivered >37 weeks' gestation may reflect in part the high incidence of elective delivery that occurred both prematurely and once 37 weeks' gestation was achieved. This very high incidence of elective preterm delivery is an interesting finding and deserves further consideration. In some of these cases, elective preterm deliveries were performed after documentation of fetal lung maturity by amniotic fluid phospholipid studies. However, in the majority of these premature deliveries there was no such documentation. Only speculation can be offered for these elective premature deliveries but possibilities include concerns over maternal well-being and pessimism over the likelihood of further prolongation of pregnancy. The frequency of elective delivery in this review underscores the impact that obstetric decision-making can have on the results found in retrospective clinical reviews.

The almost universal use of cesarean delivery is another dramatically changing trend in the management of the triplet gestation. The cesarean section rate ranged from 7% to 32% in the previously mentioned reports,[1-3, 8-10] as opposed to the 94% cesarean section rate in the current series. The impact of the increasing cesarean section rate can be evaluated by consideration of the influence of birth order on perinatal mortality. Even with a smaller number of triplet pregnancies for evaluation, almost all authors have identified an increasing perinatal mortality rate for the second and third triplet.[1-3, 8, 9] These authors suggest a variety of explanations including an increased risk of delivery trauma, intrapartum cord prolapse, placental abruption, and subtle alterations in uteroplacental blood flow that occurs after delivery of the first or second triplet. In the current series the second and third triplet continues to manifest a nonsignificant increase in perinatal mortality; however, the absence of the dramatic differences seen in previous reports suggests that cesarean delivery may improve the prognosis for aftercoming triplets by avoidance of most of these potential intrapartum complications. There was no maternal mortality in this series; however, the dramatic increase in the cesarean section rate must be associated with increased maternal morbidity and longer hospital stays.

This review of the contemporary obstetric management and neonatal outcome of 198 triplet gestations indicates that the outpatient management of the triplet gestation is completely justifiable and is associated with a much better perinatal survival rate than previously reported. Without the benefit of prospective randomized trials it is impossible to clearly define the factors most responsible for this improvement. However, the current series raises the possibility that the routine use of antepartum hospitalization is unnecessary and that prophylactic therapy with tocolytic agents does not significantly contribute to the improved prognosis for triplet gestations. It is believed that intensive antepartum surveillance, aggressive therapy with tocolytic agents, and the liberal use of cesarean section were helpful in providing these women and their neonates the best possible outcome. It is hoped the findings of this series may spur further investigation with a proper randomized study design to assess which characteristics of antepartum care contribute most to successful outcome for the multifetal gestation.

REFERENCES

1. Kurtz GR, Davis LL, Loftus JB. Factors influencing the survival of triplets. Obstet Gynecol 1958;12:504-508.
2. Ron-el R, Caspi E, Schreyer P, Weinkaub Z, Arieli S, Goldberg MD. Triplet and quadruplet pregnancies and management. Obstet Gynecol 1981;57:458-463.
3. Holcberg G, Biale Y, Lewenthal H, Insler V. Outcome of pregnancy in 31 triplet gestations. Obstet Gynecol 1982; 59:472-476.
4. Loucopoulos A, Jewelewicz R. Management of multifetal pregnancies: sixteen years' experience at the Sloane Hos-

pital for Women. AM J OBSTET GYNECOL 1982;143: 902-5.
5. Berg G, Finnstrom O, Selbing A. Triplet pregnancies in Linkoping, Sweden, 1973-1981. Acta Genet Med Gemellol 1983;32:251-6.
6. Syrop CH, Varner MW. Triplet gestation: maternal and neonatal implications. Acta Genet Med Gemellol 1985; 34:81-8.
7. Katz M, Gill PJ, Initial evaluation of an ambulatory system for home monitoring and transmission of uterine activity data. Obstet Gynecol 1985;66:273-7.
8. Daw E. Triplet pregnancy. Br J Obstet Gynaecol 1978; 85:505-9.
9. Itzkowic D. A survey of 59 triplet pregnancies. Br J Obstet Gynaecol 1979;86:23-8.
10. Michlewitz H, Kennedy J, Kawada C, Kennison R. Triplet pregnancies. J Repro Med 1981;26:243-6.
11. Evans MI, Fletcher JC, Zador IE, Newton BW, Quigg MH, Strvyk CD. Selective first trimester termination in octuplet and quadruplet pregnancies: clinical and ethical issues. Obstet Gynecol 1988;71:289-96.
12. Saunders MC, Dick JS, Brown IM. The effects of hospital admission for bedrest on the duration of twin pregnancy: a randomized trial. Lancet 1985;2:793-5.
13. Hartikarnen-Sorri AL, Jouppila P. Is routine hospitalization needed in antenatal care of twin pregnancy? J Perinat Med 1984;12:31-2.
14. O'Conner MC, Murphy H, Dalrymple IJ. Double blind trial of ritodrine and placebo in twin pregnancy. Br J Obstet Gynaecol 1979;86:706-9.
15. Marivate MM, DeVilliers KQ, Fairbrother P. Effect of prophylactic outpatient administration of fenoterol on the time of onset of spontaneous labor and fetal growth in twin pregnancy. AM J OBSTET GYNECOL 1977; 128:707-8.
16. O'Leary JA. Prophylactic tocolysis of twins. AM J OBSTET GYNECOL 1986;154:904-5.
17. Katz M, Gill PJ, Newman RB. Detection of preterm labor by ambulatory monitoring of uterine activity: a preliminary report. Obstet Gynecol 1986;68:773-7.
18. Morrison JC, Martin JN, Martin RW, Gookin KS, Wiser WL. Prevention of preterm birth by ambulatory assessment of uterine activity: a randomized study. AM J OBSTET GYNECOL 1987;156:536-43.
19. Iams JD, Johnson FF, O'Shaughnessy RW. A prospective random trial of home uterine activity monitoring in pregnancies at increased risk of preterm labor. Part II. AM J OBSTET GYNECOL 1988;159:595-603.

Editors' note: This manuscript was revised after the discussions were presented.

Discussion

DR. ALLAN G. W. MCLEOD, Miami Florida. A series of 198 women with triplets collected over a 3-year period is, without question, of significance. The 63.6% incidence of preterm labor is not unexpected; however, a 5% (29:584) corrected perinatal mortality rate is impressive. Of particular note is the observation that this represents a three-fold to sevenfold improvement over outcomes in previously reported series.

It has been correctly stressed that this is not a single center study, but is one that involved numerous obstetricians in different areas, a fact that makes the perinatal outcome even more impressive.

The emphasis on outpatient care is described as unique. One might question this description in light of the fact that 63.6% of the women had premature labor and 44% required admission on one or more occasions because of other reasons. The authors reported a significant reduction in the number of hospital days compared with an average 40-day stay for routine hospital admissions; however, not all triplet pregnancies are admitted in such a manner. I wonder whether the overall cost savings would be as great as one might initially believe. I agree, however, that they have shown that more emphasis on outpatient management is justifiable. What is not quite so clear is whether it was the daily nursing contact with early prenatal care, daily telephone contact, education, reassurance, and reinforcement, or the uterine activity monitoring, or a combination of the two factors that made the difference. Did the authors have the opportunity to look at the effect of daily nursing contact alone? There is a singular lack of controlled studies in this area. This information is needed before recommendation of wholesale use of uterine activity monitoring. Also, did the use of Tokos Medical Corporation monitoring produce a significant number of unnecessary admissions for "threatened preterm labor," which would have been avoided if palpation and monitoring for cervical changes had been used?

The suggestion that the effect of careful surveillance is reduction of very early preterm labor is an important concept; it is noteworthy that only 6.6% of the mothers were delivered before 29 weeks' gestation.

Reduction of the possible complications of labor and delivery by a 94% cesarean section rate is reported; however, it is important to emphasize the need to have an experienced surgeon and anesthesiologist present, plus adequate pediatric assistance. This report also shows that a trend toward higher perinatal mortality with the second and third twins, which, although not statistically significant, exists even when a cesarean section has been performed. There was no discussion of maternal complications associated with the increased number of cesarean sections; however, I do not disagree with this approach to delivery.

In this series the higher number of white women and the increased incidence of induced or in vitro fertilization (the latter is an expensive procedure), are suggestive of a higher socioeconomic status that reflects improved nutrition and the ability to rest at home and to understand and follow instructions.

Equally important may be the suggestion that early institution and continued use of tocolysis produces significant prolongation of pregnancy, whereas prophylactic use of β-mimetic agents was not associated with any improvement in outcome. Was hydroxyprogesterone caproate (Delalutin, Squibb, Princeton, N.J.) used for prophylaxis by any of the participants?

I concur with the authors that prospective randomized trials are needed to more clearly define the factors most responsible for the improvement in outcome. Nonetheless this general report is a significant one in the attempt to improve outcome for all women with

triplets and I encourage the authors to continue their efforts in this field.

Dr. Lawrence D. Devoe, Augusta, Georgia. I think this is a remarkable tour de force to get this kind of information with this level of detail from so many centers around the country that involve so many different forces. In terms of those of us interested in multifetal gestation, I do not think there are a lot of new take-home messages because those who have studied this issue recognize the uselessness of prophylactic tocolysis and cerclage. I think what the study enables us to uncover, which I think the authors probably can present to us, is the profile of women who do well. This would then allow rational, logical, individualized care, which is what these patients really need rather than protocolized nonthoughtful care that puts all patients in one category or another. I think that would be something that would be very interesting for them to put together and share with us at a later date.

Dr. Walter Morales, Orlando, Florida. I wonder what proportion of these patients were indigent patients and I wonder whether the author would recommend the same approach to the indigent patient? I did not catch how many of these preterm gestations were complicated with stillbirth and what was the outcome of the remaining two infants in the face of a fetal death in utero?

Dr. Gene Burkett, Miami, Florida. As to what Dr. Devoe said, I wonder whether the authors would look a little bit more carefully at the respiratory distress syndrome. We have information from collaborative studies that corticosteroids do not work well for various reasons. They have a unique opportunity with this kind of volume to look not only at the risk factors that may lead to early delivery, but possibly at administration of steroids given a different way than that to which we are accustomed.

Dr. H. B. Perry, Elkin, North Carolina. I would like to emphasize that early diagnosis in any kind of planned management is significant. Would the authors comment with regard to the birth order and interval between deliveries and its effect on anoxia and infant morbidity?

Dr. Herbert Hopwood, Arlington, Virginia. When do they electively decide to deliver these patients? If it is early with a poorly developed lower uterine segment, do they do a vertical or a transverse incision in the uterus?

Dr. Robert Yowell, Durham, North Carolina. I had the privilege of discussing our experience with the use of Tokos Medical Corporation and with our high-risk preterm labors and twin gestations. I would like to ask the authors whether they have had any experience with the terbutaline pump in their patients.

Dr. Newman (Closing). Dr. Yowell, I have not had any experience with the terbutaline infusion pump with a triplet gestation. I have used it with preterm labor in other settings, and I think it is a very exciting modality for long-term therapy with tocolytic agents because of the advantage of low-dose subcutaneous infusion, but I have not used it in triplets.

Dr. Hopwood, the patients who were electively delivered were managed by several different obstetricians. As I reviewed this, I was very impressed with the different gestational ages at which they were electively delivered. Sometimes there seemed to be no rhyme or reason to it. The patients would achieve 35 weeks' gestation and it would be determined it was time to deliver. At other times, the patients were assessed by amniotic fluid phosolipid studies for fetal lung maturity. I think this is a mixed group of patients. I am not sure if all of these elective deliveries are a good thing or a bad thing, but they probably represent a special group of patients who need to be looked at more specifically. As to the type of cesarean sections used, I cannot say. I believe that in most instances the triplet gestation, despite frequently not having had the benefit of labor, will still have a well-developed lower uterine segment. However, you should always expect malpresentation with triplets and I would not be surprised if many people did use vertical incisions to either overcome an undeveloped lower uterine segment or more easily manage malpresention. The effect of delivery intervals is very interesting. It is something that has been discussed at length for twin gestations, particularly when vaginal deliveries were planned. I think the whole focus of that issue has changed with the introduction of continuous monitoring during the entire second stage of labor. I cannot comment on how delivery intervals may affect the triplet gestation because so many of them were delivered by cesarean section. There were three attempts at vaginal delivery that resulted in cesarean section. Two had cord prolapses and one had a placental abruption, all of which happened fairly quickly after delivery of the first infant.

Dr. Burkett raised a very interesting question about respiratory distress syndrome and corticosteroids. This is a very controversial issue and most of the evidence shows that corticosteroids probably are not of much benefit in the multifetal gestation, at least among twins. I believe this is because of acceleration of lung maturity in these infants. The respiratory distress syndrome rate that we had of 23% is not different from what is reported in some older reviews that looked at neonatal morbidity. Therapy with corticosteroids, however, is very popular and was used extensively by obstetricians when confronted with the triplet gestation in preterm labor. Its wide use precludes any meaningful evaluation of its effectiveness. Once again, I think it is a very good point that the ones who had respiratory distress syndrome may be a group of patients to look at with regard to what risk factors they may have had.

Dr. Morales mentioned that this is probably a socioeconomically privileged population. I know there are both indigent and Medicaid patients involved in this series, but the majority are in the middle to upper class socioeconomic strate. However, I wound not change my recommendations for management, even for an indigent care population. Obviously efforts at outpatient management may have to be altered depending on the individual circumstances involved.

The effect of a single stillbirth among three I cannot recall in specific detail. We had several instances in which this did occur. One was a patient who we managed who had a stillbirth ultimately explained by a transfusion syndrome with vascular thrombosis. We hospitalized the patient at that point and she was followed-up another 2 weeks until she spontaneously ruptured her membranes. I do not know of any instances in which a single loss was followed at a later date by another loss.

Dr. Devoe mentioned the excellent data collection, and I must applaud Ms. Cheryl Hamer, who was of incredible assistance in this project. Ms. Hamer is the manager for international research for Tokos Medical Corporation and was responsible for the coordination of the data-collection efforts. Dr. Devoe also raised another excellent point with regard to the profile of those patients who did very well, whether you define that by laboring at term or by birth weight. This is something we will investigate.

Maternal mortality in North Carolina: A forty-year experience

W. Joseph May, MD, and Frank C. Greiss, Jr., MD
Winston-Salem, North Carolina

An analysis of 67 maternal deaths from January 1, 1981, through December 31, 1985, are reported in detail and in the perspective of 3780 maternal deaths previously reviewed in North Carolina since January 1, 1946. Although the direct obstetric mortality rate has decreased 95% over the 40 years, within causal mortality groups the rates have changed variably during the past 5 years. For the first time, no deaths from obstetric infection occurred. Whereas deaths from toxemia continue to decline, those from hemorrhage, embolism, and anesthetic complications remain unchanged. Within the hemorrhage causal group, deaths from ectopic pregnancy have risen to 70%. The maternal death rate after 20 weeks' gestation is almost 10 times that associated with pregnancy interruptions. Of particular concern is the relative risk of death between the nonwhite and white patients. The North Carolina data are reviewed in the light of those in the South Atlantic region and in the nation as a whole. (Am J Obstet Gynecol 1989;161:555-61.)

Key words: Maternal mortality, direct obstetric deaths, abortion-related deaths, relative risks

A Maternal Mortality Committee was constituted by the North Carolina State Medical Society in 1945. During the succeeding 40 years, the records of 3851 maternal deaths have been reviewed and classified. Previous studies have analyzed North Carolina's maternal mortality rates before 1981.[1-4] The present report details deaths during 1981 to 1985 and compares their incidence and cause with those of previous studies. Findings of concern are compared with those of the South Atlantic region* and of the United States as a whole from 1981 to 1985. Despite differences in data collection and reporting, the North Carolina experience appears to reflect that of the South Atlantic region and the United States.

From the Department of Obstetrics and Gynecology, Bowman Gray School of Medicine of Wake Forest University.
Presented at the Fifty-first Annual Meeting of the South Atlantic Association of Obstetricians and Gynecologists, Hot Springs, Virginia, January 29–February 1, 1989.
Reprint requests: W. Joseph May, MD, Department of Obstetrics and Gynecology, Bowman Gray School of Medicine, 300 S. Hawthorne Road, Winston-Salem, NC 27103.
**Virginia, North Carolina, South Carolina, Georgia, Florida, and Washington, DC.*
6/6/12997

Material and methods

The records of 71 deaths that occurred from January 1, 1981, through December 31, 1985, were reviewed and analyzed. With the cooperation of the North Carolina State Health Department, birth certificates were cross-checked with death certificates to assure completeness of the data collection. The classification of obstetric causes of death recommended by the Committee on Maternal and Child Care of the council on Medical Service, American Medical Association,[5] was used. This classification defines a maternal death as "the death of any woman dying of any cause whatsoever while pregnant or within 90 days of the termination of the pregnancy, irrespective of the duration of the pregnancy at the time of termination or the method by which it was terminated." A direct obstetric death is a death that results from complications of the pregnancy itself, from intervention elected or required by the pregnancy, or from the chain of events initiated by the complications or the intervention. An indirect obstetric

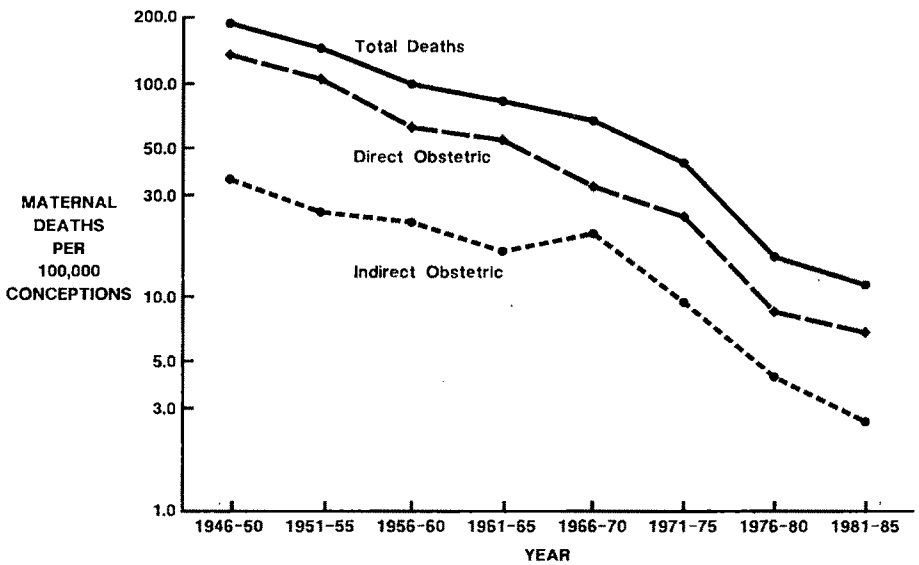


Fig. 1. Semilogarithmic graph of maternal death rates in North Carolina according to major classification from 1946 to 1985 by 5-year cohorts.

Table I. Maternal deaths in North Carolina by classification, 1981 to 1985

	Number
Direct obstetric	40
Indirect obstetric	15
Insufficient information	1
Nonobstetric	11
TOTAL	67

death is a death that results from disease that existed before or developed during pregnancy (not a direct effect of the pregnancy) that was obviously aggravated by the physiologic effects of the pregnancy and caused the death.

Autopsy confirmation of the cause of death was obtained in 88% of cases.

Of the 71 deaths, 67 met the criteria for a maternal death, 56 of which were from direct and indirect causes. Since the advent of elective abortions, our maternal mortality rates have been expressed as a function of the sum of reported live births plus elective abortions. This permits realistic comparisons with earlier data. For comparison with regional and United States data, North Carolina maternal mortality rates were expressed as a function of live births alone.

Results

During the 5-year study period (1981 to 1985), 428,891 live births and 155,498 elective abortions occurred. A total of 54 direct and indirect maternal deaths were unrelated to elective abortion (12.6:100,000 live births) and two deaths succeeded one elective and one indicated abortion (1.3:100,000 abortions). The distribution of maternal deaths by classification is shown in Table I and compared with prior experience in Fig. 1. Over the 40-year period, maternal deaths from obstetric causes have decreased 95%.

Specific causes of the direct obstetric deaths are presented in Table II and compared with prior experience in Fig. 2. For the first time, no deaths primarily attributable to obstetric infection ccurred. Similar to the experience since 1965, toxemia, embolism, and hemorrhage were the leading causes of maternal deaths with anesthestic complications a distant fourth but consistent cause. In contradistinction with deaths from toxemia that have maintained a steady decline during the study period, death rates from embolism, hemorrhage, and anesthetic complications were essentially unchanged from the 1976 to 1980 period. It is significant that deaths from ectopic pregnancy accounted for an increasingly larger proportion of all deaths from hemorrhage, rising from 16% in 1975 to 33% in the 1976 to 1980 period and to 70% in the current period. The four deaths attributed to anesthetic complications were all associated with difficult endotracheal intubations during induction of anesthesia for cesarean section, and three women were markedly obese.

Two deaths were possibly of iatrogenic origin. One patient was hypotensive from profuse bleeding and an attempted right subclavian venocentesis resulted in an arterial perforation, hemopericardium, and death. In the second patient, tocolysis to control premature labor was complicated by congestive heart failure with subsequent death from adult respiratory distress syndrome.

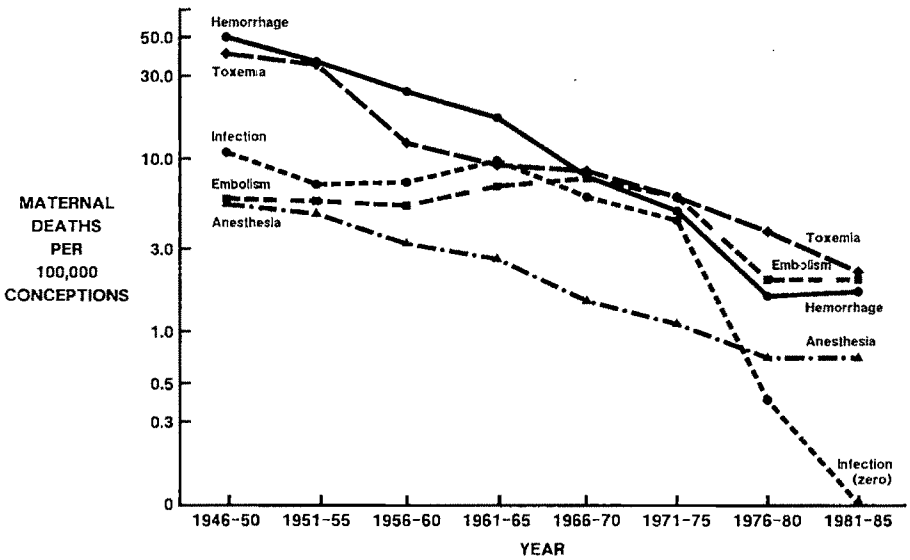


Fig. 2. Semilogarithmic graph of major direct obstetric death rates by cause in North Carolina from 1946 to 1985 by 5-year cohorts.

Specific causes of indirect obstetric deaths are presented in Table III. As in previous reviews, cardiac disease and nonobstetric infections accounted for the majority of deaths.

Two deaths complicated interruption of pregnancy. One patient with coronary artery disease died of a myocardial infarction. The second patient merits special attention. Two weeks after an "elective first-trimester abortion" she had abdominal pain and a low-grade fever and was treated by a second physician because of pelvic infection. She died 10 hours later, and autopsy confirmed a ruptured ectopic pregnancy. It is not known whether products of conception were positively identified at the time of the abortion. Delayed diagnosis as a factor in 67% of 24 deaths caused by ectopic pregnancy has been previously reported.[6]

Although the reduction in the nonwhite (primarily black) maternal mortality rates have paralleled those of the white race in North Carolina during the past 20 years (Fig. 3), the relative risk of death among nonwhite women compared with white women was relatively constant from 1966 to 1980 at 2.6% and 2.7%, respectively. However, during the 1981 to 1985 period, that relative risk rate rose to 3.3%. In an effort to more closely define the sources of these differences, relative risk rates were developed for causal categories of maternal deaths (Table IV). It is clear that the relative risk rate of direct obstetric deaths primarily accounts for the difference; toxemic and hemorrhagic deaths are quite disparate from the other causes at 11.1% and 4.8%, respectively.

Table II. Specific causes of direct obstetric maternal deaths in North Carolina, 1981 to 1985

	Number
Toxemia	
Cerebrovascular accident	6
Disseminated intravascular coagulation-multiple organ failure	4
Coma-convulsion	3
Embolism	
Amniotic fluid	7
Blood clot	4
Air embolism	1
Hemorrhage	
Ectopic pregnancy	7
Placenta previa	1
Uterine inversion	1
Uterine rupture, spontaneous	1
Anesthetic complications	
Hypoxia	3
Aspiration	1
Adult respiratory distress syndrome	1
TOTAL	40

Comment

State and national maternal deaths are variously discovered and described statistically. In North Carolina, correlation of birth and death certificates has been used to discover pregnancy-related deaths. We believe this to be the most accurate method and our opinion is confirmed by individual state and national statistics. Demographic data taken from state Health Department vital statistics have been found to be notoriously deficient. In a previous study, eight collaborators who

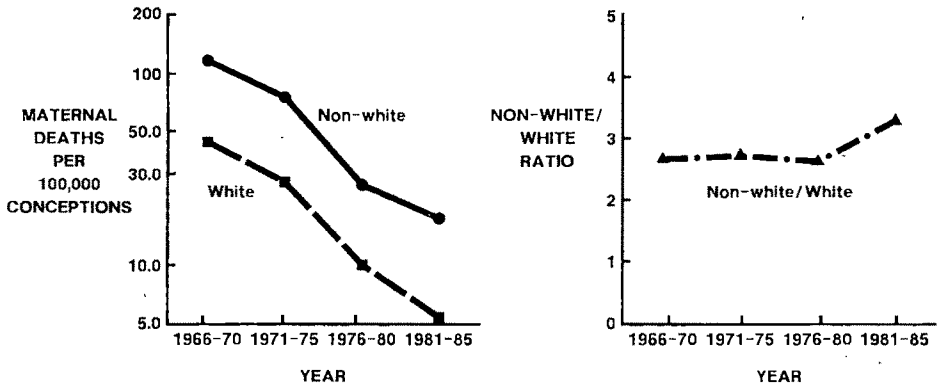


Fig. 3. Semilogarithmic graph of maternal deaths in Nort Carolina by race from 1966 to 1985 by 5-year cohorts including the nonwhite/white ratio.

Table III. Specific causes of indirect obstetric maternal deaths in North Carolina, 1981 to 1985

	Number
Cardiac disease	
Cardiomyopathy	3
Myocardial infarction	2
Rheumatic	1
Infection	
Pneumonia	3
Pyelonephritis	1
Cerebrovascular accident	2
Miscellaneous	
Hepatitis	1
Sickle cell disease	1
Diabetes	1
TOTAL	13

worked in cooperation with the American College of Obstetricians and Gynecolgoists (ACOG) and the Centers for Disease for Control (CDC) found 37% more deaths than were uncovered through current "normal" CDC channels.[7] Currently the CDC is devising a new reporting system that will be implemented uniformly throughout the country. We expect this will result in more accurate reporting. One problem may be a need for redefinition of maternal death.[8] Medical progress now permits sustentation of life well beyond the 90 days originally defined by the American Medical Association. Clearly, a system that accounts for such new achievements must be developed. In addition, a standard approach to the separation of elective abortion-related deaths from traditional maternal deaths must be used. The concept of reproductive-related deaths adds the additional obfuscating factor of contraceptive-related deaths and this also must be standardized.

Although there has been a progressive decline in pregnancy-related deaths in North Carolina during the past 40 years, within the direct obstetric causal groups varying trends have characterized the 1981 to 1985 data. As previously noted, deaths from obstetric infection have fallen precipitously since the 1971 to 1975 period and no such deaths occurred during the current report period. We believe this is a result of two main factors: the development of increasingly effective antibiotics and the legalization of elective abortion. Since 1972, no death has occurred in North Carolina from septic abortion. Deaths from toxemia of pregnancy have continued to decline at an almost unchanged rate during the past 15 years. During the past 5 years we have been unable to identify any unique characteristics in this group other than race. Lack of prenatal care was not clearly evident as a major factor in these deaths.

In contradistinction with death rates from infection and toxemia, those from embolism, hemorrhage, and anesthetic complications have remained constant from 1976 to 1980 through 1981 to 1985. Since 1970, deaths from blood clot emboli have decreased 60%, whereas those from amniotic fluid emboli show no trend in either direction. Perhaps we are approaching the irreducible minimum in this area.

As in the South Atlantic region and in the United States overall, deaths in North Carolina from ruptured ectopic pregnancies now comprise 50% or more of all deaths from hemorrhage. Review of the North Carolina experience indicates no significant change in the absolute number of patients who died from ectopic pregnancy since 1970. Therefore, reductions in the number of deaths from other causes of hemorrhage have occurred. For example, were we to delete the deaths from ectopic pregnancy from the current North Carolina data, the remaining death rate from hemorrhage would be the lowest of the direct causal groups (0.51 : 100,000 conceptions). One death

Table IV. Maternal death rates* by race, classification, and major direct cause in North Carolina, 1981 to 1985

	Nonwhite	*White*	*Nonwhite/white ratio*
Direct obstetric	13.91	3.31	4.2
Toxemia	5.67	0.51	11.1
Embolism	3.61	1.27	2.8
Hemorrhage	3.61	0.76	4.8
Anesthetic complications	1.03	0.51	2.0
Other	0.00	0.25	—
Indirect obstetric	3.61	2.03	1.8
Total obstetric	17.52	5.34	3.3

*Per 100,000 conceptions.

Table V. Maternal death rates* by race, geographic area, and select causes, 1981 to 1985

Cause	*Area*	*Death rates*			*Nonwhite/white ratio*
		Total	*Nonwhite*	*White*	
Total obstetric					
	United States	8.0	17.7	5.7	3.1
	South Atlantic	10.8	21.8	5.4	4.0
	North Carolina	13.0	25.9	7.2	3.4
Hemorrhage					
	United States	2.1	5.6	1.4	4.1
	South Atlantic	2.6	—	—	—
	North Carolina	2.3	5.2	1.0	4.8
Ectopic pregnancy					
	United States	1.0	3.5	0.5	6.9
	South Atlantic	1.4	—	—	—
	North Carolina	1.6	3.7	0.7	5.1
Toxemia					
	United States	1.3	3.04	0.96	3.4
	South Atlantic	1.6	—	—	—
	North Carolina	3.0	8.1	0.7	11.1

*Per 100,000 live births.

from ectopic pregnancy was preceded by an elective abortion. Although an ectopic and an extrauterine pregnancy may coexist, this rarely occurs. It is critically important after an elective abortion to positively identify products of conception. State laws vary on how this should be done. However, should there be any question after careful gross inspection, microscopic confirmation should be mandatory.

Deaths from anesthetic complications represent the most clearly identifiable area for prevention. Consistently these deaths result from aspiration of gastric contents or inadequate ventilation that complicate difficult endotracheal intubations in obese patients about to have a cesarean section. Such circumstances demand the best anesthesia expertise available.

The increased risk of maternal deaths in nonwhite women noted in the North Carolina data is not new and may possibly be rising. These observations prompted us to look at available data from the South Atlantic region and the United States.[9] Table V presents this data for pregnancy-related deaths and specific causal groups. It is clear that with the exception of deaths from toxemia, the North Carolina experience is typical of regional and national data that indicate at least a threefold to fourfold greater risk of death in nonwhite women. We believe that the unusually high death rate from toxemia in North Carolina reflects more precise reporting. Significant risk variations are present in causal groups that cannot be explained specifically. Perhaps the increased frequency of cardiovascular disease in the black race may be a factor in the high frequency of deaths from toxemia. Similarly, an increased frequency of pelvic inflammatory disease may predispose women to ectopic pregnancy. Irrespective of speculation, the uniformity with which the nonwhite woman remains at greater risk for maternal mortality indicates that improved education, improved access to prenatal and delivery care, and continued research into possible medical underlying factors must still be accomplished for the low socioeconomic populations.

To achieve these ends, physicians must become increasingly aggressive in their efforts to further diminish maternal deaths. That we have continually lowered maternal mortality rates over the past 4 decades indicates that quality assurance is nothing new to obstetrics. True, today's litigious climate promotes little enthusiasm for maternal mortality studies. However, lower mortality rates are attainable and our imperative to maintain medicine's pact with the public has never been greater. This study is not a negative judgment on the significant progress made through the past 50 years, but rather it shows us how far we have come and reminds us of how far we have yet to go. It should be out goal to eliminate the disparity in maternal and fetal deaths between white and nonwhite populations.

REFERENCES

1. Anderson SG, Greiss FC Jr, May WJ. Maternal mortality in North Carolina, 1946-1965. N C Med J 1968;29:367-70.
2. Anderson SG, Greiss FC Jr, May WJ. Maternal mortality in North Carolina, 1966-70. N C Med J 1972;33:949-52.
3. May WJ, Greiss FC Jr. Maternal mortality in North Carolina, 1971-75. N C Med J 1978;38:93-7.
4. May WJ, Greiss FC Jr. Maternal mortality in North Carolina: a historical survey and current update. N C Med J 1985;56:73-6.
5. Committeeon Maternal and Child Care of the Council on Medical Service. A guide for maternal death studies. Chicago: American Medical Association, 1957.
6. May WJ, Miller JB, Greiss FC Jr. Maternal deaths from ectopic pregnancy in the South Atlantic region, 1960 through 1976. AM J OBSTET GYNECOL 1978;132:140.
7. Rochat RW, Koonin LM, Atrash HK, Jewett JF, Maternal Mortality Collaborative. Maternal mortality in the United States: report from the Maternal Mortality Collaborative. Obstet Gynecol 1988;72:91-7.
8. Guidelines for perinatal care. 2nd ed. Washington, DC: American College of Obstetricians and Gynecologists 1988: 308-24.
9. Vital statistics of the United States, 1981-85, vol. I, Natality, and vol. II, Mortality, part A. Hyattsville, Maryland: U.S. Department of Health and Human Services, Public Health Service, National Center for Health Statistics.

Editors' note: This manuscript was revised after the discussions were presented.

Discussion

DR. J. BRUCE TAYLOR, Charlotte, North Carolina (Official Guest). I thank Dr. Joe May in his association with Dr. Frank Greiss for an excellent historical review and update of 40 years of obstetric mortality data from the State of North Carolina that encompasses 3851 maternal deaths. His information, presented previously, continues to provide physicians of North Carolina and elsewhere a timely update of this most catastrophic complication of our specialty. Each of these 3851 maternal deaths has undergone an extensive chart review with autopsy verification and interviews, if necessary, to elicit exact information to thereby allow discernment over those complications that are indeed preventable. A review of this information certainly provides us with the historical perspective to know we are doing far better with management of pregnancy than ever before, but there are some alarming trends on the horizon.

As you can see, Dr. May's graphic illustrations (Figs. 1, 2, and 3) show a significant (95%) decrease in the maternal mortality rate (1940, 376:100,000 live births[1]; 1980, 9:100,000 live births[2]; 1985;7.8:100,000 live births[3]). However, I seriously doubt that the objective called for in 1980 of a maternal mortality rate of 5:100,000 live births by 1990 will be attained.[4] The categorization of these obstetric nightmares summarized in Fig. 2 reflect that downward trend with a few highlights herein reiterated.

1. We may very well have reached an irreducible minimum in anesthetic-related deaths with more complications occurring now from morbid obesity than from the gravid state. (Three of four deaths were noted in markedly obese patients.)

2. The perpetual triad of toxemia, embolism, and hemorrhage continues to show essentially an unchanged status from previous data except in the alarming increase in incidence of deaths from ectopic pregnancy.

In review of an article published in December 1987,[5] the death rate from ectopic pregnancy has shown an impressive sevenfold decline (35.2 to 5.3 deaths per 10,000 cases) in the period from 1970 to 1983. During that same time, however, the number of ectopic pregnancies has shown a fourfold increase (17,900 to 69,000). This increased incidence tends to obscure the fact that we are doing a better job of preventing death from ectopic pregnancy. The simultaneous decrease in maternal deaths from other causes (hemorrhage, placenta previa, uterine rupture, infection, and embolism) has resulted in a relative increase in the maternal death rate from ectopic gestation. Unfortunately, the risk of death from ectopic pregnancy is larger for black women (16.1:10,000 cases), other minorities (7.4:10,000 cases), and teenagers (9.5:10,000 cases).[5] Further breakdown of this data reveals that a substantial proportion of fatal ectopic pregnancies occurred in women before they sought obstetric care in our health care system. Consequently, a significant number of these deaths may be preventable. Perhaps the political football being passed about in Congress with regard to maternal care for all women, regardless of economic status, may provide some means to make a distinct improvement in the death rate from ectopic pregnancy.

3. The most impressive category for highlight in Dr. May's statistics is the absence of a maternal death from an infectious source in his most recently collected data. The advancement in antibiotic therapy and life support systems technology has progressed remarkably in the past 10 years, making this an impressive statistic.

However, the most significant infectious disease epidemic in our medical careers, that of acquired immunodeficiency syndrome (AIDS), has come home. Until recently the AIDS problem has been distinctly absent from this, the ultimate heterosexual population, the gravid woman. A total of 20 cases of AIDS-related maternal deaths have been reported (unpublished data)

to augment the six previously reported cases. Although not yet a "problem" in North Carolina, we all will face this epidemic in the next few years if reported projections become reality. Unfortunately, the available information continues to implicate these same high-risk categories: minority, bisexual, and intravenous drug abuse patients. The most pertinent observation made to date in this population of pregnant patients with AIDS has been a shorter diagnosis-to-death interval (113 days versus 187 days for pregnant versus nonpregnant women with AIDS).[6] Whether pregnancy will have an adverse effect on AIDS will only be discernable by further epidemiologic analysis as this crisis unfolds.

4. Dr. May's most astute analysis of these 67 maternal deaths discerned two that were considered to be of iatrogenic origin. This 3% of maternal deaths is prime material for that segment of the legal profession that seeks fast remuneration. Unfortunately, these deaths are a significant inhibition to an effective, nationwide reporting system of maternal deaths. I commend Dr. May in his most diligent efforts of correlation of birth and death certificates to discover pregnancy-related deaths. I fully expect that these two deaths were only discerned by his analysis of this data. Fortunately, the CDC has now devised a new reporting system through the National Center for Health Statistics that it is hoped will eliminate this particular problem and will allow unbiased reporting without fear of potential medicolegal inquiry.

In conclusion, I agree with Dr. May's assessment that we have come a long way since 1946 to improve our care of the gravid patient, but there is more attainable progress, unless AIDS becomes a"bubonic plague" for us as well as for ensuing generations. I commend Dr. May in his association with Dr. Greiss for their diligent efforts in this most unglamorous and, at times, frustrating collection of information. My hope is to see us attain the irreducible minimum in all obstetric complications but, most importantly, in maternal death.

REFERENCES

1. Vital statistics of the United States, 1940, part II. Washington, DC: Bureau of the Census, 1941.
2. National Center for Health Statistics. Advance report of final mortality statistics, 1980. Monthly Vital Statistics Report 1983;32(suppl):35.
3. National Center for Health Statistics. Advance report of final mortality statistics, 1985. Monthly Vital Statistics Report 1986;35(suppl):41.
4. U.S. Public Health Service. Objectives for the nation. Atlanta, Georgia: Public Health Service, 1980.
5. Atrash HK, Hughes JM, Hogue CJ. Ectopic pregnancy mortality in the United States, 1970-1983. Obstet Gynecol 1987;70:817.
6. Koonin L, et al. Pregnancy-associated deaths due to AIDS in the United States. JAMA [In press].

DR. STEPHEN ANDERSON, Winston-Salem, North Carolina. Dr. May, I want to congratulate you and also observe that it is a lot easier to review 5 years of maternal death than it used to be. I sat thinking about the dramatic reduction in deaths from infection and would merely comment that I think most of this is probably related to legalized abortion. At this time it is not particularly popular to speak for medically available abortion, but I think that is probably the case. It behooves all of us to think about that in view of the decisions that are before the Supreme Court. There were dramatic improvements in antibiotic therapy before 1970 that did not have an impact on infection-related deaths as has occurred in the past 10 years.

DR. MAY (Closing). I wish to express my appreciation to Dr. Anderson for making his strong, positive observation on the decline of "infection deaths" as related to the advent of legalized abortion. I hope that Dr. Pearse will transmit this observation through ACOG to our representatives in Washington, D.C. I will provide him with Fig. 2, which supports the contention that medically performed abortion is much safer than is criminal abortion.

I thank Dr. Taylor for his salient observations, especially with respect to the AIDS plague in pregnant women. Possibly, even more remote unrealized causes of maternal deaths may loom over the horizon. This gives us a *raison d'être* for maternal mortality reporting. An irreducible minimum number for specific causes of maternal death is frequently mentioned. Any irreducible minimum number above zero as a cut-off point for maternal mortality study is unacceptable.

The ACOG has provided program time during the past four to five annual meetings for a group of obstetricians who are interested in maternal mortality study. This program has led to a meaningful collaboration between the ACOG group and the CDC. The collaborative efforts have made tremendous progress in the formulation of a uniform maternal mortality report system.

Maternal mortality study and reporting are less than exciting, but like all epidemiology, they are necessary to more precisely know where our attention must be directed to prevent the occurence. I solicit the help of all obstetricians throughout the United States and its territories to participate in the maternal mortality report system through the CDC.

Risk factors for intraamniotic infection: A prospective epidemiologic study

David E. Soper, MD, C. Glen Mayhall, MD, and Harry P. Dalton, PhD
Richmond, Virginia

To determine the cumulative incidence of intraamniotic infection in our hospital and to identify potential risk factors for infection, 408 consecutive patients were followed up prospectively to determine development of intraamniotic infection. The clinical diagnosis of intraamniotic infection was made in 43 (10.5%) patients. Patients with intraamniotic infection were younger, of lower gravidity and parity, more likely to require oxytocin augmentation, and more likely to be monitored internally than were patients who were not infected. They also had longer durations of labor, ruptured membranes, and hospitalization before delivery, had significantly more vaginal examinations, and were more likely to be delivered of infants by cesarean section, as compared with patients without infection. Logistic regression analysis identified four variables independently associated with intraamniotic infection: the number of vaginal examinations, duration of ruptured membranes, use of internal monitors, and duration of total labor. Further study is necessary to clarify the role of these risk factors in the pathogenesis of intraamniotic infection so that infection control strategies can be developed. (Am J Obstet Gynecol 1989;161:562-8.)

Key words: Intraamniotic infection, vaginal examinations, ruptured membranes, internal monitors, length of labor

It has been reported that chorioamnionitis or intraamniotic infection develops in between 0.7% and 1.3% of women who are in labor or who have ruptured amniotic membranes.[1-4] This rate of intraamniotic infection is based on retrospective reviews. In addition, potential risk factors that may predispose patients to the development of intraamniotic infection have not been prospectively studied. Our labor suite nurses perceived a high rate of infection in our patient population and a retrospective review of patient records revealed a higher than expected rate of intraamniotic infection. This prompted us to carry out a prospective study to determine the incidence of intraamniotic infection in our hospital. Because we suspected that the increased rate of intraamniotic infection may be related to iatrogenic factors, additional data were collected to identify risk factors associated with intraamniotic infection.

Material and methods

From January 15 to February 22, 1987, 408 consecutive patients admitted to the Medical College of Virginia's Labor and Delivery Suite were followed up prospectively to determine development of intraamniotic infection. The population is indigent and predominantly black.

Definition of intraamniotic infection. The diagnosis of intraamniotic infection was based on the following criteria. The patient had to have ruptured membranes and a temperature >100° F on two occasions at least 1 hour apart or a single temperature >101° F. In addition, the patient had to have one of the following clinical findings: (1) maternal tachycardia (>100 beats/min), (2) fetal tachycardia (>160 beats/min), (3) maternal leukocytosis (>11000/mm^3), or (4) foul-smelling amniotic fluid. Other sources of intrapartum fever such as urinary tract infection, community-acquired respiratory tract infection, intravenous catheter-associated infection, and substantial dehydration were excluded. After the criteria for the diagnosis of intraamniotic infection were fulfilled, patients were started on parenteral ampicillin and gentamicin. These antibiotics were continued during labor and in the postpartum period until the patient was afebrile for 24 hours.

Epidemiologic data. The following data were collected from each patient's record: age, race, gravidity, parity, estimated gestational age, and date and time admitted and delivered of infants. Data were collected on the use of internal monitors, induction of labor, oxytocin augmentation, use of intrapartum antibiotics, route of delivery, duration of ruptured membranes, number of vaginal examinations, and duration of the latent and active phases of labor. The duration of internal monitoring was recorded only for

From the Department of Obstetrics and Gynecology, Hospital Epidemiology Unit, Division of Infectious Diseases, Department of Medicine, and the Department of Pathology, Medical College of Virginia, and the Medical College of Virginia Hospitals, Virginia Commonwealth University.
Presented by invitation at the Fifty-first Annual Meeting of the South Atlantic Association of Obstetricians and Gynecologists, Hot Springs, Virginia, January 29–February 1, 1989.
Reprint requests: David E. Soper, MD, Box 34, MCV Station, Richmond, VA 23298.
6/6/12995

patients with the clinical diagnosis of intraamniotic infection.

Laboratory data. A complete blood count and two pretreatment blood cultures were obtained for all patients who met the criteria for the clinical diagnosis of intraamniotic infection. A catheterized urine specimen was obtained for culture. An attempt was made to aspirate amniotic fluid from the intrauterine catheter for culture.

Microbiologic techniques. Blood was cultured by the injection of 10 ml of blood into a diphasic medium that consisted of trypticase soy agar as the solid phase and 90 ml of brain heart infusion broth with 0.2% agar as the liquid phase. With a calibrated loop, urine was inoculated onto trypticase soy agar with 5% sheep blood (blood agar) and eosin methylene blue agar. Amniotic fluid was inoculated onto blood agar and chocolate agar for aerobic microorganisms and onto prereduced anaerobically sterilized peptone—yeast extract—glucose media, blood agar, and chocolate agar for anaerobic microorganisms. All media were incubated at 35° C under aerobic and anaerobic atmospheres. Several specimens were cultured for genital mycoplasmas by inoculation onto Mycotrim diphasic media (Hana Biologicals, Inc., Berkeley, Calif.) that was incubated 7 days. All organisms were identifed with standard methods as previously outlined.[5]

Statistical analysis. Discrete data were tested for significance with the χ^2 test or Fisher's exact test. Continuous data were tested for significance with a two-tailed Student's t test. Logistic regression analysis was performed with the diagnosis of intraamniotic infection as the dependent variable. All p values were based on two-tailed tests with $p < 0.05$ considered to be significant.

Results

During the study period, 45 (11.3%) of 408 patients had fever during labor. One patient had a positive urine culture that yielded >1 million colonies per milliliter of *Proteus mirabilis* and she was treated for pyelonephritis. One patient had signs and symptoms compatible with an upper respiratory tract infection and was observed but was not treated. The clinical diagnosis of intraamniotic infection was made in the remaining 43 (10.5%) patients. Urine cultures were negative in all but one of the patients who had intraamniotic infection. *P. mirabilis* and enterococci were recovered from her urine, but an amniotic fluid culture was positive for >1 million colonies per milliliter of *Escherichia coli.*

All patients with intraamniotic infection were febrile and had ruptured membranes. The mean temperature for infected patients was 101.5 ± 0.8° F, compared with 99.0 ± 0.7° F for uninfected patients ($p \leq 0.0001$). The mean white blood cell count was 14,617 ± 4907/mm³ in patients with intraamniotic infection and 11,877 ± 3737/mm³ in the uninfected patients ($p = 0.0026$). Most patients (34/43, 79.1%) met at least four criteria for the diagnosis of intraamniotic infection. All patients with leukocytosis as the only additional criterion for the diagnosis of intraamniotic infection had a white blood cell count >15,700/mm³.

Table I. Demographic data

Characteristic	*Intraamniotic infection (n = 43)*	*No intraamniotic infection (n = 365)*	*p*
Age* (yr)	21.3 ± 3.3	23.3 ± 5.5	0.002
Race, n (%)			
Black	30 (69.8)	211 (57.8)	
White	13 (30.2)	148 (40.6)	
Other	0 (0)	6 (1.6)	NS
Gravidity*	1.9 ± 1.1	2.4 ± 1.6	0.04
Parity*	0.4 ± 0.6	0.9 ± 1.3	0.005
Estimated gestational age (wk)	39.0 ± 2.5	38.5 ± 4.5	NS

*Data expressed as mean ± SD.

Demographic data are presented in Table I. Patients with intraamniotic infection were younger and had lower gravidity and parity than did patients who were not infected. A total of 67% (29/43) of patients with intraamniotic infection were nulliparous, compared with 43.6% (159/365) of the uninfected patients ($p = 0.002$). Premature delivery (<38 weeks' estimated gestational age) occurred in 18.6% (8/43) of patients with intraamniotic infection, and 11.6% (5/43) of the infants weighed <2500 gm. This was not significantly different from the rate of premature delivery (24.9%, 91/365) in uninfected patients.

Patients with intraamniotic infection were more likely to require oxytocin augmentation and to be internally monitored. Infected patients also had longer durations of labor and ruptured membranes and had significantly more vaginal examinations when compared with patients without infection. Infected patients were also more likely to be delivered of infants by cesarean section, primarily because of failure to progress in labor, than were patients without intraamniotic infection. These data are summarized in Table II.

Patients with intraamniotic infection demonstrated a longer duration of hospitalization before delivery (59.2 ± 119.1 hours) when compared with patients without intraamniotic infection (24.3 ± 85.4 hours, $p = 0.02$). We noted that 25.3% (16/63) of patients with admission-to-delivery intervals >24 hours became infected, compared with 7.8% (27/345) with intervals <24 hours ($p = 0.002$).

Logistic regression analysis identified five variables independently associated with the diagnosis of intraamniotic infection (Table III). When the outcome variable cesarean section was eliminated from the model, inter-

Table II. Intrapartum data

Characteristic	*Intraamniotic infection (n = 43)*	*No intraamniotic infection (n = 365)*	*p*
Induction*	8 (18.6)	46 (12.6)	NS
Oxytocin augmentation*	34 (79.1)	122 (33.4)	<0.0001
Internal monitor*	42 (97.7)	254 (69.6)	0.0001
Cesarean section*	25 (58.1)	58 (15.9)	<0.0001
Latent phase (hr)†	16.7 ± 11.0	7.2 ± 6.1	0.0001
Active phase (hr)†	6.8 ± 4.2	4.7 ± 4.5	0.009
Total labor (hr)†	22.8 ± 9.7	11.3 ± 8.5	0.0001
Duration of ruptured membranes (hr)†	24.3 ± 18.1	9.5 ± 21.2	0.0001
Vaginal examinations (n)†	6.6 ± 2.4	3.7 ± 2.4	0.001

*Number, (%).
†Mean ± SD.

Table III. Results of logistic regression analysis

Variable	*β*	*SEM*	χ^2	*p*	*R*
Internal monitors	3.652	1.698	5.77	0.016	0.117
Cesarean section	1.813	1.519	17.38	0.001	0.237
Total labor	0.089	0.434	13.46	0.001	0.205
Vaginal examinations	0.231	0.107	4.66	0.031	0.099
Duration of ruptured membranes	0.016	0.008	3.89	0.049	0.083

nal monitoring, duration of ruptured membranes, number of vaginal examinations, and total duration of labor remained significantly associated with intraamniotic infection (Table IV).

An amniotic fluid specimen was obtained for culture by aspiration of the intrauterine pressure catheter in 18 (41.9%) of 43 patients. All but one yielded at least one microorganism on culture. Aerobic gram-positive cocci were the bacteria most frequently isolated. Group B streptococci were cultured in five cases. Nine of 18 (50%) patients had more than one organism isolated from their amniotic fluid. Five patients had anaerobic microorganisms (*Peptostreptococcus,* 3; *Fusobacterium symbiosum*; and *Bacteroides fragilis*) isolated, and all five had concomitant isolation of aerobic organisms (Table V). One maternal blood culture yielded diphtheroids. This patient also had diphtheroids isolated from the amniotic fluid.

Comment

Determination of the cumulative incidence of clinical intraamniotic fluid infection with prospective patient surveillance has not been previously reported. The intraamniotic infection rate of 10.5% in our patients was considerably higher than the previous estimates based on retrospective record reviews.[1-4] There may be several reasons for this difference. First, data generated by retrospective study may significantly underestimate the true cumulative incidence of intraamniotic infection. Second, our definition of intraamniotic infection was somewhat different from those used in the retrospective studies; only one of those studies used criteria more stringent than ours. Third, the populations studied were also different. Our population was predominantly indigent and black, whereas the populations in the other studies were predominantly Hispanic or undefined.

We observed that the durations of rupture of the membranes and of labor, nulliparity, and oxytocin augmentation were significantly associated with intraamniotic infection. In a retrospective study, Ferguson and associates[3] observed a significant relationship between duration of ruptured membranes and intraamniotic infection. Gibbs and coworkers[6] observed that durations of labor and ruptured membranes were significantly associated with intraamniotic infection in a study of genital mycoplasmas, but it was not stated whether that study was prospective or retrospective.

Use of internal monitors was significantly associated with intraamniotic infection. Two studies[6,7] have previously examined the association between intraamniotic infection and duration of internal monitoring. In the most recent of these two studies,[7] internal monitoring was independently associated with the development of intraamniotic infection. In our series, all patients who had intraamniotic infection with ruptured membranes for <24 hours were internally monitored. These monitors were in place for an average of 12 hours before

Table IV. Results of logistic regression analysis with cesarean section deleted

Variable	*β*	*SEM*	χ^2	*p*	*R*
Vaginal examinations	0.324	0.100	10.42	0.0012	0.175
Duration of ruptured membranes	0.020	0.008	6.12	0.0134	0.123
Internal monitors	3.550	1.601	4.92	0.0265	0.103
Total labor	0.101	0.023	18.87	0.0001	0.248

the clinical diagnosis of intraamniotic infection. Logistic regression analysis confirmed an independent association of internal monitoring with intraamniotic infection.

The number of vaginal examinations was also significantly associated with intraamniotic infection by both univariate and multivariate statistical analysis. Data on the impact of the number of vaginal examinations on postpartum infection are in conflict.[8-10] Our data indicate that internal monitoring and the number of vaginal examinations are important contributors to the occurrence of intraamniotic infection during labor. This relationship with internal monitoring and number of vaginal examinations is strengthened by the results of multivariate analysis that allowed us to control for the duration of ruptured membranes and the total duration of labor. Table VI shows the cumulative probability of the occurrence of intraamniotic infection in monitored patients as the number of vaginal examinations and the durations of ruptured membranes and total labor increases.

The disproportionate number of patients with intraamniotic infection admitted to the hospital >24 hours before delivery also suggests a nosocomial source of these infections. Most of these patients were admitted with either premature rupture of the membranes or pregnancy-induced hypertension. Although the duration of hospitalization before delivery did not emerge from the logistic regression model as a risk factor independent of all of the other variables, further study of this variable is warranted because duration of hospitalization before surgery is a risk factor of postoperative wound infection.[11] This may be a result of the increased exposure to hospital flora or to the underlying condition that requires longer hospitalization.

We found cesarean section to be significantly associated with intraamniotic infection by both univariate and multivariate analysis (Tables II and III). However, because cesarean section occurred after infection, it was considered an outcome variable and not a risk factor of infection. Thus, in further analysis to identify risk factors, cesarean section was excluded from the model (Table IV). Although we observed a nearly fourfold increase in the cesarean section rate of patients with intraamniotic infection, other studies conflict with respect to the association of intraamniotic infection with the need for abdominal delivery.[12, 13] It is unclear whether dysfunctional labor leads to intraamniotic infection and cesarean section or whether intraamniotic infection causes dysfunctional labor that leads to abdominal delivery. Further prospective studies are needed to determine the nature of this relationship.

Table V. Bacteria isolated from amniotic fluid (n = 18)

Microorganisms	*Number of isolates*
Aerobic bacteria	
Group B streptococci	5
Staphylococcus, coagulase negative	4
Escherichia coli	3
Alpha streptococci	3
Enterococci	3
Neisseria gonorrhoeae	2
Proteus mirabilis	1
Gardnerella vaginalis	1
Lactobacillus	1
Diphtheroids	1
Group D streptococci	1
Anaerobic bacteria	
Peptostreptococcus asaccharolyticus	2
Peptostreptococcus anaerobius	1
Bacteroides fragilis	1
Fusobacterium symbiosum	1
Genital mycoplasms	
Mycoplasma hominis	1
Ureplasma urealyticum	2
Total isolates	33

Number of patients with >1 isolate = 9 (50%).

The bacterial species isolated from the amniotic fluid specimens of our patients are similar to those reported by others for patients with intraamniotic infection.[13-15] Isolation of these bacterial species is consistent with the currently held theory that intraamniotic infection develops after bacteria normally found in the lower vagina and cervix ascend to the amniotic cavity.[16] Controversy remains with regard to how these organisms gain access to the intrauterine cavity. Amniocentesis performed in patients with and without ruptured membranes has documented ascending spread of microorganisms without the introduction of a foreign body.[13, 17-20] However, fetal scalp electrodes, internal pressure catheters, and vaginal examinations, especially

Table VI. Probability of intraamniotic infection developing in patients monitored internally with increasing numbers of vaginal examinations and increasing duration of rupture of membranes and total labor

Risk factors			
No. of vaginal examinations	*Duration of ruptured membranes (hr)*	*Total labor (hr)*	*Probability*
2	5.25	6.0	0.03
5	12.00	16.0	0.16
6	16.00	21.0	0.32
8	21.75	29.0	0.63
13	88.00	29.0	0.95

when performed repeatedly, may increase the inoculum of potentially pathogenic bacteria in the amniotic cavity and increase the risk of clinical infection. Our data suggest that this is indeed the case.

In summary, we found a surprisingly high rate of intraamniotic infection in our hospital. Although related to the intrinsic risk factors, duration of labor, and ruptured membranes, this high rate also appears to be related to iatrogenic risk factors such as internal fetal monitoring and multiple vaginal examinations. To our knowledge, these parameters have never been examined as possible risk factors of intraamniotic infection in prospective, comparative epidemiologic studies. Further study is necessary to clarify the role of these risk factors in the pathogenesis of intraamniotic infection. Infection control strategies can then be developed in an attempt to decrease our intraamniotic infection rate.

REFERENCES

1. Gibbs RS, Castillo MS, Rodgers PJ. Management of acute chorioamnionitis. Am J Obstet Gynecol 1980;136:709-13.
2. Looff JD, Hager WD. Management of chorioamnionitis. Surg Gynecol Obstet 1984;158:161-6.
3. Ferguson MG, Rhodes PG, Morrison JC, Puckett CM. Clinical amniotic fluid infection and its effect on the neonate. Am J Obstet Gynecol 1985;151:1058-61.
4. Hauth JC, Gilstrap LC, Hankins GDV, Connor KD. Term maternal and neonatal complications of acute chorioamnionitis. Obstet Gynecol 1985;66:59-62.
5. Lennette EH, Balows A, Hausler WJ, Shadomy HJ, eds. Manual of clinical microbiology. 4th ed. Washington, DC: American Society for Microbiology, 1985.
6. Gibbs RS, Cassell GH, Davis JK, St. Clair PJ. Further studies on genital mycoplasmas in intra-amniotic infection: blood cultures and serologic response. Am J Obstet Gynecol 1986;154:717-26.
7. Newton ER, Prihoda TJ, Gibbs RS. Logistic regression analysis of risk factors for intra-amniotic infection. Obstet Gynecol 1989;73:571-5.
8. Hagen D. Maternal febrile morbidity associated with fetal monitoring and cesarean section. Obstet Gynecol 1975; 46:260-2.
9. Lewis JF, Johnson P, Miller P. Evaluation of amniotic fluid for aerobic and anaerobic bacteria. Am J Clin Pathol 1976;65:58.
10. Gibbs RS, Jones PM, Wilder CJY. Internal fetal monitoring and maternal infection following cesarean section: a prospective study. Obstet Gynecol 1978;52:193-7.
11. Bruun JN. Post-operative wound infection. Predisposing factors and the effect of a reduction in the dissemination of staphylococci. Acta Med Scand 1970;514(suppl):1.
12. Duff P, Sanders R, Gibbs RS. The course of labor in term patients with chorioamnionitis. Am J Obstet Gynecol 1983;147:391-5.
13. Garite TJ, Freeman RK. Chorioamnionitis in the preterm gestation. Obstet Gynecol 1982;59:539-45.
14. Gibbs RS, Blanco JD, St. Clair PJ, Castaneda YS. Quantitative bacteriology of amniotic fluid from women with clinical intraamniotic infection at term. J Inf Dis 1982;145:1-8.
15. Yoder PR, Gibbs RS, Blanco JD, Castaneda YS, St Clair PJ. A prospective, controlled study of maternal and perinatal outcome after intraamniotic infection. Am J Obstet Gynecol 1983;145:695-701.
16. Benirschke K. Routes and types of infection in the fetus and the newborn. Am J Dis Child 1960;99:714-21.
17. Miller JM, Pupkin MJ, Hill GB. Bacterial colonization of amniotic fluid from intact fetal membranes. Am J Obstet Gynecol 1980;136:796-804.
18. Miller JM, Hill GB, Welt SI, Pupkin MJ. Bacterial colonization of amniotic fluid in the presence of ruptured membranes. Am J Obstet Gynecol 1980;137:451-8.
19. Broekhuizen FF, Gilman M, Hamilton PR. Amniocentesis for gram stain and culture in preterm premature rupture of the membranes. Obstet Gynecol 1985;66:316-21.
20. Gravett MG, Hummel D, Eschenbach DA, Holmes KK. Preterm labor associated with subclinical amniotic fluid infection and with bacterial vaginosis. Obstet Gynecol 1986;67:229-37.

Editors' note: This manuscript was revised after the discussions were presented.

Discussion

Dr. Billy D. Burk, Rome, Georgia (Official Guest). There is confusion in the literature as to the definition of intraamniotic infection and as a result the true incidence is hard to discern. It has been reported to range from 0.5% to 1.75% and up to 10.5% at the Medical College of Virginia. The diagnosis is confirmed when bacteria or their breakdown products are demonstrated in the amniotic fluid. Cultures may be obtained by means of catheters or by amniocentesis guided by ultrasonography. Rapid confirmation of the presence of organisms can be accomplished by gram stains or by gas-liquid chromatography.

Before labor, bacteria are seldom found in the amniotic fluid. Inhibitory factors in cervical mucus, placental membranes, and amniotic fluid prevent entry of bacteria or their replication. When labor begins and the membranes rupture, bacteria ascend from the vagina into the amniotic cavity where they, by numbers or by their virulence, overcome the inhibitory factors and rapidly multiply.

When the bacteria invade the decidua, the membranes, the placenta, and the fluid in quantity, infection and inflammation lead to maternal fever, maternal and fetal tachycardia, maternal leukocytosis, and foul-smelling amniotic fluid.

Dr. Soper has identified and applied statistical analysis to incriminate: (1) the total length of labor, (2) duration of rupture of the membranes, (3) the number of vaginal examinations and, (4) the use of internal monitoring as risk factors for intraamniotic infections.

I believe the true risk factors are the number and virulence of bacteria that are in the vagina and cervix when labor begins. The increasing and decreasing pressure caused by uterine contraction in labor acts as a pump to enhance the ascension of bacteria into the amniotic cavity. Vigorous vaginal examinations and the introduction of intraamniotic pressure catheters and scalp electrodes add to the inoculation of bacteria into the cavity.

Intraamniotic infection becomes symptomatic when 10^3 colony forming units per milliliter of bacteria are present. The virulence or toxin production of the bacteria determine the severity of the infection.

Young nulliparous patients who had long labors, prolonged rupture of the membranes, numerous vaginal examinations, and internal monitoring had a greater incidence of intraamniotic infection. The bacteria recovered by culture confirmed the theory of ascending infection. Patients who had intraamniotic infection were more likely to require augmentation of labor and had a fourfold greater chance of delivery by cesarean section because of failure to progress in labor.

I would like to ask Dr. Soper the following questions: (1) What is the induction and augmentation policy at the Medical College of Virginia? and (2) When the diagnosis of intraamniotic infection was made, were antibiotics begun?

Dr. Gene Burkett, Miami, Florida. I am a little confused by some of the data presented here as to whether infection preceded manipulations or resulted from manipulations. The author uses premature rupture of the membranes as part of his definition. Is not this study really a study of what happens when rupture of the membranes occurs? About 6 to 8 years ago Ledger showed that there is intraamniotic infection before rupture of the membranes. I would ask the author to define this very clearly so that we can appreciate his point.

Dr. J. Terrell Pope, Macon, Georgia. Were the membranes examined pathologically to determine whether there was evidence of infection or inflammation?

Dr. Herbert G. Hopwood, Arlington, Virginia. I wonder whether Dr. Soper may have considered that he might be using the wrong stimulatory agent. Instead of oxytocin, perhaps he might have used prostaglandins.

Dr. Allan G. W. McLeod, Miami, Florida. A few years ago we had a problem with infection and we went back very bascially to some simple techniques. In the process of vaginal examination in labor, we sponged the vulva carefully with Betadine and used careful separation of the labia before the insertion of the examining fingers each time an examination was done. Infection rate dropped dramatically.

Dr. John W. C. Johnson, Gainesville, Florida. I congratulate the authors on a beautifully designed and executed study. We have been interested in premature rupture of the membranes for a few years. One of the cofactors we found associated with increased intraamniotic infection is gestational age, an inverse relationship. Would Dr. Soper comment on that?

Dr. Soper (Closing). Thank you for your comments. The augmentation and induction policies at the Medical College of Virginia were similar to those used across the country; those patients with hypotonic dysfunctional labor were augmented with oxytocin. Indications for induction included patients at term with ruptured membranes who were not in labor, post dates, and pregnancy-induced hypertension. We administered oxytocin, not prostaglandins, for augmentation and induction. When the diagnosis of intraamniotic infection was made, the studies that I mentioned were performed and antibiotics were started. A combination of ampicillin and gentamicin was administered at our institution.

Dr. Burkett commented on infections that either preceded manipulation or caused ruptured membranes. The great majority of patients in this study had no clinical indication of infection on admission. Subclinical intraamniotic infection may have played a role in premature rupture of the membranes and may have predisposed the patient to the development of clinical intraamniotic infection. Our study did not address this issue. It is important, however, to make the distinction between clinical intraamniotic infection, as defined in our study, and histologic chorioamnionitis, which may not be assoicated with intrapartum fever. We believe that clinical intraamniotic infection may be nosocomial in some cases. Patients admitted to the hospital at term with intact membranes who do not have a fever for at least 2 hours after admission but who later have clinical manifestations of infection may be categorized as having a nosocomial intraamniotic infection. Infections that occur after admission may be related to the iatrogenic risk factors reported in this study. In this subset of patients some type of intervention may decrease the rate of intraamniotic infection. We have designed a new prospective study to further investigate potential risk factors for intraamniotic infection. About 60% of our patients with intraamniotic infection appear to have acquired it in the hospital. In our study, patients rarely became infected before they were instrumented. A second category would be those patients with a community-acquired intraamniotic infection. These are patients admitted to the hospital who already meet the criteria for the diagnosis of intraamniotic infection. These patients make up $<10\%$ of our patients with intraamniotic infection. Finally, a third category would encompass the rest of the patients who have intraamniotic infection. The third category includes patients with premature rupture of the membranes or premature delivery who are afebrile at the time of admission. In these cases the relationship between admission and infection is unknown.

In this study, we did not routinely evaluate the placenta pathologically. However, of those placentas examined by our pathologists, 85% of the placentas from patients with intraamniotic infection showed histologic evidence of chorioamnionitis.

In response to Dr. Johnson's question with regard to gestational age, the majority of our patients with intraamniotic infection were at term. We were concerned that patients in preterm labor would have a higher cumulative incidence of intraamniotic infection, but that was not the case. At the Medical College of Virginia the diagnosis of intraamniotic infection is a clinical entity predominantly seen in term pregnancies.

Dr. McLeod commented that he studied the institution of careful aseptic technique while performing vaginal examinations and found that intraamniotic infection rates were decreased. This information would further strengthen our position that iatrogenic factors do play a role in the overall rate of intraamniotic infection. After we have completed our prospective study, we will use the data to develop preventive strategies.

In conclusion, we were the first to look at overall intraamniotic infection rates prospectively. The currently accepted rate of intraamniotic infection may be an underestimate of the true rate. Additional studies in similar institutions need to be performed to determine accurate rates of intraamniotic infection in various obstetric populations.

Short course of antibiotic therapy in treatment of postpartum endomyometritis

Walter J. Morales, MD, PhD, Evan M. Collins, MD, Jeffrey L. Angel, MD, and Robert A. Knuppel, MD, MPH

Orlando, Florida

To evaluate the safety and efficacy of an abbreviated course of antibiotic therapy in postpartum endomyometritis, 109 patients with endomyometritis were randomized to three study groups. All were treated with clindamycin and tobramycin until afebrility and clinical signs of disease were absent. Patients in group I received antibiotics for ≥24 hours, group II received therapy for ≥48 hours, and group III received antibiotic therapy for ≥48 hours that preceded a 7-day course of oral Augmentin. The groups were similar in size and in demographic and clinical parameters. Two patients from each group required a third antibiotic, and no patient required rehospitalization. Group III required more days of antibiotic therapy than did group I, 2.9 versus 2.1 days ($p < 0.01$), and cost $412.00 more per patient. This data strongly suggest that a short course of antibiotic therapy is efficacious and safe and would result in substantial monetary savings. (Am J Obstet Gynecol 1989;161:568-72.)

Key words: Endomyometritis, short-course antibiotic therapy

Postpartum endomyometritis remains the most common infectious complication of the puerperium. Incidence varies depending on the study cited, but ranges from 20% to 38.5% of patients after cesarean section, and 1.2% to 2.5% of patients after vaginal delivery.[1-3] Because of the polymicrobial nature of the infection, a combination antibiotic regimen of clindamycin and an aminoglycoside has been accepted as standard therapy, and its efficacy has been well documented in the literature.[4-6]

Many studies have proposed various antibiotic regimens, but few have focused on the proper duration of therapy. Many advocate the use of intravenous antibiotics for a minimum of 48 hours of afebrility,[7-9] whereas others recommend 7 to 10 days of oral antibiotics on completion of parenteral therapy.[5, 6, 10-12] Recent studies have advocated the use of shorter courses of antibiotic therapy,[13-15] but none has evaluated the efficacy of clindamycin-aminoglycoside in an indigent population in a prospective randomized fashion. To evaluate the safety and efficacy of abbreviated courses of antibiotics, we studied a group of service patients with the diagnosis of postpartum endomyometritis.

From the University of South Florida, Orlando Regional Medical Center, Department of Obstetrics and Gynecology, Division of Maternal-Fetal Medicine.

Presented by invitation at the Fifty-first Annual Meeting of the South Atlantic Association of Obstetricians and Gynecologists, Hot Springs, Virginia, January 29–February 1, 1989.

Reprint requests: Walter J. Morales, MD, PhD, 85 W. Miller St., Suite 303, Orlando, FL 32806.

6/6/13439

Material and methods

The study was conducted at Tampa General Hospital's postpartum unit from July 1987 through April 1988. Patients were enrolled in the study after they were assigned the diagnosis of postpartum endomyometritis. This diagnosis was established on the basis of a combination of clinical and laboratory findings that included the following: (1) febrile morbidity defined as temperature >100.4° F taken twice at least 6 hours apart, or an isolated temperature spike of 101° F that excluded the first day postpartum, (2) uterine tenderness, (3) leukocytosis, and (4) absence of other foci of infection. Patients who had received prophylactic antibiotics were considered eligible for the study. A catheterized urine specimen with culture and sensitivity, a complete blood count with differential, and two sets of blood cultures (in antibiotic removal device bottles if prophylactic antibiotics had been administered) were obtained before initiation of therapy. All antipyretics were removed from the "standard postpartum orders" and were given only at the discretion of the house officer.

The antibiotic regimen consisted of intravenous administration of clindamycin 900 mg every 8 hours and tobramycin 95 to 140 mg loading dose, then adjusted to 80 to 120 mg every 8 hours based on lean body weight. Tobramycin dosages were adjusted based on serum peak and trough levels, which usually were evaluated after the fifth dose. Tobramycin levels were also adjusted on the basis of serum creatinine levels, which were evaluated at the initiation of therapy and every other day therafter until discontinuation of therapy.

Patients were excluded from the study protocol if they had a known allergy to either clindamycin or tobramycin. Other patients excluded were those being treated for any other infectious process, and those who had immunosuppressive disorders. Patients with bacteremia before the initiation of therapy also were excluded from the final data analysis. Eligible patients were randomized to one of the three treatment regimens: (1) clindamycin-tobramycin for 24 hours of afebrility and asymptomatic, (2) clindamycin-tobramycin for 48 hours of afebrility and asymptomatic, (3) clindamycin-tobramycin for 48 hours of afebrility and asymptomatic, plus 7 days of oral Augmentin (Beecham Laboratories, Bristol, Tenn.).

Therapy was considered to have failed in patients with temperature elevations ≥100.4° F 72 hours after initiation of therapy and in those who displayed persistent uterine tenderness. Thus a third antimicrobial agent, usually a broad spectrum penicillin, was added to the antibiotic regimen. Therapy also was considered to have failed in instances in which sequelae of endomyometritis (bacteremia, abscess, septic pelvic thrombophlebitis) developed, and the treatment regimen was adjusted accordingly.

Table I. Conditions that resulted in exclusion

	Group		
	*I**	*II†*	*III‡*
Positive blood culture	3	3	3
Allergic reaction	1	—	—
Urinary tract infection	—	—	2
Failure to adhere to protocol	2	3	2
	6	6	7

*Clindamycin + tobramycin × at least 24 hours of afebrility.

†Clindamycin + tobramycin × 48 hours of afebrility.

‡Clindamycin + tobramycin × 48 hours of afebrility + oral augmentin × 7 days.

Successful therapy was defined as a complete resolution of fever and symptoms at the completion of the specified therapy. If stable, patients were discharged after completion of therapy, and were instructed to monitor their temperatures at home and to call Tampa General Hospital because of temperatures ≥100.4° F or abdominal pain. Patients who underwent cesarean section were examined 2 weeks after discharge, and were evaluated for signs of persistent disease or sequelae.

The charts of patients who underwent vaginal delivery were reviewed for evidence of rehospitalization or emergency room visits. The data were analyzed with the χ^2 test and p value of ≤0.05 was considered statistically significant.

Results

During the period of study, 5317 deliveries were recorded at Tampa General Hospital and, of these, 843 (15.8%) were delivered by cesarean section. Postpartum endomyometritis was diagnosed in 128 (2.4%) of these deliveries with 81 (63%) of these infections diagnosed after cesarean section. A total of 128 patients with the diagnosis of postpartum endomyometritis were randomized to one of the three study groups; 19 of these patients were excluded from the analysis because of a variety of reasons (Table I). Of the remaining 109 patients, 36, 36, and 37 were included in study groups I, II, and III, respectively.

The demographic characteristics of the study groups are summarized in Table II, which demonstrates that no difference among the groups in terms of maternal age, parity, gestational age, length of labor, length of rupture of membranes, number of digital cervical examinations after rupture of membranes, use of fetal scalp electrode, internal pressure catheter, and the frequency of meconium-stained fluid. The groups were also similar in terms of predelivery and postpartum hemoglobin, use of prophylactic antibiotics, type of anesthetic agent, and incidence of cesarean section, as well

Table II. Demographic characteristics of study group

	Group			
	I (*n* = 36)	*II* (*n* = 36)	*III* (*n* = 37)	*p*
Age (yr)*	23.4 ± 3.8	21.8 ± 5.1	24.6 ± 4.6	NS
Parity (median)*	1.0	1.0	1.5	NS
Gestational age (wk)*	38.6 ± 3.7	37.1 ± 3.3	37.5 ± 3.4	NS
Labor (hr)*	7.7 ± 5.7	9.5 ± 4.8	7.3 ± 5.1	NS
Rupture of membranes*	11.8 ± 6.9	8.4 ± 6.1	10.4 ± 8.3	NS
Cervical examination after rupture of membranes*	3.8 ± 4.3	3.2 ± 5.2	3.2 ± 4.5	NS
Fetal scalp electrode *n* (%)	23 (64)	20 (56)	18 (49)	NS
Internal pressure catheter *n* (%)	19 (53)	16 (44)	12 (32)	NS
Meconium-stained fluid *n* (%)	8 (22)	7 (19)	8 (22)	NS

*Values are mean ± SD.

Table III. Delivery characteristics of study group

	Group			
	I	*II*	*III*	*p*
Predelivery hemoglobin*	11.8 ± 1.1	11.2 ± 1.8	11.3 ± 1.6	NS
Postpartum hemoglobin*	9.6 ± 1.0	9.2 ± 1.3	9.3 ± 1.1	NS
Prophylactic antibiotics, *n* (%)	24 (67)	19 (53)	23 (62)	NS
Cesarean section, *n* (%)	28 (78)	29 (81)	24 (65)	NS
Failure to Progress	13	14	9	
Breech	3	7	5	
Fetal Distress	4	2	6	
Repeat	8	6	4	

*Values are mean ± SD.

as indications for abdominal delivery (Table III). No difference was observed among the groups in terms of time from delivery to diagnosis of endomyometritis, maximum white blood cell count, and maximum temperature (Table IV).

Although there was no difference among the three groups in terms of total postpartum hospital days, patients from group I required statistically significantly fewer days of antibiotic administration than did those from group III, 2.1 versus 2.9 days ($p < 0.01$). Two patients from each group required the addition of a third antibiotic to achieve satisfactory resolution of the infectious process. Thus there was no difference among the three study groups in terms of success of therapy. One patient was readmitted with complaint of abdominal tenderness 2 weeks after discharge from the hospital. She was discharged without antibiotics after 24 hours and did not have further complaint. Finally, with regard to antibiotic and hospital room costs, the average cost of patients from groups II and III was $233.35 and $412.45 higher, respectively, compared with the average cost of patients from group I.

Comment

Postpartum endomyometritis continues to be a major cause of infectious morbidity in the obstetric patient. Careful timing of amniotomy, limited vaginal examinations, and prophylactic antibiotics for cesarean section delivery may help to reduce the incidence and severity of postpartum endomyometritis. A number of antibiotic regimens have been studied with varying degrees of success, but few clinical trials have addressed the proper duration of therapy.

Three recent articles in the literature have advocated the use of shorter courses of antibiotics for postpartum endomyometritis. In 1986, Duff[15] recommended cessation of antibiotics after 24 to 48 hours of afebrility, although there was little in the literature to support such a recommendation. In 1987, Soper et al.,[13] with historic controls, demonstrated a favorable response to abbreviated therapy with clindamycin-gentamicin in a dependent military population. They agreed that the application of such therapy to an indigent population would need further investigation, and the limitations of such a control group are obvious. Cabbad et al.[14] were successful in the treatment of a group of indigent patients with short courses of imipenem. The study is limited by not only the size of the study group (25), but also by the lack of a control group.

The clindamycin-aminoglycoside regimen was chosen for the following reasons. It is the antibiotic regimen that has been tested most extensively, with cure

Table IV. Comparative characteristics of endomyometritis of study groups

	Group			
	I	*II*	*III*	*p*
Time from delivery to diagnosis (days)*	1.7 ± 0.9	1.5 ± 0.7	1.5 ± 0.7	NS
Maximum white blood cell count*	14.4 ± 1.7	14.6 ± 1.4	14.9 ± 1.5	NS
Maximum temperature, (° F)*	101.6 ± 1.1	101.7 ± 1.0	101.9 ± 0.9	NS
Administration of antibiotics (days)*	2.1 = 1.1*	2.6 ± 0.7	2.9 ± 1.2†	$p < 0.05$
Hospital days postpartum*	5.0 = 1.6	5.6 ± 1.7	5.8 ± 1.8	NS
Success, *n* (%)	34 (94)	34 (94)	34 (92)	NS
Readmission	0	1	0	NS

*Values are mean ± SD.

†Difference between groups I and III by Duncan's multiple range test.

rates quoted as high as 90% to 95%. Of the patients who received this antibiotic regimen, <0.5% had serious complications of endomyometritis.[15] A commonly quoted disadvantage of aminoglycoside therapy is the need to monitor serum peak and trough levels. In our study, 17 of 36 patients in group I completed therapy by 1.7 days of intravenous administration of antibiotics and thus alleviated the need to monitor serum levels. The rapid elimination of aminoglycosides by the obstetric patient makes toxicity an infrequent complication.[16] Furthermore, Duff and associates,[17] and Blanco et al.[18] in separate studies reported successful therapy with aminoglycosides in significant percentages of patients despite "subtherapeutic" serum levels.

One potential weakness in our study should be noted. No endometrial cultures were obtained before therapy to document infection with resistant or high-virulence organisms. Although these cultures may be helpful to test various antibiotic regimens in vitro, their reliability and practicality in the clinical management of patients with postpartum endomyometritis has been disputed.[19]

In this first prospective randomized study that uses short courses of clindamycin-tobramycin for postpartum endomyometritis, data suggest that the abbreviated regimen may be applicable to an indigent population. Patients in group I had shorter hospitalizations and fewer days of intravenous administration of antibiotics than did those in groups II and III. Of more importance, no increase in morbidity was noted in those who received the abbreviated regimen. Data from this study strongly suggest that a short course of antibiotic therapy is both efficacious and safe, and would result in substantial monetary savings to a busy obstetric service.

REFERENCES

1. Swartz WH, Grolle K. The use of prophylactic antibiotics in cesarean section. J Reprod Med 1981;26:595-9.
2. Gibbs RS, Listwa HM. The effect of internal fetal monitoring on maternal infection following cesarean section. Obstet Gynecol 1976;48:653-8.
3. Gibbs RS. Clinical risk factors for puerperal infection. Obstet Gynecol 1980;55(suppl):178s-84s.
4. Gibbs RS, Blanco JD, Casteneda YS, St. Clair PJ. A double-blind, randomized comparison of clindamycin-gentamicin versus cefamandole for treatment of post-cesarean section endomyometritis. Am J Obstet Gynecol 1982;144:261-7.
5. Gibbs RS, Blanco JD, Duff P, Casteneda YS, St. Clair PJ. A double-blind randomized comparison of moxalactam versus clindamycin-gentamicin in treatment of endomyometritis after cesarean section deilvery. Am J Obstet Gynecol 1983;146:769-72.
6. DiZerega G, Yonekura L, Roy S, Nakamura RM, Ledger WJ. A comparison of clindamycin-gentamicin and penicillin-gentamicin in the treatment of post-cesarean endomyometritis. Am J Obstet Gynecol 1979;134:238-42.
7. Sorrell TC, Marshall JR, Yoshimori R, Chow AW. Antimicrobial therapy of postpartum endomyometritis. Am J Obstet Gynecol 1981;141:246-51.
8. Duff P, Keiser JF, Strong SL. A comparative study of two antibiotic regimens for the treatment of operative site infections. Am J Obstet Gynecol 1982;142:996-1003.
9. Gilstrap LC, Maier RC, Gibbs RS, Connor KD, St. Clair PJ. Piperacillin versus clindamycin plus gentamicin for pelvic infections. Obstet Gynecol 1984;64:762-6.
10. Gibbs RS, Jones PM, Wilder PJ. Antibiotic therapy in endometritis following cesarean section. Obstet Gynecol 1978;52:31-7.
11. Hemsell DL, Cunningham FG, De Palma RT, Nobles BJ, Heard M, Hemsell PG. Cefotaxime sodium therapy for endomyometritis following cesarean section: dose-finding and comparative studies. Obstet Gynecol 1983;62:489-97.
12. Donowitz LG, Wenzel RP. Endometritis following cesarean section. Am J Obstet Gynecol 1980;137:467-9.
13. Soper DE, Kemmer CT, Conover WB. Abbreviated antibiotic therapy for the treatment of postpartum endometritis. Obstet Gynecol 1987;69:127-30.
14. Cabbad M, Sijin O, Minkoff M. Short course of antibiotics for post-cesarean section endometritis. Am J Obstet Gynecol 1987;157:908-9.
15. Duff P. Pathophysiology and management of post-cesarean endomyometritis. Obstet Gynecol 1986;67:269-76.
16. Zaske DE, Cipolle RJ, Strate RG, Malo JW, Koszalka MF.

Rapid gentamicin elimination in obstetrics patients. Obstet Gynecol 1980;56:559-64.
17. Duff P, Jorgensen JM, Gibbs RS, Blanco JD, Alexander G, Casteneda YS. Serum gentamicin levels in patients with post-cesarean endomyometritis. Obstet Gynecol 1983;61: 723-7.
18. Blanco JD, Gibbs RS, Duff P, Casteneda YS. Serum tobramycin levels in puerperal women. AM J OBSTET GYNECOL 1983;147:466-8.
19. Ledger WJ. Management of postpartum cesarean section morbidity. Clinical Obstet Gynecol 1980;23:621-31.

Editors' note: This manuscript was revised after the discussions were presented.

Discussion

DR. ROBERT BOWDEN, Lynchburg, Virginia. I would like to know the timing of the determination of your leukocyte count inasmuch as you have exertional leukocytosis that may exist for 24 to 48 hours after delivery. The second point is why did you choose the aminoglycoside rather than one of the more straightforward antibiotics in that early period?

DR. GUY BENRUBI, Jacksonville, Florida. I want to thank Dr. Morales for something that sorely needs to be done. Was there any difference between the groups who had endomyometritis after cesarean section and those who had it after normal vaginal delivery, and were they identical in the three groups they were randomized to in terms of total numbers? The other question is, did you determine whether the Augmentin was, in fact, taken by your patients? Augmentin is a very emetogenic type of medication and I wonder how many of those patients did, in fact, take it? Finally, did your patients who underwent cesarean section have their placentas cultured at the time of cesarean section for any particular reason? Do you have any information of what organisms were on the placenta when you were doing the study?

DR. WILLIAM MIXSON, Gables, Florida. I have not done obstetrics in many years, but it seems to me that an old basic concept was neglected here. A lot of times these patients just need drainage. An intrauterine culture was not done and nothing was done to try to improve drainage in these patients.

DR. MORALES (Closing). Leukocytosis is a very minor criterion, and we did not require leukocytosis to be part of the diagnosis. This was obtained at a time of the diagnosis of endometritis. Why the aminoglycoside? We wanted to use what I consider to be the "gold standard" of therapy for endometritis. We now are conducting studies with single agents, but first I wanted to answer the question of duration; thus the use of the most accepted antibiotic regimen—aminoglocoside and clindamycin. In terms of the difference between the patient who had endometritis after vaginal delivery and cesarean section, they were essentially not different on the basis of the various parameters studied. In terms of whether the patients indeed took the Augmentin, I cannot assure you that they did. This is basically an indigent population. However, no patient enrolled in the study groups required readmission or evaluation in the emergency room because of sequelae of postpartum endomyometritis.

At the time of diagnosis of endometritis, we try to get an endometrial scraping, but of course this was not done 100% of the time. I do have the information on the microbiology. We did culture a number of placentas. In terms of the drainage of the uterus with diagnosis of endometritis, I do not have sufficient experience with endometrial cavity drainage to answer this question.

Subsequent fertility in women who undergo cardiac surgery

Wallace C. Nunley, Jr., MD,[a] Lisa A. Kolp, MD,[a] Laura N. Dabinett, MD,[a] James D. Kitchin III, MD,[a] Bruce G. Bateman, MD,[a] and George B. Craddock, Jr., MD[b]
Charlottesville, Virginia

A retrospective review was undertaken on all pediatric and reproductive-aged females who underwent cardiac surgery and required cardiopulmonary bypass from 1958 through 1986. The purpose of this study was to define the fertility of these patients after surgery and to compare their reproductive performance with that in the general population. Analysis was complete for 208 patients. These patients make up the following cardiac surgical categories: septal defect repairs, 92; commissurotomies, 60; valve replacements, 46; tetralogy of Fallot repairs, 6; and coronary artery bypass procedures, 4. Of 208 patients, 68 (32.7%) attempted pregnancy after surgery. Infertility was defined in five women as follows: endometriosis, 3; ovulatory dysfunction, 1; unknown, 1. A total of 64 patients conceived 121 pregnancies with the following outcomes: live births, 98; spontaneous abortion, 9; ectopic pregnancy, 2; therapeutic abortion, 12. Our results suggest that infertility is not an apparent disorder after cardiac surgery and that subsequent pregnancy outcomes are similar to those in the general population. (AM J OBSTET GYNECOL 1989; 161:573-6.)

Key words: Reproductive performance, cardiac surgery, infertility

Women with congenital and acquired heart disease are able to live healthier lives and to have improved reproductive outcomes because of advancements in cardiovascular surgical technique, refinements in prosthetic valve design, and improvements in medical management of cardiac disease throughout pregnancy. Several published reports detail pregnancy management and outcome after cardiac valve replacement surgery[1-5] and other types of cardiac surgery.[6-8] Additional articles, including a review by Bernal and Miralles,[9] contain information on the outcomes of gestations in patients who required cardiac surgery during pregnancy.[10-15]

Information is lacking with regard to infertility as a possible disorder in women who have had cardiac surgery that required extracorporeal circulation. The purpose of this study was to tabulate the reproductive performance of women after cardiac surgery, including those who attempt pregnancy unsuccessfully. If infertility (defined as the couple's perception of difficulty with conception) was identified, the specifics of the problem were defined as completely as possible.

From the Division of Reproductive Endocrinology, Department of Obstetrics and Gynecology,[a] and Division of Cardiology, Department of Internal Medicine,[b] University of Virginia Health Sciences Center.

Presented at the Fifty-first Annual Meeting of the South Atlantic Association of Obstetricians and Gynecologists, Hot Springs, Virginia, January 29–February 1, 1989.

Reprint requests: Wallace C. Nunley, Jr., MD, Division of Reproductive Endocrinology, Box 387, Department of Obstetrics and Gynecology, University of Virginia Health Sciences Center, Charlottesville, VA 22908.

6/6/12988

Material and methods

All cardiovascular surgical procedures performed at the University of Virginia Health Sciences Center from 1958 to 1986 were reviewed. A total of 470 pediatric-adolescent girls and reproductive-aged women <40 years of age who underwent cardiac surgery that required extracorporeal circulation were identified. The surgical procedures selected were atrial and ventricular septal defect repairs, valvular commissurotomies, valvular replacements, coronary artery bypass, and tetralogy of Fallot repairs.

To be included in the analysis of this study, the patient must have been 16 years of age by 1988 and must have had a minimum 2-year follow-up if surgery occurred during her reproductive years. Consequently, 95 patients were eliminated secondary to the age restriction and 23 were deleted from analysis because of lack of follow-up. Questionnaires were mailed to 181 patients from whom additional information was required. A total of 37 completed and returned the questionnaires. Therefore, the sample population totaled 208.

Information extracted from hospital records and the questionnaire included the type of surgical procedure, the year of surgery, and reproductive performance before and after surgery. The hospital record information was deemed adequate in 171 patients, and no questionnaire was mailed. Reproductive performance included information with regard to pregnancy attempts before and after surgery and specific outcomes of all known pregnancies. If difficulty with conception after surgery was acknowledged, the specifics of this infertility diagnosis and management were solicited.

Table I. Age distribution of 208 women at time of cardiac surgery

Age (yr)	*No. of patients*
0-9	41
10-14	17
15-19	27
20-24	27
25-29	28
30-34	33
35-39	35

Results

From the original study population of 470 patients, information was judged to be adequate for analysis in 208 patients. The age distribution of these individuals at the time of their cardiac surgery is listed in Table I. Table II details the different cardiac surgeries performed. Approximately 75% of these patients underwent operative procedures to repair septal defects or valves. The mean age of the patients who underwent septal defect repairs and commissurotomies was approximately 10 years less than the mean age of those who underwent valve replacements.

After cardiac surgery, 140 (67.3%) patients never attempted pregnancy; 16 underwent sterilization; and 68 (32.7%) attempted pregnancy. Five (7.3%) patients acknowledged infertility and listed the following causes: endometriosis, 3; ovulatory dysfunction, 1; unknown, 1. One of the three patients with endometriosis had three live births after management of her reproductive disease. Table III lists the outcomes of all known pregnancies after cardiac surgery as well as conceptions per cardiac procedure. There were no maternal deaths.

More conceptions occurred in patients after septal defect repairs than in those who underwent valve replacements. The mean age of the former group was 18.0 years, compared with 30.2 years in the latter group. Table IV details the relationship of conceptions to the cardiac procedure.

Comment

The incidence of cardiac disease in pregnancy is 1% to 4%.[15] Because of improved medical management of the heart disease, many patients are able to be delivered of healthy infants without significant maternal morbidity. However, if maternal condition deteriorates significantly, cardiac surgery that requires extracorporeal circulation can be performed during pregnancy without an increase in mortality different from that in nonpregnant women.[9] Alterations that involve the uteroplacental-fetal unit during this surgical procedure may include decrease in uterine blood flow and fetal bradycardia with or without the onset of uterine contractions.[16]

However, the purpose of this article is not to detail the management of pregnancy in women after cardiac surgery that required extracorporeal circulation. Rather, our intent is to provide information with regard to the reproductive performance of women who have previously undergone various types of cardiac surgical procedures with an emphasis on the incidence of infertility. Certainly with the physiologic changes, such as transient decrease in blood flow and hypothermia, succinctly described by Lake,[17] one might theorize a potential adverse effect on the neuroendocrine axis that could alter ovulatory status. To attempt to define any effect on ovulation would require serial investigations at different intervals after surgery, a task not done in this study. Only one patient with infertility described the need for ovulatory agents.

Because of the nature of retrospective studies, we concede that it may be difficult to ascertain the absolute incidence of infertility in the population examined. The data suggest that infertility, with an incidence of 7.3%, is not an apparent disorder in women who undergo cardiac surgery that requires cardiopulmonary bypass. Mortensen and Ellsworth[18] found in a series of 168 patients in the childbearing age that 11% were "involuntarily sterile" before their cardiovascular surgery, whereas only 3.6% were "involuntarily sterile" postoperatively.

Maternal age may be a factor that could influence the reproductive performance of patients included in this study. Several patients who previously underwent septal defect and tetralogy of Fallot repairs are still in their reproductive years. Conception may not be as important an issue to older patients who had undergone valve replacement surgeries or coronary artery bypass grafting. Furthermore, the type of cardiac surgery may influence conception and embryo development because anticoagulation therapy would be rec-

Table II. List of cardiac operations

Procedure	*No. of patients (%)*	*Mean age (yr)*	*Age range (yr)*
Septal defect repairs	92 (44.2)	18.0	2-37
Atrial	73		
Ventricular	17		
Atrial and ventricular	2		
Commissurotomies	60 (28.8)	22.4	0.16-39
Mitral	37		
Pulmonary	14		
Aortic	9		
Valve replacements	46 (22.2)	30.2	16-38
Mitral	27		
Aortic	14		
Mitral, aortic	5		
Tetralogy of Fallot	6 (2.9)	11.2	5-30
Coronary artery bypass grafting	4 (1.9)	37.3	36-38
TOTAL	208 (100)		

Table III. Fertility of 208 patients after undergoing cardiac surgery

Procedure	No. patients (%)		Infertility (%)	Reproductive outcome			
	Never attempted pregnancy	Attempted pregnancy		Live births	Spontaneous abortion	Ectopic	Therapeutic abortion
Septal defect repairs	55 (59.8)	37 (40.2)	3 (8.1) 2 Endometriosis 1 Unknown	55	4	2	6
Commissurotomies	38 (63.3)	22 (36.7)	2 (9.1) 1 Endometriosis 1 Ovulation	36	4	0	3
Valve replacements	40 (86.9)	6 (13.1)	0	2	1	0	3
Tetralogy of Fallot	3 (50)	3 (50)	0	5	0	0	0
Coronary artery bypass grafting	4 (100)	0	0	0	0	0	0
TOTAL	140 (67.3)	68 (32.7)	5 (7.3)	98 (89.9%)	99 (8.3%)	2	12

ommended in those patients after valve replacements. The risks of such treatment to both mother and fetus have been reviewed by Ueland.[8] More recently Deviri and colleagues[5] reported more favorable pregnancy outcomes for those patients who had a valve replacement with porcine xenograft prosthesis (instead of a mechanical device) and were not treated with coumarin derivatives.

The significance of the cardiac disease on decisions with regard to fertility is difficult to define in this study. Certainly many patients had improved quality of life after surgery but possibly not to the point of being comfortable with an attempt at pregnancy. Improved quality of life after heart transplantation has resulted in a vaginal delivery of a healthy female infant as reported by Lowenstein and colleagues.[19] Physician advice to the patient with regard to attitude toward conception was not always apparent from chart review or from the questionnaire.

The pregnancy outcomes detailed in Table III indicate that approximately 90% of pregnancies resulted in live births. Our data suggest that infertility is not an apparent disorder in women after cardiac surgery that required extracorporeal circulation. Furthermore, there is not an increase in pregnancy wastage postoperatively in these patients compared with the general population.

We express gratitude to Dr. Stanton Noland, head of cardiovascular surgery at the University of Virginia Health Sciences Center, for permission to review the cardiovascular operative records. Appreciation is given to Ms. Brenda Kennedy for her assistance with the questionnaire.

Table IV. Relationship of conceptions to cardiac operations

Procedure	No. of patients who conceived		
	Before surgery	After surgery	Before and after surgery
Septal defect repairs	19	31	3
Commissurotomies	23	16	5
Valve replacements	27	5	1
Tetralogy of Fallot	0	3	0
Coronary artery bypass grafting	4	0	0
TOTAL	73 (53.3%)	55 (40.1%)	9 (6.6%)

REFERENCES

1. Ueland K, Tatum HJ, Metcalfe J. Pregnancy and prosthetic heart valves: report of successful pregnancies in 2 patients with Starr-Edwards aortic valves. Obstet Gynecol 1966;27:257-60.
2. Johnson AS, Meyers MP, Eckhous AS, Bacher B, Limia A. Successful pregnancies in patients with prosthetic mitral valves. Mich Med 1966;65:718-9.
3. Lutz DJ, Noller KL, Spittell JA, Danielson GK, Fish CR. Pregnancy and its complications following cardiac valve prostheses. Am J Obstet Gynecol 1978;131:460-8.
4. Denbow CE, Matadial L, Sivapragasam S, Spencer H. Pregnancy after open-heart surgery. West Indian Med J 1982;31:191-3.
5. Deviri E, Levinsky L, Yechezkel M, Levy MJ. Pregnancy after valve replacement with porcine xenograft prosthesis. Surg Gynecol Obstet 1985;160:437-43.
6. Whittemore R, Hobbins JC, Engle MA. Pregnancy and its outcome in women with and without surgical treatment of congenital heart disease. Am J Cardiol 1982;50:641-51.
7. Tanaka T, Miyakoshi Y. Pregnancy and delivery following cardiac operations. Nippon Junkankigaku Shi 1986;50:918-22.
8. Ueland K. Cardiac surgery and pregnancy. Am J Obstet Gynecol 1965;92:148-62.
9. Bernal JM, Miralles PJ. Cardiac surgery with cardiopulmonary bypass during pregnancy. Obstet Gynecol Surv 1986;41:1-6.
10. Knapp RC, Arditi LI. Closed mitral valvulotomy in pregnancy. Clin Obstet Gynecol 1968;11:978-91.
11. Meffert WG, Stansel HC. Open heart surgery during pregnancy. Am J Obstet Gynecol 1968;102:1116-20.
12. Zitnik RS, Brandenburg RO, Sheldon R, Wallace RB. Pregnancy and open-heart surgery, Circulation 1969;39:257-62.

13. Salomon J, Yortner R, Levy MJ. Open heart surgery during pregnancy: case report. Vasc Surg 1975;9:257-60.
14. Bahary CM, Ninio A, Gorodesky IG, Neri A. Tococardiography in pregnancy during extracorporeal bypass for mitral valve replacement. Isr J Med Sci 1980;16:395-7.
15. Eilen B, Kaier IH, Becker RM, Cohen MN. Aortic valve replacement in the third trimester of pregnancy: case report and review of the literature. Obstet Gynecol 1981;57:119-21.
16. Farmakides G, Schulman H, Mohtashemi M, Ducey J, Fuss R, Mantell P. Uterine-umbilical velocimetry in open heart surgery. Am J Obstet Gynecol 1987;156:1221-2.
17. Lake CL. Cardiopulmonary bypass: anatomy, physiology, and pharmacology. In: Lake CL, ed. Cardiovascular anesthesia. New York: Springer-Verlag Inc., 1985;311.
18. Mortensen JD, Ellsworth HS. Pregnancy and cardiac surgery. Circulation 1963;28:773.
19. Lowenstein BR, Vain NW, Perrone SV, Wright DR, Boullon FJ, Favaloro RG. Successful pregnancy and vaginal delivery after heart transplant. Am J Obstet Gynecol 1988;158:589-90.

Fertility after ectopic pregnancy

Dorothy E. Mitchell, MD, Helen F. McSwain, MD, and Herbert B. Peterson, MD
Atlanta, Georgia

In the period July 1983 to March 1985, 264 women had surgery for ectopic pregnancy at Grady Memorial Hospital; 76 had postoperative hysterosalpingograms. Of these, 55 (76.4%) women were followed up for 3 to 41 months (mean, 23.8) to determine subsequent fertility. During the follow-up period, 30 pregnancies occurred among the 55 patients; 24 were intrauterine and 6 were repeat ectopic pregnancies. In the surgical group of 39 patients with salpingectomy, 60.8% of those desiring pregnancy achieved an intrauterine pregnancy. Of the 12 patients with salpingostomy, the three who desired pregnancy achieved it (100%). In the tubal abortion group, the two women desiring pregnancy conceived (100%). There were six repeat ectopic pregnancies (10.9%). Of the ectopic pregnancies, one occurred in the salpingectomy group (2.6%), four in the salpingostomy group (33.3%), and one in the tubal abortion group (25%). Five of the six ectopic gestations were found in the contralateral fallopian tube. Hysterosalpingographic evidence of contralateral tubal patency was a good prognostic indicator for subsequent intrauterine pregnancy. By contrast, one half of study patients with findings suggesting tubal occlusion still achieved an intrauterine pregnancy. (Am J Obstet Gynecol 1989;161:576-80.)

Key words: Ectopic pregnancy, postoperative hysterosalpingograms, subsequent fertility

The incidence of ectopic pregnancy has increased dramatically over the past decade, making this condition an increasingly important individual and public health concern. At Grady Memorial Hospital, the ratio of ectopic pregnancies to live births in 1986 was 1:34. To better characterize factors associated with reproductive outcome after ectopic pregnancy in our patient population, we conducted a prospective study of women with surgical management of ectopic pregnancy during the period 1983 to 1985. Previously we reported on the correlation between clinical characteristics of these women and postoperative hysterosalpingography.[1] In this article we detail the reproductive experience of study participants through September 1986. We correlate clinical circumstances, including initial surgical procedure and postoperative hysterosalpingogram, with subsequent fertility.

From the Department of Gynecology and Obstetrics, Emory University School of Medicine.
Presented by invitation at the Fifty-first Annual Meeting of the South Atlantic Association of Obstetricians and Gynecologists, Hot Springs, Virginia, January 29–February 1, 1989.
Reprint requests: Dorothy E. Mitchell, MD, Department of Obstetrics and Gynecology, Emory University School of Medicine, Thomas K. Glenn Memorial Building, 69 Butler St., S.E., Atlanta, GA 30303.
6/6/13345

Material and methods

This study was conducted over two time periods. The first involved the enrollment of all women who underwent tubal operation for an ectopic pregnancy at Grady Memorial Hospital between July 1, 1983, and March 31, 1985. The methods for that portion of this study were described in detail elsewhere.[1] In brief, during the study period 264 women underwent operation for ectopic pregnancy. All women with ectopic pregnancy

Table I. Percentage and length of follow-up of women with ectopic pregnancy by surgical management

	Surgical management		
Follow-up	*Salpingectomy (includes partial salpingectomy)*	*Salpingostomy*	*Tubal abortion*
Total No. of patients	52	13	5
Patients with follow-up	39 (72%)	12 (92%)	4 (80%)
Follow-up time (mo)	25 ± 11.2	21.3 ± 9.2	19.3 ± 11.2

were considered for conservative surgical management. Salpingectomy was performed only if more conservative options were not clinically feasible. For isthmic pregnancies, partial salpingectomy was performed. For ampullary pregnancies, linear salpingostomy was attempted when feasible. Although surgical procedures were performed by various surgeons, all used microsurgical techniques without magnification. Seventy-six (29%) of the 264 women underwent postoperative hysterosalpingography with water-based medium as closely as possible to the sixth postoperative week.

In the second period of the study, the subsequent fertility of the 264 women with ectopic pregnancy was evaluated. After medical record review, all study participants who could be located were interviewed by telephone; no women refused interviews. Follow-up interviews continued until September 1986. Information regarding desire for pregnancy and occurrence of pregnancy was obtained during the interview. When pregnancy was reported, detailed information was obtained when available. In all cases when ectopic pregnancy was reported, the occurrence was verified by review of the medical record. The length of follow-up was established for each individual as the time from the initial surgery for ectopic pregnancy to the time of the telephone interview or the date of the last pertinent entry into the medical record. For ascertaining the interval from the previous ectopic pregnancy until a subsequent conception, a calculation was performed using the date of the original surgery for ectopic pregnancy and a presumed conception based on a 40-week term gestation, a 12-week spontaneous or therapeutic abortion, and a 6-week repeat ectopic pregnancy.

Results

Two hundred and sixty-four patients had surgery for ectopic pregnancy during the study period July 1983 to March 1985. Seventy-six patients in the surgical groups evaluated had postoperative hysterosalpingograms. Fifty-two had salpingectomy (total and partial), 13 had salpingostomy, and 5 had mechanical tubal abortion. Six women had other procedures. Fifty-five (76.4%) of these patients had follow-up longer than 3 months. The follow-up time ranged from 3 to 41 months (mean, 23.8 months). Follow-up times for the three operative groups were not significantly different: 25.0, 21.3, and 19.3 months, respectively (Table I). The mean age of the patients was 23.4 years (25.2 years for women undergoing salpingectomy, 21.8 years for women undergoing salpingostomy, and 24.3 years for the tubal abortion group). The salpingostomy patients were significantly younger than the salpingectomy patients ($p = 0.027$).

The mean parity of the study patients was 1.09. At the time of their initial ectopic pregnancy, 60% of the patients were parous as follows: 25 (64.1%) of the salpingectomy group, 6 (50%) of the salpingostomy group, and 4 (100%) of the mechanical tubal abortion group.

A total of 209 women were lost to follow-up. To assess characteristics of these women in relation to our study population, we randomly sampled 80 of those who were lost to follow-up. The mean age of those women was 25.7 years, which is comparable with a mean age of 23.4 years for those women who have been followed up. The distribution of procedures for management of ectopic pregnancy was comparable between those women followed up and those lost to follow-up. Specifically, in the former group 31% underwent salpingostomy and 52% had salpingectomy. In the latter group, 35% had salpingostomy and 59% had salpingectomy.

During the follow-up period, 30 pregnancies occurred among the 55 patients. Twenty-four were intrauterine pregnancies and six were repeat ectopic gestations. Twenty-nine of the patients (52.7%) desired pregnancy. Of these, 20 patients (69%) achieved pregnancy (19 pregnancies were intrauterine and 18 were delivered at term). Nine of the 29 women desiring pregnancy (31.1%) have remained infertile. Ten patients who did not desire pregnancy became pregnant; five of the resultant ten pregnancies were intrauterine and five were ectopic (Table II).

Table II. Fertility status after previous ectopic pregnancy by desire for pregnancy

Desire for pregnancy	*Fertility status*	
	Pregnancy achieved	*No pregnancy achieved*
Pregnancy planned (29 patients)	20 (69%)	9 (31%)
Pregnancy unplanned (26 patients)	10 (39%) (5 IUP, 5 ectopic)	16 (62%)

IUP, Intrauterine pregnancy.

Table III. Subsequent fertility when pregnancy was desired after previous ectopic pregnancy by surgical management

Fertility status	*Surgical management*		
	Salpingectomy (n = 39)	*Salpingostomy (n = 12)*	*Tubal abortion (n = 4)*
Total pregnancies	15	3	2
Intrauterine pregnancies	14	3	2
Mean time to conception (mo)	12.2 ± 7.4	8.3 ± 3.0	10.7 ± 4.5

Table IV. Surgical findings among women with repeat ectopic pregnancy by previous surgical management and prior hysterosalpingographic findings

Previous surgical management	*Hysterosalpingographic findings*	*Findings at surgery for ectopic gestation*
Left salpingectomy	Contralateral patency	Right[2] tubal pregnancy
Left salpingostomy	Bilateral patency	Right tubal pregnancy
Left salpingostomy	Contralateral patency	Right tubal pregnancy
Right salpingostomy	Contralateral patency but located	Left tubal pregnancy
Left salpingostomy	Contralateral patency	Left tubal pregnancy
Left tubal abortion	Ipsilateral patency	Right tubal pregnancy

To evaluate pregnancy potential, we performed a subgroup analysis on that group of women desiring pregnancy (excluding those not desiring pregnancy, most of whom intermittently used barrier contraception). Among the 39 patients initially undergoing salpingectomy, 14 (60.8%) of the 23 women desiring pregnancy achieved an intrauterine pregnancy. Of the 12 patients who had salpingostomy, three desired and achieved pregnancy (100%). Hysterosalpingographic findings indicated at least one patent tube in 28 of 39 (71.8%) women in the salpingectomy group, 11 of 12 (91.7%) in the salpingostomy group, and of the women in the tubal abortion group, the 2 women desiring pregnancy conceived (100%) (Table III).

Six of the 55 women who were followed up experienced a repeat ectopic pregnancy. One such pregnancy occurred in a woman who had salpingectomy and who desired pregnancy. The other five ectopic gestations occurred among women not desiring pregnancy. It is noteworthy that five of the six ectopic pregnancies were found in the contralateral fallopian tube (Table IV).

Patients who had an intrauterine pregnancy without regard to desire for pregnancy (24) were compared with those unable to achieve a desired pregnancy (9), and those with repeat ectopic pregnancy (6) to determine whether any important differences could be discerned. The mean patient ages were not significantly different (24 ± 3.7, 23.6 ± 5.6, and 23 ± 2.1 years, respectively, for the three groups).

In each group similar numbers of women were parous: 16 (66%) of those with intrauterine pregnancies, 6 (66%) of those not achieving pregnancy, and 5 (83%) of those with repeat ectopic pregnancy. Follow-up times for the groups were 25.6 months for the intrauterine pregnancy group, 28.4 months for the infertility group, and 22.5 months in the repeat ectopic pregnancy group. The repeat ectopic pregnancy patients had significantly shorter follow-up time ($p = 0.31$).

Hysterosalpingographic patency was different for the pregnancy outcome groups. Patients with an intrauterine pregnancy had 83.4% patency. Those who did not become pregnant but desired pregnancy had 55% patency, and patients with repeat ectopic pregnancies had 100% patency. Intrauterine contraceptive device

Table V. Fertility status of women with prior ectopic pregnancies of history of intrauterine contraceptive device use and history of pelvic inflammatory disease

History	*Fertility status*		
	Intrauterine pregnancy ((n = 24)	*Not pregnant but desiring pregnancy (n = 9)*	*Repeat ectopic pregnancy (n = 6)*
IUD use	3 (13%)	1 (11%)	—
PID	8 (33%)	2 (22%)	2 (33%)
Both	5 (21%)	2 (22%)	—
Neither	8 (33%)	3 (33%)	4 (67%)
Unknown	1 (.5%)	1 (11%)	—

IUD, Intrauterine contraceptive device; *PID*, pelvic inflammatory disease.

Table VI. Fertility status of women with prior ectopic pregnancies by previous surgical management

Previous surgical management	*Fertility status*		
	Intrauterine pregnancy (n = 32)	*Not pregnant but desiring pregnancy (n = 9)*	*Repeat ectopic pregnancy (n = 6)*
Salpingectomy	18 (75%)	9 (100%)	1 (17%)
Salpingostomy	4 (17%)	0	4 (66%)
Tubal abortion	2 (8%)	0	1 (17%)

Table VII. Fertility status after prior ectopic pregnancy by hysterosalpingographic findings

	Intrauterine pregnancy achieved whether or not planned	*No pregnancy achieved when pregnancy desired*
Hysterosalpingogram patent	20 (83%)	5 (55%)
Hysterosalpingogram nonpatent	4 (17%)	4 (45%)
Total	24 (100%)	9 (100%)

use and pelvic inflammatory disease history was not significantly different for the three groups (Table V). However, there was a difference noted by type of surgical procedure performed (Table VI). All of the women not pregnant but desiring pregnancy during the follow-up period had salpingectomy. Those women experiencing ectopic pregnancy were most likely to have had salpingostomies.

We evaluated the usefulness of hysterosalpingography in predicting fertility status in the 33 patients who achieved an intrauterine pregnancy or were desirous of a pregnancy (Table VII). Of the 25 women with a patent hysterosalpingogram, 20 (80%) achieved an intrauterine pregnancy. Of the eight patients with nonpatent hysterosalpingographies, four (50%) become pregnant. Although patency is a good prognostic indicator of ability to conceive, nonpatency is not necessarily a predictor of poor outcome. Of the women not pregnant but desiring pregnancy in the following period, five (55.5%) had patency.

Comment

Our findings suggest that most women desiring pregnancy after previous ectopic gestations can achieve an intrauterine pregnancy within a short time interval regardless of initial surgical management.

A substantial percentage (38.6%) of women who did not plan pregnancy nevertheless became pregnant. Of those women, a relatively higher percentage (50%) had ectopic gestations. A total of six (10.9%) repeat ectopic pregnancies were experienced by our 55 study patients. Other reports have identified comparable rates of 6% to 27%.[2-7]

Women desiring but unable to achieve pregnancy were more likely to have undergone salpingectomy, but those who were experiencing repeat ectopic pregnancy were more likely to have undergone salpingostomy. These findings suggest that salpingostomy is associated with an increased risk of subsequent ectopic pregnancy. Three of 12 women in our series who had salpingostomy desired and achieved an intrauterine pregnancy.

However, four of the nine women not desiring pregnancy experineced ectopic gestations. Only one of these had bilateral patency on postoperative hysterosalpingography. Three had contralateral patency; two of these patients and one with bilateral patency had their ectopic gestations in the contralateral tube. Whether linear salpingostomy on one tube might increase the risk of ectopic pregnancy in the contralateral tube must therefore be questioned. All in our series who underwent salpingostomy had unruptured ampullary pregnancy. By contrast, women with salpingectomy had isthmic or ruptured ampullary pregnancies. Although we are not aware of any plausible mechanism to provide a unifying hypothesis, it is possible that some determinant of the indications for salpingostomy is associated with an increased risk of ectopic pregnancy in the contralateral tube. Alternatively, salpingostomy itself might somehow result in increased risk in the contralateral tube. Possible mechanisms include operative site hemorrhage or postoperative adhesions.

One of the objectives of our study was to determine the usefulness of postoperative hysterosalpingography in predicting subsequent fertility potential. Contralateral tubal patency demonstrated by hysterosalpingography appears to be a good prognostic indicator for achieving intrauterine pregnancy. Approximately 80% of women with tubal patency on hysterosalpingography achieved an intrauterine pregnancy; in a report by DeCherney et al.,[2] 50% of patients did achieve intrauterine pregnancy. By contrast, in our study one half of those with findings of nonpatency still achieved an intrauterine pregnancy. Whether this difference is a result of tubal spasm, technique, or other factors requires additional investigation.

Our findings may not be generalizable to all women experiencing ectopic gestations. The 264 women initially enrolled in our follow-up investigation were primarily indigent, urban residents, many of whom were transient residents and most of whom were black. However, we do have some confidence that our findings are generalizable to the Grady Hospital population. Although only 76 women returned for postoperative hysterosalpingographies as advised, motivation, (as opposed to other selection factors), appears to be reasonable. We have no indication of important selection biases. Furthermore, we were able to follow up 75% of these women by telephone. Those we did not interview could not be located; no woman refused the interview. The lack of apparent bias in selection of those followed up gives us further confidence in generalizing our findings to the Grady Hospital population. Further studies elsewhere should determine whether findings in our population are generalizable to other racial and socioeconomic groups.

REFERENCES

1. Mitchell DE, McSwain HF, McCarthy JA. Hysterosalpingographic evaluation of tubal patency after ectopic pregnancy. Am J Obstet Gynecol 1987;157:618-220.
2. DeCherney AH, Slidker JS, Mezer HC, Tarlatzis BC. Reproductive outcome following two ectopic pregnancies. Fertil Steril 1985;43:82-5.
3. Dadak CH, Feiks A, Deutinger J, Reinthaller A, Janisch H. Fertility after conservative surgery. Gerburtshilfe Frauenheilkd 1985;45:559-62.
4. DeCherney AH, Kase N. The conservative surgical management of unruptured ectopic pregnancy. Obstet Gynecol 1979;54:451.
5. Timonen S, Nieminen U. Tubal pregnancy, choice of operative method of treatment. Acta Obstet Gynecol Scand 1967;46:327.
6. DeCherney AH, Maheux R. Modern management of tubal pregnancy. Curr Probl Obstet Gynecol 1983;6:4-38.
7. DeCherney AH, Maheaux R, Naftolin F. Salpingostomy for ectopic pregnancy in the sole patent oviduct: reproductive outcome. Fertil Steril 1982;37:619.

Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. Wallace Nunley, Charlottesville, Virginia. Your findings were similar to those that we reported last year regarding the higher repeat ectopic rate in the salpingotomy patients versus those with salpingectomy. Although the numbers were small, do you have additional information about the repeat ectopic pregnancy rate in those patients who had conservative tubal surgery on the sole remaining tube? When you said that the hysterosalpingography revealed nonpatent tubes and that there was a 16% intrauterine pregnancy rate, I assume that we are discussing complete nonfilling of the tube as opposed to a distal obstruction.

Dr. H. Oliver Williamson, Charleston, South Carolina. Did you reexamine the hysterosalpingograms for salpingitis isthmica nodosa on those patients who had ectopic pregnancies?

Dr. Mitchell (Closing). Dr. Nunley, most of the obstructions were distal occlusions. In reviewing the hysterosalpingograms, if we did find a cornual occlusion, it might have been caused by spasm or technique, and we could not really ascertain this since we had different individuals performing the hysterosalpingography. We could not verify one specific reason for our good pregnancy rate. We cannot tell the patient that sheis not going to achieve a pregnancy, and we have been advising patients of that information. I really do not have an answer for your other question.

Dr. Williamson, we did attempt to restudy the hysterosalpingograms. We found that the patients who had salpingectomies and then had a repeat ectopic pregnancy primarily cornual ectopic pregnancies correlating with the diagnosis of salpingitis isthmica nodosa. Essentially, the majority of those who had salpingectomies had cornual occlusion, and the repeat ectopic pregnancies in that particular group were interstitial ectopic pregnancies.

Effects of clomiphene citrate on episodic luteinizing hormone secretion throughout the menstrual cycle

David F. Archer, MD, Glen Hofmann, MD, PhD, Robert Brzyski, MD, PhD, Barbara Ross, RN, BSN, Richard T. Scott, Jr., MD, Christine B. Philput, PhD, Sergio Oehninger, MD, and J. Catesby Ware, PhD

Norfolk, Virginia

Pituitary luteinizing hormone secretory dynamics (pulse frequency and amplitude) were evaluated in eight normal women administered clomiphene citrate or placebo. After 5 days of treatment clomiphene citrate was found to increase mean luteinizing hormone secretion by increasing the amount of pituitary luteinizing hormone released per pulse. No change in pituitary luteinizing hormone interpulse interval was found in the follicular phase of the cycle, although a faster pulse frequency in the luteal phase was found after clomiphene citrate when compared with placebo. No differences were found in the luteal phase (pulse frequency, amplitude, and mean luteinizing hormone level) after clomiphene citrate versus placebo in the volunteers. These data suggest that the principal mechanism of action of clomiphene citrate is an increase in the concentration of hypothalamic gonadotropin releasing hormone into the hypothalamic-pituitary portal circulation, with a resultant increase in pituitary luteinizing hormone secretion. (Am J Obstet Gynecol 1989;161:581-92.)

Key words: Clomiphene citrate, pituitary luteinizing hormone secretion, normal women

Clomiphene citrate, an antiestrogen, has been used extensively to initiate follicular development in anovulatory women. The principal mechanism of action appears to be via the hypothalamic-pituitary axis resulting in an increase in serum follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels.[1] Recent evidence suggests that clomiphene citrate increases the frequency of episodic pituitary LH secretion in normal women.[2] An alteration in pituitary LH pulse frequency implies a hypothalamic site of action of clomiphene citrate.

The use of clomiphene citrate in the treatment of anovulatory infertility and/or luteal phase deficiency has been shown to result in the persistence or occurrence of luteal phase deficiency.[3] The administration of clomiphene citrate in large doses (200 mg/day for 5 days) also has been associated with a short luteal phase in normal women.[4] An increase in the follicular phase pituitary LH pulse frequency has been described in women with histologic evidence of luteal phase deficiency.[5]

The current study was undertaken because of the potential relationship between clomiphene citrate administration, an increase in pituitary LH pulse frequency, and the occurrence of luteal phase deficiency. The study design was to determine pituitary LH episodic release in the follicular and luteal phases of normal women who were administered clomiphene citrate. Any alterations in the parameters of pituitary LH episodic release would then be correlated with histologic and hormonal evidence of luteal phase deficiency.

Material and methods

Volunteers. Eight female volunteers who were between the ages of 18 and 35 and had regular cyclic menstrual bleeding episodes were asked to volunteer for this study. The protocol and informed consent had been approved by the Institutional Review Board of Eastern Virginia Medical School. A written informed consent was obtained from each volunteer before enrollment in the study. None of the women had used any exogenous steroid in the preceding 3 months. None of the volunteers had any evidence of gynecologic problems by history or physical examination, and they were

From the Howard and Georgeanna Jones Institute for Reproductive Medicine, Department of Obstetrics and Gynecology, and the Academic Computer Center, Eastern Virginia Medical School, and the Sleep Disorders Center, Sentara Norfolk General Hospital.

This work is supported partially by Basic Research Support Grant No. 87-36 from Eastern Virginia Medical School, and the Contraceptive Research and Development (CONRAD) Program at Eastern Virginia Medical School, under a Cooperative Agreement with the United States Agency for International Development (#DPE-2044-A-00-6063-00).

Presented by invitation at the Fifty-first Annual Meeting of the South Atlantic Association of Obstetricians and Gynecologists, Hot Springs, Virginia, January 29–February 1, 1989.

The views expressed by the authors do not necessarily reflect the views of the United States Agency for International Development.

Reprint requests: David F. Archer, MD, The Howard and Georgeanna Jones Institute for Reproductive Medicine, Hofheimer Hall, Eastern Virginia Medical School, 825 Fairfax Ave.—6th Floor, Norfolk, VA 23507.

6/6/13910

Table I. Demographic information and menstrual cycle lengths in days in volunteers who were administered either placebo or clomiphene citrate 100 mg/day

Volunteer No.	Age (yr)	Gravidity	Parity	Menstrual interval						
				Before treatment	Placebo			Clomiphene citrate		
					Follicular*	Luteal†	Total‡	Follicular	Luteal	Total
1	35	2	2	28-32	12	15	27	14	12	26
2	28	0	0	31	13	14	27	15	15	30
3	24	1	0	28-30	13	10	23	21	15	36
4	30	1	1	28	12	14	26	14	15	29
5	30	3	3	28	14	12	26	14	11	25
6	25	2	2	29	10	14	24	14	15	29
7	(Dropped out after one cycle)									
8	26	0	0	28-30	16	14	30	15	13	28
9	26	0	0	28	17	14	31	15	14	29

*Follicular interval is the number of days from onset on menstruation to the urinary LH surge.
†Luteal interval is the number of days from a urinary LH surge until the onset of the next bleeding episode.
‡Total is the number of days from onset of menstruation to onset of next menses.

all in good health. Their demographic backgrounds and menstrual cycle data are given in Table I.

Study design and medication schedule. The study design was a double-blind randomized administration of clomiphene citrate (50 mg twice a day) versus placebo (one tablet twice a day) taken orally for 5 days beginning on day 5 after the onset of menstruation. The oral medication, both clomiphene citrate and placebo, was supplied by a registered pharmacist from a local pharmacy to the Clinical Research Coordinator in similar packages in a random order. The composition of each package was blinded to the study coordinator and the other investigators. Each volunteer, on entering the study, was assigned a sequential number that was related to the medication dispensed. Each volunteer was administered a course of medication in one menstrual cycle, then was not treated in the next menstrual cycle. In the third cycle the alternate medication was used.

Throughout each investigative cycle, a basal body temperature recording was performed. The midcycle urinary LH surge was determined by assessing morning urine specimens from menstrual days 12 to 18 with a commercial kit (Ovustik, Monoclonal Antibodies, Inc., Mountain View, Calif.). Daily blood samples were obtained from menstrual day 5 to the onset of the next menstrual bleeding episode in all volunteers.

The volunteers were randomized into either the follicular or the luteal phase investigation of episodic LH secretion at the time of their entry into the study. Four of the volunteers were evaluated on the last day of medication administration (day 9 of the cycle) in each treatment cycle. The other four volunteers were evaluated on day 10 after a urinary LH surge in the luteal phase of the two treatment cycles.

All volunteers underwent an endometrial biopsy on day +11 after the urinary LH surge in both treatment cycles.

Sampling schedule for the assessment of episodic LH secretion. Each volunteer was admitted to the Sleep Disorders Center of Sentara Norfolk General Hospital at 6 PM on the selected menstrual cycle day on the basis of her group assignment. An indwelling intravenous catheter was inserted into an arm vein and the patency was maintained with a slow infusion of normal saline solution containing 10,000 IU of heparin per 1000 ml of fluid. An extension catheter that was led through a wall porthole allowed sampling to take place from an adjacent room without disturbing the volunteers' sleep. Peripheral venous samples were obtained every 10 minutes for 12 hours from each volunteer. Each blood sample was allowed to clot at room temperature; then serum was collected by centrifugation and stored at −20° C until analyzed.

Evaluation of the menstrual cycle. Daily peripheral venous blood samples were obtained from each volunteer from day 5 of menstruation until the onset of the next menstrual period in each treatment cycle for analysis of 17β-estradiol, progesterone, and LH. Each blood sample was handled as described above.

During the treatment cycles each volunteer collected a morning urine specimen beginning on day 12 of the menstrual cycle; this specimen was tested at home for LH with the Ovustik LH test kit. This procedure was performed until the LH surge was demonstrated or day 20 was reached.

Each volunteer also underwent an endometrial biopsy on day 11 after the identified urinary LH surge. The endometrial tissue obtained was examined histologically and a date assigned according to published criteria.[6]

Table II. Pituitary LH secretory dynamics during a 12-hour sampling interval in normal women receiving a placebo or clomiphene citrate 100 mg/day for 5 days

	Mean LH (ng/ml)		*Mean amplitude of pulses (ng/ml)*		*Mean interpeak interval*	
Volunteer No.	*Placebo*	*Clomiphene citrate*	*Placebo*	*Clomiphene citrate*	*Placebo*	*Clomiphene citrate*
Follicular phase						
4	14.77 ± 2.09	18.79 ± 7.14	3.44 ± 1.74	10.68 ± 4.63	33.68 ± 11.65	33.89 ± 18.19
5	22.42 ± 4.32	19.55 ± 6.14	6.66 ± 2.65	9.37 ± 5.32	39.44 ± 14.74	41.81 ± 19.65
6	14.80 ± 1.77	41.03 ± 9.83	2.84 ± 1.28	14.41 ± 9.77	30.87 ± 13.45	44.67 ± 77.48
9	18.50 ± 6.38	30.94 ± 7.76	9.63 ± 5.76	11.03 ± 6.07	45.33 ± 21.00	41.76 ± 22.70
Mean	19.37 ± 5.31[a,b]	31.11 ± 12.13[a,c]	5.28 ± 4.03[d]	11.27 ± 3.22[d,e]	36.53 ± 15.81[f]	40.15 ± 21.91
Luteal phase						
1	7.77 ± 2.77	5.87 ± 2.86	3.69 ± 5.22	2.99 ± 2.76	88.57 ± 75.15	36.84 ± 18.87
2	4.22 ± 1.93	7.19 ± 2.15	2.55 ± 2.93	3.11 ± 1.85	65.56 ± 49.02	33.68 ± 14.61
3	5.77 ± 3.08	9.90 ± 2.55	3.65 ± 3.52	3.73 ± 2.35	34.50 ± 13.95	37.78 ± 13.09
8	16.59 ± 9.69	9.87 ± 2.79	7.57 ± 10.74	3.06 ± 2.93	45.33 ± 32.04	40.59 ± 18.09
Mean	10.50 ± 8.26[b]	9.23 ± 3.27[c]	4.59 ± 6.73	3.22 ± 2.46[e]	50.58 ± 42.16[f]	37.12 ± 16.20

Multiple venous sampling was performed on day 9 to day 10 of the cycle and day +10 to +11 after a urinary LH rise between 6 PM and 7 AM.

Superscripts represent differences between means (*a, b, c, d, e*, $p = 0.0001$; *f*, $p = 0.02$).

Estimation of 17β-estradiol, progesterone, and LH. Peripheral levels of 17β-estradiol, progesterone, and LH were assessed with commercially available radioimmunoassay kits. Serum 17β-estradiol levels were determined with a kit obtained from Radioassay Systems Laboratory (Carson, Calif.); the within-assay coefficient of variation was 11% and the between-assay variation was 7%. Progesterone was estimated with a radioimmunoassay kit from Diagnostic Products, Inc. (Los Angeles). The within-assay coefficient of variation was 12.5%, 10.0%, and 6.7%, respectively, for low, mid, and high controls, while the between-assay coefficient of variation was 17%, 13.5%, and 7.0% for low, mid, and high controls. Serum LH was determined with a kit from Leeco Co. (Southfield, Mich.); the within-assay coefficient of variation was 17.7% and 9.8% for the low and high controls, and the between-assay coefficient of variation was 29.4% and 17.7%, respectively, for low and high controls.

Evaluation of episodic LH secretion. Episodic release of LH was evaluated with the program PULSAR, edition 1983.1 of Merriam and Wachter,[7] as modified for the IBM-PC by Gitzen and Ramirez.[8] We modified the program as described by Mais et al.[9] by determining the coefficient of variation at different dose levels of LH and defined an LH pulse as an increment in LH concentration exceeding the preceding nadir value by 3.8 times the coefficient of variation.

Statistical analysis. The parameters of the episodic pituitary LH secretion were compared between placebo and clomiphene citrate–treated cycles in both the follicular and the luteal phases. An analysis of variance was used to determine if there were any significant interactions. When significant interactions were found with the analysis of variance, this was followed with Student's *t* test for significant differences. These computations were performed with the SAS program under the general linear model on a VAX/VMS computer. A paired analysis was performed on the daily serum 17β-estradiol, progesterone, and LH levels with STAT Graphics statistical software (Statistical Graphics Corporation, Rockville, Md.) on an IBM-PC.

Results

Episodic pituitary LH release

Effects of clomiphene citrate

FOLLICULAR PHASE. Mean peripheral LH levels on the fifth day of active therapy (day 9 to 10 of the cycle) during the 12-hour sampling interval were significantly increased during the administration of clomiphene citrate compared with placebo ($p = 0.0001$) (see Table II, Fig. 1, *A*). The mean amplitude of each identified LH pulse was statistically increased during clomiphene citrate administration compared with placebo ($p = 0.0001$, Table II, Fig. 1, *A*). The frequency of the pituitary LH pulse(s) was expressed as the interpeak interval and was not significantly different between placebo and clomiphene citrate (36 vs. 40 minutes, respectively, Table II). There was no significant difference in the duration of the identified LH peaks with either treatment (data not shown).

LUTEAL PHASE. Mean LH levels on luteal days +10 to +11 after the identified urinary LH rise during the 12-hour sampling interval were not significantly different between the placebo and clomiphene citrate–treated cycles (see Table II, Fig. 1, *B*). There was no difference in the amplitude of the identified LH peaks in the luteal investigation with either treatment (see

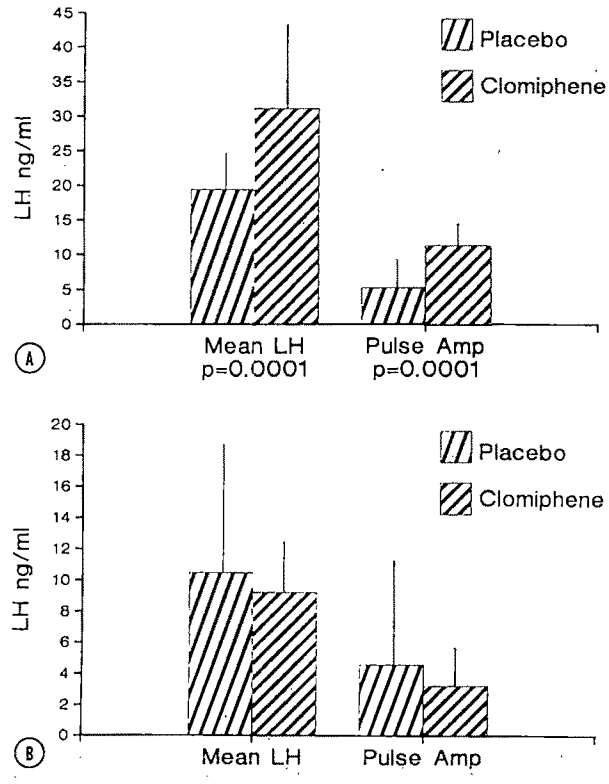


Fig. 1. A, Mean LH levels and mean pulse amplitude of identified peaks in normal women receiving placebo or clomiphene citrate 100 mg/day from day 5 to day 9 of menstrual cycle. See text for details of sampling, which was performed in the follicular phase (day 9) of the cycle. **B,** Mean serum LH levels and mean pulse amplitude of identified LH peak in normal women while receiving either a placebo or clomiphene citrate 100 mg/day from day 5 to day 9 of cycle. These values were obtained on luteal day +10 after urinary LH rise. See text for details of sampling.

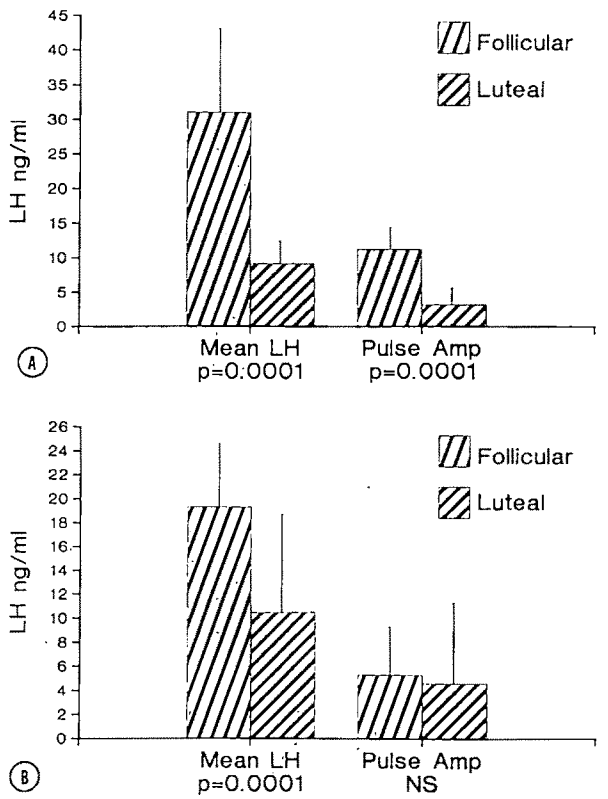


Fig. 2. A, Comparison of mean LH levels and pulse amplitude between follicular day 9 and luteal day +10 after the LH rise in normal women while receiving placebo from days 5 to 9 of menstrual cycle. **B,** Comparison of mean LH amplitude of identified LH pulses between follicular and luteal phases of women who were administered clomiphene citrate 100 mg/day between days 5 and 9 of menstrual cycle.

Table II, Fig. 1, *B*). The interpeak interval was shorter in the volunteers who had received clomiphene citrate (37 minutes) compared with placebo (50 minutes) during the luteal phase sampling (Table II, $p = 0.03$).

A comparison of the mean LH levels between the follicular and luteal phases was performed for both placebo and clomiphene citrate. The mean LH levels were significantly higher in the follicular phase than in the luteal phase for both placebo (19.37 vs. 10.50 ng/ml; $p = 0.0001$) and clomiphene citrate (31.11 vs. 9.23 ng/ml; $p = 0.0001$) (see Table II, Fig. 2). This finding was associated with a decreased LH peak amplitude only in the clomiphene citrate volunteers in the luteal phase ($p = 0.0001$) compared with the follicular phase (Table II, Fig. 2, *B*), whereas there was no difference in pulse amplitude between follicular and luteal phases of the placebo cycle (Table II, Fig. 2, *A*). The interpeak intervals were significantly different between follicular and luteal phases in the placebo treatment groups. There was no difference in the interpeak interval in the clomiphene citrate volunteers between the follicular and luteal phases. There was a significant difference in the interpeak interval in the clomiphene citrate group (37 minutes) compared with the placebo group (50 minutes) during the luteal phase ($p = 0.02$, Table II).

Menstrual cycle hormonal changes. Each volunteer had daily blood samples drawn beginning on day 5 of the cycle and proceeding until the onset of the next menstrual bleeding episode. These serum samples were analyzed for 17β-estradiol, progesterone, and LH. The identified midcycle serum LH peak was used as the reference point for comparison of the steroid hormone levels in both follicular and luteal phases in all volunteers. The daily serum LH levels were significantly increased during clomiphene citrate administration compared with placebo in all of the volunteers ($p = 1 \times 10^{-6}$, Fig. 3). The height of the LH midcycle peak between placebo and clomiphene citrate cycles was not statistically different (see Fig. 4, *A*). Clomiphene

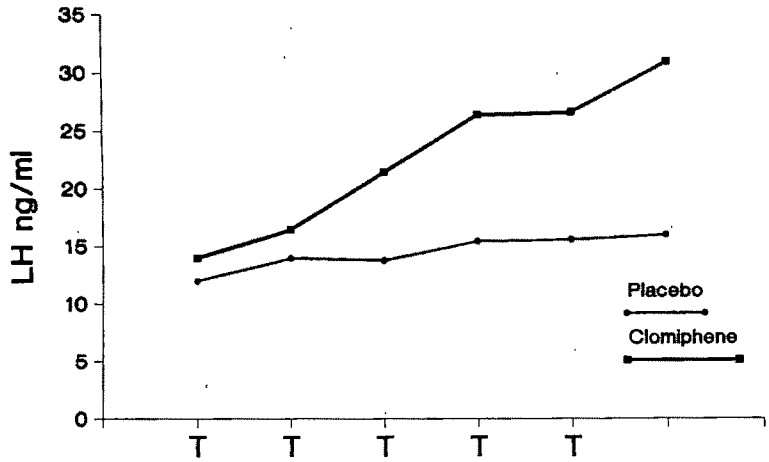


Fig. 3. Serum LH (nanograms per milliliter) levels during 5 days of treatment, placebo or clomiphene citrate 100 ml/day, and the day after medication was stopped.

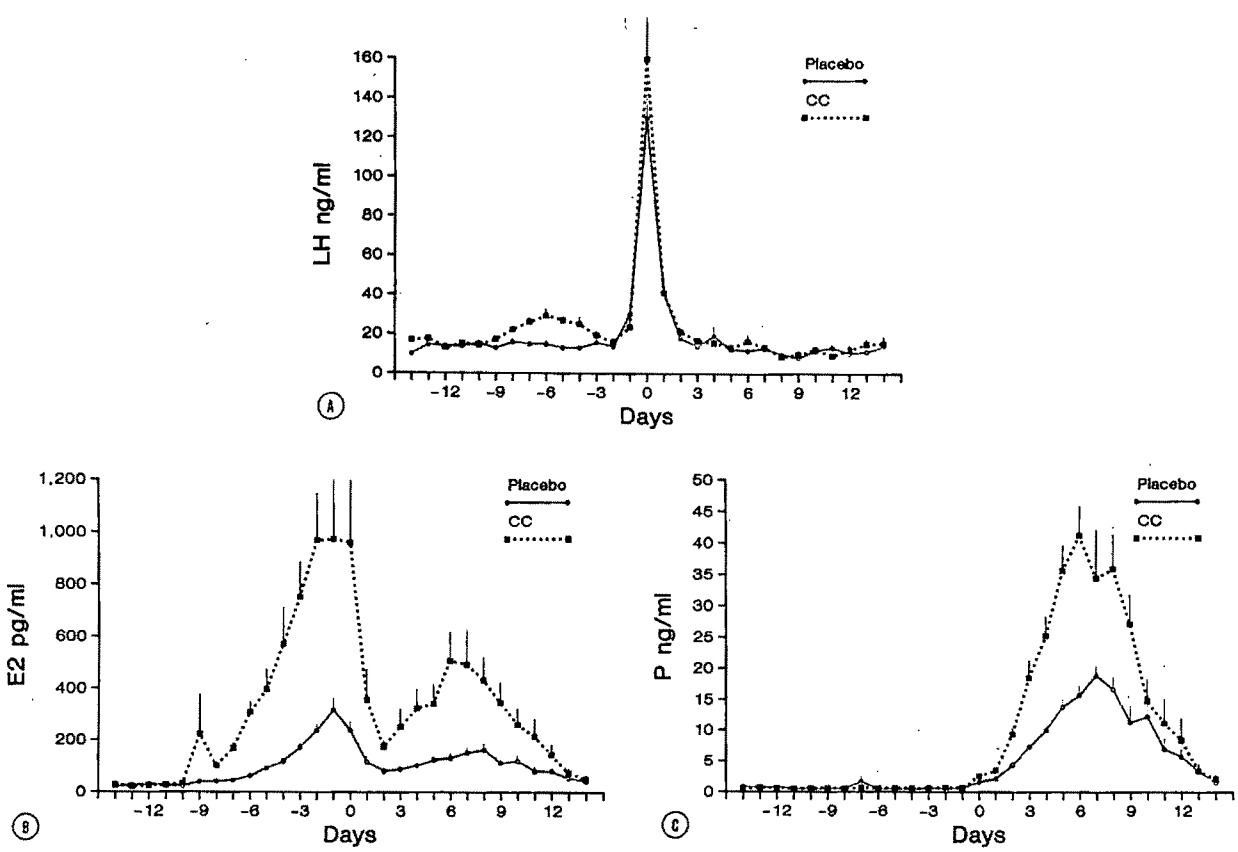


Fig. 4. A, Daily serum LH (nanograms per milliliter) levels from day 2 of cycle in women who received either placebo or clomiphene citrate 100 mg/day from days 5 to 9 of menstrual cycle. Data have been normalized to LH peak. **B,** Daily serum 17β-estradiol (picograms per milliliter) levels in normal volunteers, who took either placebo or clomiphene citrate 100 mg/day from days 5 to 9 of cycle. All values have been normalized to day of serum LH surge (day 0). **C,** Daily serum progesterone *(P)* (ng/ml) levels in normal volunteers who took either placebo or clomiphene citrate 100 mg/day from day 5 to 9 of cycle. All values have been normalized to day of serum LH surge (day 0).

citrate administration also significantly increased the mean 17β-estradiol levels from day −8 until the LH peak, when compared with the placebo treatment (see Fig. 4, *B*). A paired analysis of these data demonstrated a statistically significant difference in the follicular phase ($p < 1 \times 10^{-7}$) and luteal phase ($p < 2 \times 10^{-12}$, Fig. 4, *B*). Because there were occasional missing data points in one cycle of a volunteer, on comparison with her second cycle, the area under the curve for 17β-estradiol was then estimated by the trapezoidal method. When this estimate was performed, the area under the 17β-estradiol curve for clomiphene citrate was still significantly greater than placebo ($p = 0.02$). Serum progesterone levels were also markedly elevated during the luteal phase in the clomiphene citrate volunteers compared with placebo ($p < 1 \times 10^{-7}$) (see Fig. 4, *C*).

Menstrual cycle length and luteal phase adequacy. There were no significant changes in the menstrual cycle interval in the volunteers during the placebo or clomiphene citrate administration (Table I). All volunteers had cycle intervals of 27 to 31 days before treatment. During treatment, one volunteer had a menstrual interval of 36 days while receiving clomiphene citrate; this was secondary to a prolonged follicular phase (Table I). None of the other volunteers had any evidence of follicular phase dysfunction. There was no discernible difference in the length of the luteal phase (LH urinary rise to menses) in any of the volunteers with either treatment (Table I). The cycle day of the serum and urinary LH rise was not different between the two treatments although the standard deviation of the mean interval from onset of menses to peak LH levels was three times larger in the placebo group than in the clomiphene citrate group (data not shown).

Histologic examination of endometrial biopsy specimens obtained in both the placebo and clomiphene citrate treatment cycles on the morning of day +11 after the urinary LH rise were assigned dates according to previously reported criteria.[6] All biopsy specimens were read as day 22 to 25 of the cycle. There was no histologic evidence of luteal phase deficiency in any of these biopsy specimens.

Comment

This study has found that there is a significant increase in pituitary LH secretion in women who are taking clomiphene citrate between days 5 and 9 of the follicular phase of the menstrual cycle. These data are in agreement with other reports that the mode of action of clomiphene citrate in stimulating the initiation of folliculogenesis is via a hypothalamic-pituitary mechanism, which results in an increased gonadotropin secretion.[1, 10, 11] The increase in serum LH levels could be brought about through at least three different mechanisms. These are: (1) an increase in the total amount of LH released per pulse, (2) an increase in the frequency of pulses per unit time, and (3) a change in the pituitary sensitivity to the endogenous gonadotropin releasing hormone. These parameters should be considered assuming that there are no significant changes in peripheral metabolism or excretion of the hormone under investigation.

It appears that the total amount of LH released per pulse is the explanation for the increased serum LH level found during clomiphene citrate administration. The amplitude of the identified LH pulses was higher in the follicular phase of the cycle with clomiphene citrate treatment (Fig. 1, *A*). Increased pituitary hormonal secretion has been found to be related to an increase in the quantitative amount of hormone released per episode on the basis of deconvolutional analysis.[12] Our data support this concept that the increase in serum hormone concentration is due to an increased release of hormone with each secretory episode.

We were unable to discern a significant difference in pituitary interpeak interval between the women receiving placebo and those given clomiphene citrate. This is in contradistinction to a previous report that demonstrated an increase in pulse frequency in the follicular phase of the cycle in women.[2] In this study pulse frequencies were compared before and after ingestion of clomiphene citrate and placebo in the volunteers. These authors did not find a significant difference in the pulse frequency on the same cycle day between women receiving placebo and those given clomiphene citrate. Other investigators have described a small but significant increase in pulse frequency in women who were administered clomiphene citrate.[13]

In the current study we were not able to show any difference in pulse frequency on the basis of interpeak interval between clomiphene citrate and placebo on cycle days 9 to 10. These data suggest that clomiphene citrate does not have an effect on the hypothalamic pulse generator. The reason for the differences between our study and the other may be explained by the fact that we were sampling overnight. A slowing of LH pulse frequency has been described to occur in the follicular phase of the cycle with sleep. We have not specifically addressed the issue of pituitary LH pulse frequency in the awake state versus the asleep state in this study, although the data are under analysis. Alternatively, the longer duration of sampling (12 hours) in our study compared with 4 and 9 hours, respectively, and the more frequent venous sampling interval (10 minutes) could contribute to a more accurate assessment of pulse frequency.[13, 14, 21]

We found a high frequency of pituitary LH pulses on days 9 to 10 of the menstrual cycle. The mean interpeak interval in the follicular phase of the cycle was 40 minutes in the clomiphene citrate–treated volunteers; this is not different from that with placebo (Table II). We believe that the high frequency of identified

LH pulses was due to the sensitivity of the PULSAR program. Because of the large coefficient of variation in the radioimmunoassay associated with the low levels of LH, we determined the within-assay coefficient of variation with low, medium, and high LH control and constructed a binomial equation from these data points.[9] Using these coefficients of variation we reran the data and used the criterion that an increase of LH over the preceding nadir value of >3.8 times the coefficient of variation was needed to identify a LH peak. When this modification was performed, the increased number of peaks presented in the current study were identified. We believe that the increased pulse identification is related both to the sensitivity of the PULSAR program and to the duration and frequency of the sampling. These data are in accordance with the reported paradigm indicating that glandular hormonal secretion is characterized by a short-lived rapid burst of secretory activity.[12]

The increased pituitary LH secretion per pulse may be secondary to an increase in the amount of endogenous hypothalamic gonadotropin releasing hormone released. Clomiphene citrate has been shown to increase the in vitro release of gonadotropin releasing hormone from isolated perfused rat hypothalami.[15] Increased quantities of circulating immunologic gonadotropin releasing hormone have been detected in peripheral sera of women who were administered clomiphene citrate.[16] The quantity of pituitary LH released was found to be directly correlated with the amount of exogenous gonadotropin releasing hormone administered in men with hypogonadotropic hypogonadism associated with a gonadotropin releasing hormone deficiency.[17] Other investigators have found that exogenous gonadotropin releasing hormone administration (10 μg) does not result in a significant difference in the quantity of pituitary LH release in normal women studied at the same time in the menstrual cycle, either not receiving treatment or receiving clomiphene citrate.[13] We believe that these data and our own from the current study are compatible with the hypothesis that there is an increased quantity of hypothalamic gonadotropin releasing hormone being released during clomiphene citrate administration in normal women.

Another possibility is that clomiphene citrate could alter the pituitary sensitivity to endogenous gonadotropin releasing hormone. Clomiphene citrate was found to increase the pituitary secretion of LH only minimally with an in vitro superfusion technique.[15] However, pituitary cells exposed to clomiphene citrate in vitro were shown to have an increased sensitivity to gonadotropin releasing hormone.[1] Further, clomiphene citrate, an antiestrogen, could result in endocrine changes similar to those present in a postmenopausal woman. Increased pituitary LH secretion with large-amplitude LH pulses is known to occur in women with ovarian failure.[18] Further, studies in castrated rats have found that there is a decrease in the hypothalamic-pituitary portal concentration of gonadotropin releasing hormone, with an increase in both the number and the sensitivity of pituitary gonadotropic cell gonadotropin releasing hormone receptors.[19] Although these reports do indicate a direct effect of clomiphene citrate on the pituitary gonadotropic cell, they imply that the effect, if present, is minimal.

We did not find any significant differences in luteal phase mean serum LH levels and LH amplitude between the placebo and clomiphene citrate cycles with sampling performed on LH surge day +10 during the luteal phase. The effect of progesterone on hypothalamic-pituitary function is believed to be a slowing in the hypothalamic pulse generator with a concomitant increase in pulse amplitude.[21] Our data, however, are at variance with other reports of investigations in both subhuman primates and humans relative to the increased pulse amplitude in the luteal phase of the cycle.[22, 23] There does appear to be a reduction in both mean LH levels and peak amplitude in the late luteal phase of the cycle in these studies.[22, 23] Our luteal sampling was performed on the tenth day after the urinary LH surge and should be considered late luteal phase. There also appears to be a decrease in the LH pulse amplitude at night in women during the late luteal phase, although the published data are too sparse to make any meaningful conclusions.[23] We believe that the timing of our investigation of pituitary secretory dynamics i.e., at night and in the late luteal phase, explains our current findings.

The pituitary LH interpeak interval was less in the clomiphene citrate volunteers than in those receiving placebo in the luteal phase (Table II). The cause of this latter finding is not known but could be related to an increased retention of clomiphene citrate in the body.[1] Studies have indicated that clomiphene citrate can be present for up to 6 weeks after administration.[1] The administration of clomiphene citrate in the luteal phase also has been found to result in an increased LH pulse frequency.[20] These data suggest that the decreased luteal phase interpeak interval in the volunteers may be secondary to the presence of clomiphene citrate.

The clinical parameters used to determine menstrual cycle interval did not demonstrate any significant differences between placebo and clomiphene citrate. Specifically we did not find any alteration in the interval in days of the follicular or luteal phases (see Table I). Other investigators who have used clomiphene citrate for initiation of follicular maturation in anovulatory or normal women report a delay in the LH surge related to the use of clomiphene citrate.[1, 10, 11] In a study of normal women undergoing clomiphene citrate therapy for in vitro fertilization the follicular phase was increased by 1 day in comparison with the untreated cy-

cle.[24] Although we looked for this, no significant difference was found in the interval from onset of menses to serum LH surge or from onset of treatment to serum LH surge. We were impressed with the finding that there was a smaller standard deviation in the interval from menses to LH surge in the clomiphene citrate–treated women compared with those given placebo. We feel that this indicates that clomiphene citrate results in a more concordant midcycle LH release, possibly related to the follicular development.

High doses of clomiphene citrate administered to subhuman primates have been found to inhibit follicular response and retard the occurrence of the midcycle LH surge. Only one of the women in this study had evidence of a prolonged follicular phase and this occurred during the use of clomiphene citrate (volunteer 9, Table I).[25]

There was a highly significant increase in the serum 17β-estradiol levels in the women while they were receiving clomiphene citrate (Fig. 4, *B*). The serum 17β-estradiol levels in anovulatory women during and after administration of clomiphene citrate have been reported to be on the order of 300 to 400 pg/ml.[1, 10] An evaluation of the serum 17β-estradiol levels in normal women who were undergoing controlled ovarian hyperstimulation for in vitro fertilization indicate a large increase in their serum 17β-estradiol levels.[17, 24] These data would imply a potential follicular resistance to or dysfunction with endogenous gonadotropins in anovulatory women. The large amount of clomiphene citrate used for initiation of follicular development in some women could inhibit ovarian folliculogenesis as described above, but there may be an ovarian dysfunction to explain the lower levels of 17β-estradiol found during clomiphene citrate administration in anovulatory women.[25] Alternatively, follicular development could be retarded in the anovulatory women as an explanation for the lower 17β-estradiol levels found during clomiphene citrate treatment.

Despite the increased serum concentrations of 17β-estradiol the LH surge was not advanced in the women as determined by both the serum LH levels and the urinary LH surge. In fact, in all but two women the serum LH peak coincided with the urinary LH surge (data not shown), and there was no difference in the height of the LH midcycle peak between the two treatments.

There were no discernible differences in the luteal phase lengths between the two treatments. The use of clomiphene citrate in high doses in normal women along with exogenous 17β-estradiol has been reported to cause a shortened luteal interval. The luteal interval on the basis of the day of the urinary LH surge to the onset of the next bleeding episode ranged from 10 to 15 days. Two of the women had luteal lengths of 10 and 11 days, respectively (volunteers 3 and 5), during a placebo and a clomiphene citrate–treated cycle.

The serum progesterone levels during the luteal phases were significantly higher in the women who had received clomiphene citrate. On the basis of the serum progesterone levels there was no evidence for either a luteal phase inadequacy or a shortened luteal interval. The endometrial biopsies that were performed on day +11 after the urinary LH surge were compatible with day 23 to day 25 of the cycle, and there was no histologic evidence of luteal deficiency. The exact incidence of luteal phase deficiency is difficult to assess but appears to be on the order of 2% to 3% in infertile women.[3] Clomiphene citrate has been thought to induce a luteal phase deficiency in both normal and infertile women. However, we were unable to find any evidence of luteal phase deficiency in the normal volunteers in this small series.

In summary, we have shown that clomiphene citrate results in an increased serum LH level in the follicular phase of the cycle in normal women. The increase appears to be related to an increased quantitative release of pituitary LH per pulse, since we found an increased amplitude of the identified peaks but no discernible change in pulse frequency.

Our data also indicate that luteal phase pituitary LH episodic secretion is not independent of the follicular phase administration of clomiphene citrate. We found a statistically different interpeak interval in the luteal phase of the women who received clomiphene citrate when compared with those given placebo, implying a long duration of effect of clomiphene citrate.

We acknowledge the assistance of Cindy Anderson, RN, MSN, FNC, Janice Hammond, J-G Hsui, MD, and Ms. Pauline Clynes.

REFERENCES

1. Adashi EY. Clomiphene citrate-initiated ovulation: a clinical update. Semin Reprod Endocrinol 1986;4:255-76.
2. Kerin JF, Lui JH, Phillipou G, Yen SSC. Evidence for a hypothalamic site of action of clomiphene citrate in women. J Clin Endocrinol Metab 1985;61:265-8.
3. Kubik CJ. Luteal phase dysfunction following ovulation induction. Semin Reprod Endocrinol 1986;4:293-9.
4. Vaitukaitis JL, Bermudez JA, Cargille CM, Lipsett MB, Ross GT. New evidence for an anti-estrogenic action of clomiphene citrate in women. J Clin Endocrinol Metab 1971;32:503-9.
5. Soules MR, Steiner RA, Clifton DK, Bremner WJ. Abnormal patterns of pulsatile luteinizing hormone in women with luteal phase deficiency. Obstet Gynecol 1984;63:626-9.
6. Noyes RW, Hertig AT, Rock J. Dating the endometrial biopsy. Fertil Steril 1950;1:3-17.
7. Merriam GW, Wachter KW. Algorithm for the study of episodic hormone secretion. Am J Physiol 1982; 243:E310-8.
8. Gitzen JF, Ramirez VD. PC-pulsar—pulsar pulse analysis for the IBM-PC. Life Sci 1986;38:17-27.
9. Mais V, Cetel NS, Muse KN, Quigley MK, Reis RL, Yen

SSC. Hormonal dynamics during luteal follicular transition. J Clin Endocrinol Metab 1987;64:1109-14.
10. Wu Ch. Plasma hormones in clomiphene citrate therapy. Obstet Gynecol 1977;49:443-8.
11. Bohnet HG, Kato K, Trapp M, Weise HC, Leidenberger FA. Different hormonal patterns in human menopausal gonadotropin treated, clomiphene citrate-treated and untreated conception cycles. Fertil Steril 1986;45:469-74.
12. Veldhuis JD, Carlson ML, Johnson ML. The pituitary gland secretes in bursts: appraising the nature of glandular secretory impulses by simultaneous multiple-parameter deconvolution of plasma hormone concentrations. Proc Natl Acad Sci USA 1987;84:7686-90.
13. Judd SJ, Alderman J, Bowden J, Michailov L. Evidence against the involvement of opiate neurons in mediating the effect of clomiphene citrate on gonadotropin-releasing hormone neurons. Fertil Steril1987;47:574-8.
14. Veldhuis JD, Evans WS, Rogol AD, et al. Intensified rates of venous sampling unmask the presence of spontaneous, high-frequency pulsations of luteinizing hormone in man. J Clin Endocrinol Metab 1984;59:96-102.
15. Miyake A, Tasaka K, Sakumoto T, Kawamura Y, Nagahara Y, Aono T. Clomiphene citrate induces luteinizing hormone release through hypothalamic luteinizing hormone in vitro. Acta Endocrinol 1983;103:289-92.
16. Miyake A, Aono T, Minagawa J, Kawamura Y, Kurachi K. Changes in plasma luteinizing hormone-releasing hormone during clomiphene-induced ovulatory cycles. Fertil Steril 1980;34:172-4.
17. Spratt DI, Finkestein JS, Badger TM, Butler JP, Crowley WF Jr. Bio- and immunoactive luteinizing hormone responses to low doses of gonadotropin-releasing hormone (GnRH): dose response curves in Gn-RH-deficient men. J Clin Endocrinol Metab 1986;63:143-50.
18. Albert JL, Kubik CJ, Archer DF. Effect of exogenous luteinizing hormone-releasing hormone on hypothalamic pituitary function in women with ovarian failure. Fertil Steril 1987;48:198-203.
19. Valenca MM, Ching M, Masotto C, Negro-Vilar A. How does the gonad affect LH-RH secretion? Effects of gonadectomy on LH-RH release from median eminence nerve terminals incubated in vitro and on LH-RH concentrations in hypophyseal portal blood. In: Mahesh VB, Anderson E, Dhindsa D, Kalra S, eds. Regulation of ovarian and testicular function. New York: Plenum Press, 1987:617-21.
20. Maruncic M, Casper RF. The effect of luteal phase estrogen antagonism on luteinizing hormone pulsatility and luteal function in women. J Clin Endocrinol Metab 1987; 64:148-52.
21. Soules MR, Steiner RA, Clifton DK, Cohen NL, Aksel S, Bremner WJ. Progesterone modulation of pulsatile luteinizing hormone secretion in normal women. J Clin Endocrinol Metab 1984;58:378-83.
22. Filicori M, Santoro N, Merriam GR, Crowley WF Jr. Characterization of the physiologic pattern of episodic gonadotropin secretion throughout the human menstrual cycle. J Clin Endocrinol Metab 1986;62:1136-44.
23. Reame N, Sauder SE, Kelch RP, Marshall JC. Pulsatile gonadotropin secretion during the human menstrual cycle: evidence for altered frequency of gonadotropin-releasing hormone secretion. J Clin Endocrinol Metab 1984;59:328-37.
24. Thorneycroft IH, Bustillo M, Simon JA, Buster JE. The effects of superovulatory doses of clomiphene citrate on spontaneous luteinizing hormone peaks in regularly ovulating women. Am J Obstet Gynecol 1984;149:375-8.
25. Marut EL, Hodgen GD. Antiestrogenic action of high-dose clomiphene in primates: pituitary augmentation but with ovarian attenuation. Fertil Steril 1982;38:100-4.

Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. Mary G. Hammond, Chapel Hill, North Carolina. Although clomiphene citrate has been in use for the induction of ovulation for almost 30 years, the mechanism of action of this very useful drug continues to be debated. This antiestrogen, which was initially studied as a contraceptive and then introduced for the induction of ovulation in anovulatory women, has found increasing utility in treatment of women with evidence of ovulation. It is being used in the treatment of luteal phase dysfunction, and it is finding increasing usefulness in induction of multiple follicular development, in in vitro fertilization, and at present in some centers for multiple follicle development and washed intrauterine insemination in the treatment of male factor and unexplained infertility.

Essentially three mechanisms have been suggested as possible mode of action for clomiphene.[1] The first is by an interaction with hypothalamic estrogen receptors, leading to an increase in gonadotropin releasing hormone stimulation of the pituitary, resulting in a rise in circulating levels of FSH and LH, and stimulating follicle recruitment. The second is by increasing sensitivity of the pituitary gland to gonadotropin releasing hormone secretion, also leading to an increase in FSH and LH concentrations. The third proposed that clomiphene interferes with steroidogenesis in the ovary, reducing concentrations of circulating estrogens and again, through the hypothalamic mechanism, leading to an increase in gonadotropin releasing hormone production and a subsequent rise in gonadotropins. Two out of three of these mechanisms postulate a change in gonadotropin releasing hormone secretion at the hypothalamic level.

It would theoretically be possible to increase gonadotropin releasing hormone secretion by two methods. One would be by increasing the amplitude of the gonadotropin releasing hormone release. The second would be by increasing the frequency of gonadotropin releasing hormone pulses. An increase in pulsatility of gonadotropin releasing hormone would lead to an increased frequency of LH peaks in the circulation. An increase in the amplitude of the gonadotropin releasing hormone stimulation would lead to an increased amplitude of LH peaks in the serum.

The second mechanism, an effect on the pituitary gland, would lead to an increased sensitivity to the gonadotropin releasing hormone pulses and therefore to an increase in amplitude, but not frequency, of LH pulses.

The intent of this article was to evaluate further the effect of clomiphene citrate on LH pulse characteristics in normal women. These characteristics have been previously evaluated in the follicular phase. However, there are no reports of an evaluation in the luteal phase. In addition, the authors looked at the period of sleep as opposed to the daylight hours that had been previously studied. They concluded that the rise in daily LH concentration seen during the follicular phase is associated with an increased amplitude of LH pulses

rather than an increase in LH pulse frequency. This conclusion is based on the observations of LH peak frequency in serum sampling obtained during a 12-hour evening and sleep period in normal women who were studied in either the follicular or the luteal phase of a placebo cycle and a clomiphene citrate cycle. Four women were studied in each arm. They were evaluated on cycle day 9, the last day of administration of clomiphene, and on luteal day 10 after a urinary LH surge. In addition to studying the variety of hormones in the serum, the authors also performed an endometrial biopsy 11 days after the urinary LH surge.

The authors confirmed that there was an increase in the mean peripheral follicular phase LH levels in patients receiving clomiphene citrate and that there was no change in the mean LH levels of patients in the luteal phase; these findings support the concept that the action of clomiphene is primarily in the immediate early follicular phase during and immediately after the clomiphene dose. The authors also confirmed an increase in the mean estradiol level in the follicular and luteal phases and essentially a doubling of the progesterone level in the luteal phase of patients receiving clomiphene citrate. These findings have been previously reported in the literature and would seem to reflect multiple follicle and corpus luteal development in normal women receiving clomiphene.[2] The authors also explored the question as to whether clomiphene citrate might affect luteal phase adequacy on the basis of luteal phase length and endometrial biopsy. They noted no difference between the cycle day of urinary LH rise or the length of the luteal phase. They also demonstrated no histologic evidence of luteal phase deficiency in association with clomiphene treatment. This is an interesting finding in that they have examined eight biopsy specimens, all of which were in phase. The literature suggests that, even in normally ovulating women, approximately 20% of cycles show some endometrial abnormality. However, in this small sampling it is not surprising that this was not detected. Statistical significance would not have been shown, however, had a single abnormal endometrial biopsy specimen been detected in the clomiphene group, because the frequency of the abnormality is sufficiently high in normal cycles as to make it difficult to attribute to the drug.

The major focus of the article has to do with the effect of clomiphene on LH pulsatility. In this regard there are several comments to be made. The first is that there is an unusually high frequency of LH pulses or, stated another way, an extremely low LH interpulse interval reported in this study. Pulses are reported to occur between 30 and 50 minutes in both follicular and luteal phases. This is quite contrary to the literature in which the normal interpulse interval reported is approximately every 90 minutes in the follicular phase and every 2 to 3 hours in the luteal phase. Previous sleep studies have suggested that this pulse frequency actually decreases further during the night by an average of approximately 25 minutes.[3] In sleep, midluteal LH peaks have been reported to be as infrequent as every 5 hours. In the analysis of the LH peaks by the method described, the authors were unable to detect an increased frequency of LH peaks after clomiphene citrate administration, in contrast with other studies. They did, however, note an increase in LH amplitude. Their interpretation is that this eliminates an increase in gonadotropin releasing hormone pulse frequency at the hypothalamic level as a cause of clomiphene activity. It does not, however, rule out an increase in the amplitude of gonadotropin releasing hormone pulses or an increase in the sensitivity of the pituitary to gonadotropin releasing hormone pulses as the cause of clomiphene's action.

Of major import to interpretation of the results is the selection of a computer program for the analysis of the episodic release of LH.[4] A variety of methods have been used in the past to identify LH peaks and there are a number of programs available for performing this analysis. This is an intricate and controversial area of neuroendocrinology, and the selection of the program PULSAR used in this study clearly appears to have had a significant impact on the results.

I have several questions for the authors.

1. Why did they select the evening for performing their studies? Do they feel the nocturnal LH peaks are more important in neuroendocrine function?
2. Did they look at pelvic ultrasonograms to confirm the number of follicles in the patients responding to clomiphene therapy?
3. How did they interpret their endometrial biopsy specimens? The endometrial biopsies were timed on the basis of a midcycle LH peak. However, the analysis of the histologic features is based on time to the subsequent menses. Which method did they use for analysis and was there a discrepancy noted between the two methods of analysis?
4. How did they explain the fact that all of their endometrial biopsy specimens were normal in light of previous studies that detected approximately 20% discrepancy in endometrial biopsy specimens, even in normal women?
5. Have they submitted their LH pulsatility data to analysis by another computer program besides PULSAR? If so, did they get the same results and how do they interpret these very frequent LH peaks that have not been reported previously? Do they feel that these are physiologically significant? Is there a method by which they can analyze their data and report the peak frequencies previously noted by other authors?

REFERENCES

1. Adashi EY. Clomiphene citrate: mechanism(s) and site(s) of action. Fertil Steril 1984;42:331.
2. Kerin JF, Liu JH, Phillipow G, Yen SSC. Evidence for a hypothalamic site of action of clomiphene citrate in women. J Clin Endocrinol Metab 1985;61:265.
3. Rossmanith WG, Yen SSC. Sleep associated decrease in luteinizing hormone pulse frequency during the early follicular phase of the menstrual cycle: evidence for an opioid-

Table I. Pituitary LH pulse dynamics during a 12-hour sampling interval by Cluster Analysis

	LH amplitude (ng/ml)*		*Interpulse interval (min)*	
Volunteer No.	*Placebo*	*Clomiphene citrate*	*Placebo*	*Clomiphene citrate*
Follicular phase				
4	17.8	22.2	125.0	58.3
5	24.8	24.6	56.3	80.0
6	17.4	50.4	107.5	102.0
9	23.4	37.4	67.8	71.7
Mean ± SD	20.85 ± 3.29	33.65 ± 11.26	89.15 ± 28.09	76.75 ± 17.48
Luteal phase				
1	10.3	6.4	51.0	50.9
2	4.5	8.7	102.0	73.3
3	8.1	11.1	57.8	68.9
8	25.4	11.2	210.0	76.7
Mean ± SD	12.07 ± 7.96	9.35 ± 1.97	105.20 ± 63.59	67.45 ± 9.94

*Amplitude is from nadir to peak of identified pluses.

ergic mechanism. J Clin Endocrinol Metab 1987;65:715.
4. Urban RJ, Evans WS, Rogol AD, Kaiser DL, Johnson ML, Veldhuis JD. Contemporary aspects of discrete peak-detection algorithms. I. The paradigm of the luteinizing hormone pulse signal in men. Endocr Rev 1988;9:3.

Dr. Archer (Closing). I want to thank Dr. Hammond for what I thought was a very penetrating and cogent discussion of this article, touching on the highlights and pointing out some problems that we might have that could be rectified.

First, why we did the study in the evening is unique because we did not start out to look at evening samples in normal menarchal women. Several years ago I became very interested in the fact that some menopausal women wake up between 4 and 5 AM for no apparent reason and stay awake. I thought that some phenomenon occurring at night should be investigated. This was coupled with data regarding a nocturnal, sleep-related LH secretion during puberty, and I felt that these nocturnal LH pulses might be of significance to us in terms of neuroendocrine function. There was also a suggestion that if clomiphene citrate did increase pituitary LH pulses and did so at night as opposed to day, this might be the principal mechanism of action of clomiphene in changing LH secretion. The increased nocturnal LH pulses could result in the pathophysiologic finding of luteal phase defects. Increased follicular stage LH pulse frequency has been reported to occur in women with luteal phase defects.[1]

We are continuing to analyze our data to determine if there is a change in sleep-associated LH pulses. All of these women have been studied in the sleep laboratory with sleep stages determined on a minute-to-minute or second-to-second basis, and those data are now being analyzed with Dr. Ware.

We did have problems with the endometrial biopsies. I must admit that in my enthusiasm for this study we looked at the biopsy specimens from the time of the urinary LH surge to the biopsy date. When the pathologist reported that they were within day 23 to day 25 as I expected them to be, I just assumed that that was right. After we looked again at the data base, three of these eight women have luteal phase lengths of <13 days (one 11, one 12, and one 13). It is conceivable, if we use the criteria of Noyes et al. and the onset of the next menstrual period and then count back and look critically at these biopsy specimens, that we might find one or two that are out of phase. Whether that will be significant I am not sure, but at least it will again indicate to us that clomiphene may have some effect that is picked up histologically.

Last, in terms of an LH pulse analysis program, PULSAR is the program we had readily available to us and we used it. It has some deficiencies. One of the deficiencies that is obvious to us is that it picks up a lot of background noise. We have obtained, from the University of Virginia, Dr. Johannes Veldhuis' program, Cluster Analysis.[2] We have reanalyzed these data by means of Cluster Analysis (Table I).

Luteinizing hormone pulse amplitudes are greater with the Cluster Analysis program than the PULSAR program, since the measurement is from the preceding nadir to peak. PULSAR measures peak amplitudes from a smoothed mean to the apex of the identified peak. The relationship still exists that clomiphene citrate increases mean LH pulse amplitude in the follicular phase. There is no difference in mean LH pulse amplitude in the luteal phase of either clomiphene citrate– or placebo-treated women.

The interpulse interval between clomiphene citrate and placebo is not statistically different in the follicular phase of the cycle on the basis of Cluster Analysis (76 and 89 minutes respectively). This pulse frequency is more in keeping with the published literature of approximately one pituitary LH pulse per hour. This interval is greater (slower) with Cluster Analysis than with

Pulsar. We believe that Cluster Analysis has a more accurate identification of discrete pulses since it takes into account both preceding nadir and postpeak nadirs for pulse identification. Of interest is that the luteal phase interpulse interval is longer with placebo than with clomiphene citrate. This supports our contention that clomiphene citrate persists in the body and continues to have a central effect on hypothalamic function.

REFERENCES

1. Soules MR, Steiner RA, Clifton DK, Bremner WJ. Abnormal patterns of pusatile luteinizing hormone in women with luteal phase deficiency. Obstet Gynecol 1984;63: 626-9.
2. Veldhuis JD, Johnson ML. Cluster Analysis: a simple versatile and robust algorithm for endocrine pulse detection. Am J Physiol 1986;250:E486-93.

Make researching easier with the Ten-Year Cumulative Index (1978-1987) to the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY

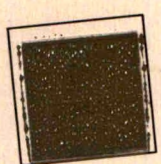
The Ten-Year Cumulative Index is a complete guide to more than 30,000 pages of original articles, case reports, letters, and editorials published in the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY from 1978 through 1987 (volumes 130 to 157). Numbering more than 800 pages, the Cumulative Index includes both a subject and an author index. This hard-cover volume is $64.50 ($69.00 international). Prices include shipping. *Payment must accompany all orders and must be in U.S. funds, drawn on a U.S. bank.*

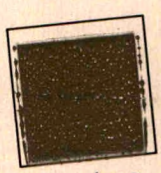
Contact The C.V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146 USA; phone (314) 872-8370, ext. 394. In the U.S., call toll-free: 1-800-325-4177, ext. 394. Please allow six weeks for delivery.

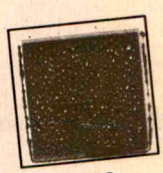
Take the OC preference test.

1.

In the United States last year, the OC group with the largest number of prescriptions was:

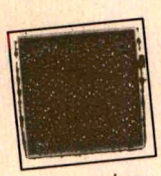

triphasic


low-dose monophasic


1/50 formulations

2.

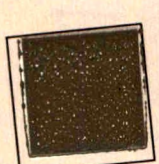
In the last 18 months, more *new* prescriptions were dispensed for:

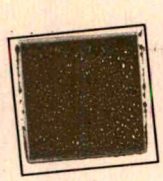

triphasics

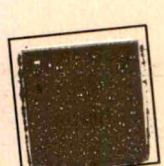

low-dose monophasics

3.

Which monophasic had an increase in new prescriptions 13 times the average of all low-dose monophasics in the last 18 months?


Demulen® 1/35*


Ortho-Novum® 1/35†


Lo/Ovral®‡

Scratch gold squares with a coin to reveal the answers.

*Demulen® 1/35 (ethynodiol diacetate 1 mg, ethinyl estradiol 35 mcg) is a registered trademark of G.D. Searle & Co.

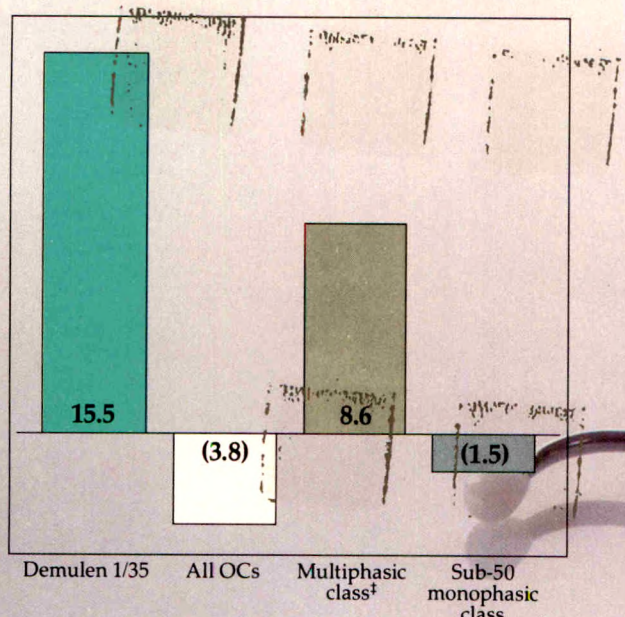
†Ortho-Novum® 1/35 (norethindrone 1 mg, .035 mg ethinyl estradiol) is a registered trademark of Ortho Pharmaceutical Corporation.

‡Lo/Ovral® (norgestrel and ethinyl estradiol tablets) is a registered trademark of Wyeth-Ayerst.

There's an OC in
and it's not

Now Demulen® 1/35 is the fastest growing monophasic*

New OC Rx's: *Percent change, October 1987 to March 1989*†



Today more and more physicians are taking an increased interest in Demulen 1/35 and prescribing it for their patients on oral contraceptive (OC) therapy. In fact, recent statistics show that new prescriptions for Demulen 1/35 rose by 16% during the last 18 months (October 1987 to March 1989).† That's a significantly greater increase than any other monophasic OC,* and nearly twice the growth rate of the multiphasic class.‡



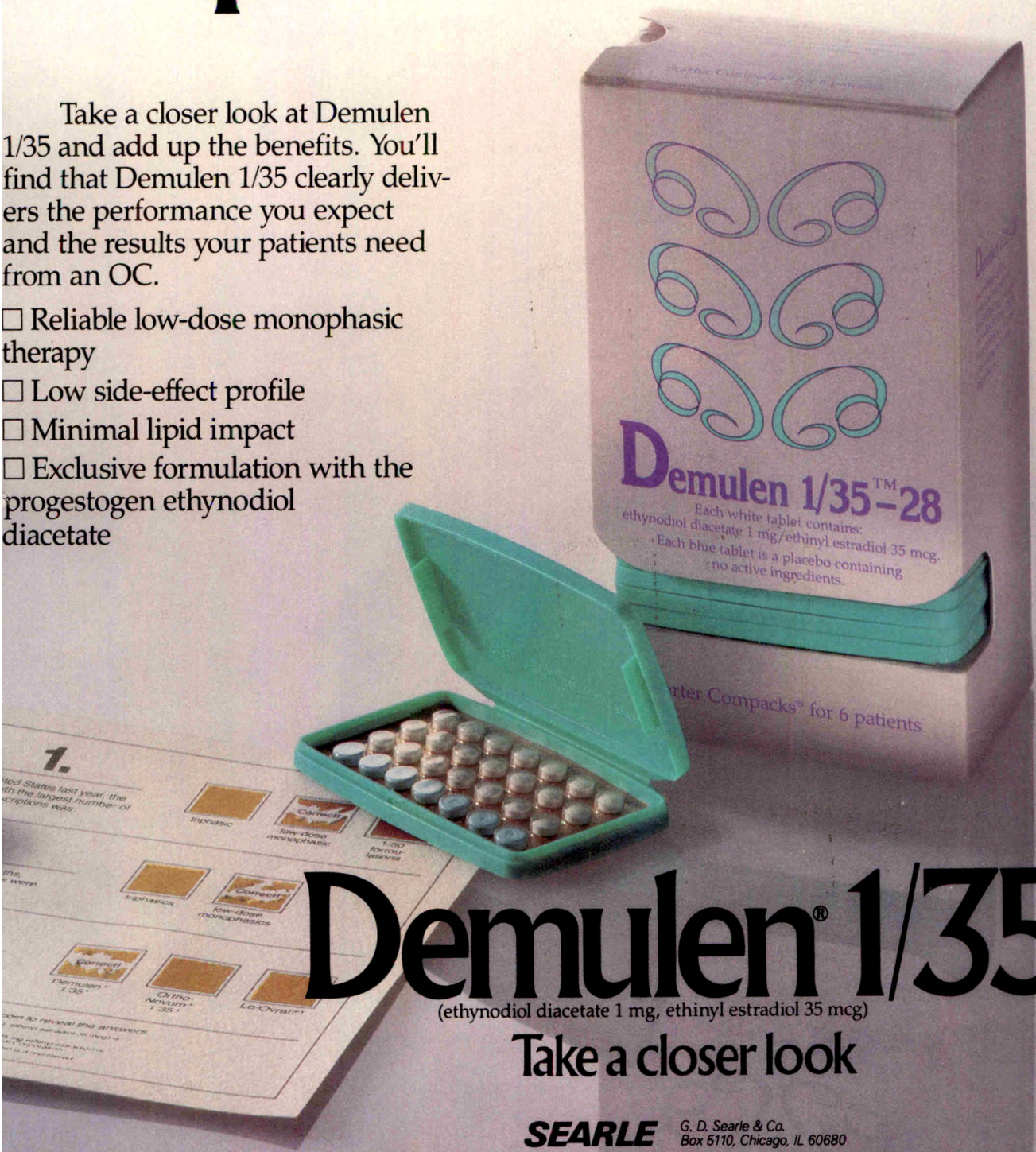
*Includes all monophasic products with 3% or greater market share.
†Pharmaceutical Data Services, Scottsdale, AZ.
‡Data derived from the change (by percentage) in the number of new prescriptions for all multiphasic products combined, from October 1987 to March 1989.

The physician should become familiar with the full prescribing information (labeling) for this product, which discusses reported risks, such as thromboembolic disorders (including strokes and heart attacks) and hepatic lesions, as well as the need to monitor patients for early symptoms of any diseases or medical conditions, so use can be discontinued where appropriate. A brief summary of the prescribing information accompanies this advertisement.

a growth phase—
a triphasic.

Take a closer look at Demulen 1/35 and add up the benefits. You'll find that Demulen 1/35 clearly delivers the performance you expect and the results your patients need from an OC.

- ☐ Reliable low-dose monophasic therapy
- ☐ Low side-effect profile
- ☐ Minimal lipid impact
- ☐ Exclusive formulation with the progestogen ethynodiol diacetate



Demulen® 1/35

(ethynodiol diacetate 1 mg, ethinyl estradiol 35 mcg)

Take a closer look

SEARLE G. D. Searle & Co.
Box 5110, Chicago, IL 60680

© 1989, G.D. Searle & Co.

Please see brief summary of prescribing information on following page.

A89-A7450T

Demulen® 1/35

(ethynodiol diacetate 1 mg, ethinyl estradiol 35 mcg)

Demulen 1/35™ Each white tablet contains ethynodiol diacetate 1 mg and ethinyl estradiol 35 mcg. Each blue tablet in Demulen 1/35-28 is a placebo containing inert ingredients.

Demulen 1/50™ Each white tablet contains ethynodiol diacetate 1 mg and ethinyl estradiol 50 mcg. Each pink tablet in Demulen 1/50-28 is a placebo containing inert ingredients. **Indication and usage:** For prevention of pregnancy. Pregnancy rate for products with ≥35 mcg ethinyl estradiol or ≥50 mcg mestranol is ≤1/100 woman-years. With ≤35 mcg ethinyl estradiol, rate is slightly higher; with progestogen-only oral contraceptives (OCs), rate is about 3. Precise comparisons cannot be made. Rates for other methods have been published. *Dose-related Thromboembolic Risk*: Studies showed positive association between OC estrogen dose and thromboembolic risk. It is in keeping with good therapeutics to minimize estrogen exposure. Prescribe product containing least amount of estrogen compatible with acceptable pregnancy rate and patient acceptance. Start new users on ≤50 mcg estrogen. **Contraindications:** Thrombophlebitis, thromboembolic disorders, past history of deep vein thrombophlebitis or thromboembolic disorders; cerebrovascular disease, myocardial infarction or coronary artery disease, or past history of these; known or suspected breast carcinoma, estrogen-dependent neoplasia, or pregnancy; undiagnosed abnormal genital bleeding; past or present, benign or malignant liver tumors developing during use of estrogenic products. **Warnings:**

Cigarette-smoking increases risk of serious cardiovascular side effects from OC use. Risk increases with age and heavy smoking (15 or more cigarettes/day) and is quite marked in women over 35. OC users should be strongly advised not to smoke. OCs are associated with increased risk of several serious conditions including venous and arterial thromboembolism, thrombotic and hemorrhagic stroke, myocardial infarction, visual disorders, hepatic tumors, gallbladder disease, hypertension, fetal abnormalities. Practitioners should be familiar with the available information relating to these and other risks.

1. *Thromboembolic disorders and other vascular problems:* Increased risk of thromboembolic and thrombotic disease associated with OCs is well established. A British study showed increased risk for fatal venous thromboembolism (TE). British and U.S. studies showed increased risk for nonfatal disease. U.S. studies showed increased risk for stroke. OC users were 2-11 times more likely to manifest these diseases without evident cause. In a study of idiopathic deep vein thrombosis and pulmonary embolism (PE), projected hospitalization rates for ages 16-40 were 47/year/100,000 users and 5 for nonusers. Overall excess mortality from PE or stroke was 1.3-3.4 deaths/year/100,000 users and increased with age. *Cerebrovascular Disorders:* In a study of stroke with and without predispositions, risk of hemorrhagic stroke was 2 times greater and thrombotic stroke 4.0-9.5 times greater in users. Mortality trends in 21 countries indicate that changes in cardiovascular mortality were associated with changes in OC prevalence. The Royal College of GPs (RCGP) reported on its prospective study: "A statistically significant higher rate of reporting of cerebrovascular accidents in Takers is evident, but the numbers are too small to justify an estimation of the degree of risk." A 1981 analysis showed a 4-fold increased mortality in users from circulatory diseases, mainly myocardial infarction (MI) and hemorrhagic stroke. Excess mortality was associated with age and smoking. A 1983 analysis showed elevated cerebrovascular disease, increasing with use to 8 yrs, including cerebral thrombosis or embolism and TIA. Peripheral arterial disease and MI were increased. Women over 30 had more arterial disease, being greatest in smokers. Arterial case-fatality was 2-3 times greater for users who smoked. Family Planning Assoc. (FPA) found significant association between OC use and stroke; increased risk for venous thrombosis and PE was 4-fold and statistically significant. Mortality from nonrheumatic heart disease was greater in ever-users. In the Walnut Creek Study, subarachnoid hemorrhage risk was associated with smoking, age, and OC use. *Myocardial Infarction:* Increased risk of MI associated with OCs was reported in Britain, as previously suspected. The morbidity study found the greater the number of risk factors for coronary artery disease (smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia) the higher the risk, regardless of OC use. OCs were an additional risk factor. Excess rate of fatal MI was 3.5/year/100,000 users aged 30-39 and 20 for ages 40-44. (These are based on British statistics with acute MI death rates 2-3 times less than the U.S. Similar estimates for the U.S. are 25.7 for users aged 30-39 with predispositions vs 1.5 without; for ages 40-44, 86.2 vs 5.1.) Excess hospitalization rate for nonfatal MI in Britain was 3.5/year/100,000 users aged 30-39 and 47 for ages 40-44. Smoking major predisposing condition to MI. In terms of relative risk, nonsmoking OC users are twice as likely to have fatal MI as nonsmoking nonusers. Smoking users have a 5-fold greater risk than nonsmoking users, but a 10-12-fold greater risk than nonsmoking nonusers. Amount of smoking is an important factor. In determining importance of these risks, give serious consideration to rates in table below. Importance of other predispositions cited above in determining relative and absolute risks has not been quantified; it is likely the same synergism exists, but perhaps to a lesser extent. U.S. studies similarly relate MI, OCs, smoking, and age.

Estimated Mortality Rate from Myocardial Infarction per 100,000 Women/Year

	Aged 30-39:	*Users*	*Nonusers*	*Aged 40-44:*	*Users*	*Nonusers*
All smokers		10.2	2.6		62.0	15.9
≥15 cigarettes daily		13.0	5.1		78.7	31.3
<15 cigarettes daily		4.7	0.9		28.6	5.7
Nonsmokers		1.8	1.2		10.7	7.4
Smokers and nonsmokers		5.4	1.9		32.8	11.7

Risk of Dose: Reports of TE after use of OCs with ≥50 mcg estrogen received by drug safety committees in Britain, Sweden, and Denmark were compared with distribution expected from market research sales estimates. A positive correlation was found between estrogen dose and reporting of TE, including coronary thrombosis, in excess of that predicted by sales. OCs with ≥100 mcg estrogen were associated with higher TE risk than those with 50-80 mcg. Estrogen quantity may not be the sole factor; influence of progestogens was not considered, which may be responsible for certain discrepancies in the data. No significant differences were noted between OCs containing same estrogen dose nor between ethinyl estradiol and mestranol. In Britain certain TE conditions were associated with progestogen or estrogen dose. In Sweden reports of TE decreased when higher estrogen doses were no longer prescribed. Studies on TE risk with progestogen-only OCs have not been done. Cases have been reported for these; they are not presumed free of risk. In a U.S. case-controlled study, relative risk of OC use 1 month before hospitalization for various types of TE was calculated. If relative estrogenic potency and possible influence of different progestogens are ignored, OCs may be divided into those with <100 mcg and those with ≥100 mcg estrogen. For all cases combined, larger doses were associated with only slightly higher risk; for idiopathic cases risk was doubled with larger doses, but confidence limits overlapped and differences were not significant. Apparently there was less increased risk for cases with predispositions. In FPA study no nonhemorrhagic strokes occurred with <50 mcg estrogen, vs 13 with higher doses. TE risk in users and nonusers increases with age. OCs have been considered an independent risk factor. *Persistence of Risk* after OC discontinuation was reported for circulatory disease, nonrheumatic heart disease, and cerebrovascular disease, including subarachnoid hemorrhage, cerebral thrombosis, and TIA. *Excess Mortality from Circulatory Diseases:* A U.K. prospective study estimated annual mortality from circulatory diseases for OC users and nonusers by age, smoking, and duration of use. Excess mortality was 23/100,000 users (2 and 10 for ages 25-34, 15 and 48 for ages 35-44, 41 and 179 for ages >44, for nonsmokers and smokers, respectively). Risk was significant only for smokers over 34. Most deaths were due to subarachnoid hemorrhage or ischemic heart disease. Risk for ever-users rose with parity. One study estimated risk of death with various contraceptive methods, each estimate including risk of method (eg, TE for OCs) plus risk due to pregnancy or abortion in cases of method failure. This latter risk varies with effectiveness. It concluded mortality with all methods is low and below that of childbirth, except for OC users over 40 who smoke. Heavy smokers have double the risk of smokers, light smokers about half. Mortality with OC use in nonsmokers over 40 is higher than with any other method in that age group. Lowest mortality is with condom or diaphragm backed up by early abortion. Risk of TE and thrombotic disease with OCs increases with age after 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, history of preeclamptic toxemia, and especially cigarette smoking. Based on current data, the chart gives gross estimates of risk of death from circulatory disorders associated with OCs:

Risk is: A—very high; B—high; C—moderate; D—low. Age:	<30	30-39	≥40
Heavy smokers (≥15/day)	C	B	A
Light smokers (<15/day)	D	C	B
Nonsmokers, no predispositions	D	C,D	C
Nonsmokers, with predispositions	C	C,B	B,A

Be alert to earliest manifestations of TE and thrombotic disorders (eg, thrombophlebitis, PE, cerebrovascular insufficiency, coronary artery disease, MI, retinal thrombosis, mesenteric thrombosis). Should any be suspected, discontinue OCs immediately. A 4-7-fold increased risk of postsurgery TE was reported in users; if feasible discontinue OCs at least 4 weeks before surgery or in prolonged immobilization. Before resuming OCs, weigh risks of postsurgery TE complications against contraceptive needs. The RCGP prospective study reported higher incidence of superficial and deep vein thrombosis in users, the former correlated with progestogen dose. The data suggest varicose veins have little effect on development of deep vein thrombosis but substantially increase risk of superficial vein thrombosis, risk depending on severity of varicosities. **2.** *Ocular lesions:* Neuro-ocular lesions such as optic neuritis or retinal thrombosis have been associated with OCs. Discontinue if there is unexplained, gradual or sudden, partial or complete loss of vision; proptosis or diplopia; papilledema; or evidence of retinal vascular lesions. Institute diagnostic and therapeutic measures. **3.** *Carcinoma:* Long-term estrogen in certain animals increases carcinomas and/or nonmalignant neoplasms, such as those of breast, uterus, cervix, vagina, ovary, liver, pituitary. Some synthetic progestogens, none currently in OCs, increase incidence of benign and malignant mammary nodules in dogs. There is evidence that estrogens increase risk of human endometrial carcinoma. In case-controlled studies, increased risk (2.2-13.9 times) associated endometrial carcinoma with prolonged estrogen for menopausal symptoms. Risk was independent of other known risk factors and depended on duration of use and dose. This is supported by increased endometrial cancer incidence since 1969 in areas with cancer-reporting systems, which may be related to rapidly expanding estrogen use. There is no evidence that natural estrogens are more or less hazardous than synthetics at equi-estrogenic doses. Of the first 30 cases endometrial carcinoma in OC users under 40, those without predispositions (eg, irregular bleeding before OCs, polycy ovaries) occurred nearly always with no-longer-marketed sequential OCs. No statistical association is reported suggest increased endometrial cancer with combination or progestogen-only OCs, though individual cases were reported. Studies sh no increased breast cancer with OCs or estrogens. However, greater risk was noted in OC users with benign breast dise or long-term (2-4 years) use. One study found greater risk of breast cancer with >4 years OC use prior to first full-te pregnancy. Risk of breast cancer with menopausal estrogens rose and further increased with duration of follow-up. OCs estrogen were related to breast cancer in connection with other risk factors. Decrease in benign breast tumors in OC users well known. Some studies suggested increased risk of cervical dysplasia, erosion, and carcinoma in long-term users. Cervi microglandular hyperplasia has been reported. In summary, there is no consistent evidence from human studies of increas cancer risk with OCs. Close surveillance is, however, essential. In undiagnosed persistent or recurrent abnormal vagi bleeding, consider nonfunctional causes and take diagnostic measures to rule out malignancy. Carefully monitor users w strong family history of breast cancer or with breast nodules, fibrocystic disease, recurrent cystic mastitis, abnorm mammograms, or cervical dysplasia. **4.** *Hepatic adenomas, etc,* have been associated with OCs. In 1 study OC formulatic with high "hormonal potency" were associated with higher risk, as was age over 30. Though benign, these lesions m rupture and cause fatal hemoperitoneum in short- or long-term users. Two studies related risk to duration of use, being mu greater after ≥4 years. Long-term OC users have an estimated adenoma incidence of 3-4/year/100,000. Consider su lesions in users with abdominal pain, tenderness, mass, or shock. About 25% present with abdominal masses, up to h with acute hemoperitoneum. Radiologic and lab studies may not be helpful; liver scans may show local defect. Hepa arteriography or CAT may be useful. Hepatocellular carcinoma risk is increased 7-20 times in users for 8 or more years. B duct cancer may occur with OC use. OCs are contraindicated in case of past or present, benign or malignant liver tumc developing during use of estrogenic products. **5.** *Use in or immediately preceding pregnancy, birth defects, malignancy offspring:* Estrogens or progestogens during early pregnancy may seriously damage offspring. Females exposed in utero DES and other nonsteroidal estrogens may have increased risk of vaginal or cervical cancer. Though OCs may not enhan this risk, monitor such patients carefully. From 30-90% of DES-exposed women develop benign vaginal or cervical adenos which could be premalignant. DES-exposed daughters have more unfavorable pregnancy outcomes. Males so exposed m develop urogenital and sperm defects. Other estrogens may mimic DES. Reports relate fetal exposure to female sex hormon and congenital anomalies, eg, VACTERL for vertebral, anal, cardiac, tracheoesophageal, renal, and limb defects. There appea preferential expression by males. There was a 4.7-fold increased risk of limb-reduction defects in infants exposed in utero sex hormones (OCs, hormonal withdrawal pregnancy tests, or treatment for threatened abortion). Some exposures were ve short, being only a few days. Cardiovascular defects in children exposed to female hormones, including OCs, during ea pregnancy occurred in 18.2/1,000 births, compared to 7.8 for those unexposed (P<.05). More twinning was reported conceptions shortly after OC discontinuance. Female sex hormones were used for threatened or habitual abortion, but the is considerable evidence estrogens are ineffective and no evidence progestogens are effective. Triploidy and possibly oth polyploidy may be increased in abortuses from women becoming pregnant soon post-OC. These almost always abc spontaneously. It is unknown if spontaneous abortion is increased soon after stopping OCs. Increased chromosome aberratio have been reported. Safety of OCs in pregnancy has not been demonstrated. Rule out pregnancy in using OCs and alway consider it if withdrawal bleeding does not occur. If 2 consecutive periods are missed rule out pregnancy before continuin If user deviates from regimen, consider pregnancy at first missed period and withhold OCs. If pregnancy is confirmed, appris patient of potential fetal risks and discuss continuation of pregnancy. Users discontinuing for pregnancy should use alternati contraception for 3 months before attempting to conceive, though this is not based on precise information. Do not us progestogen-only or progestogen-estrogen drugs as pregnancy tests. **6.** *Gallbladder disease* risk, surgically confirme appeared after 2 years' use and doubled after 4-5 years in 1 study; it appeared in 6-12 months' use in another. **7.** *Carbohydra and lipid metabolism:* Decreased glucose tolerance occurs in a significant number of users; carefully observe diabetic an prediabetic patients. Triglycerides, total phospholipids, and lipoproteins may increase. **8.** *Elevated blood pressure* (BP) occu in some users in a few months, and degree may relate to progestogen dose. Hypertension prevalence in users is not highe in the first year, but increases with longer exposure; in the 5th year it is 2½—3 times the first year. Age is strongly correlate with hypertension in users. Those with history of elevated BP (hypertension), preexisting renal disease, history of toxemia elevated BP in pregnancy, familial tendency to hypertension or its consequences, or history of excessive weight gain or flu retention during menstrual cycles may be more likely to develop elevated BP on OCs and should be monitored closely. Eve though elevated BP may be in "normal" range, do not ignore implications. Follow closely, particularly those with other ris factors for cardiovascular disease or stroke. High BP may or may not persist after stopping OCs. **9.** *Headache:* Onset exacerbation of migraine or development of headache of a new pattern that is recurrent, persistent, or severe requires stoppir OCs and evaluating cause. **10.** *Bleeding irregularities:* Breakthrough bleeding (BTB), spotting, and amenorrhea are freque reasons for discontinuing. In BTB, as in all cases of irregular bleeding, consider nonfunctional causes. In undiagnose persistent or recurrent bleeding, rule out pregnancy or malignancy. If pathology is excluded, time or change to another produ may suffice. Change to a higher-estrogen OC only when necessary as this may increase thromboembolic risk. Those wit history of oligomenorrhea or secondary amenorrhea or without established regular cycles may be anovulatory or amenorrhe after discontinuing. Those with such problems should be so informed and other methods encouraged. Post-use anovulatio possibly prolonged, may occur in women without previous irregularities. Higher incidence of galactorrhea and of pituitar tumors has been associated with postpill amenorrhea. One study reported 16-fold increased prevalence of pituitary prolactir secreting tumors in patients with postpill amenorrhea when galactorrhea was present. **11.** *Ectopics:* In failures with combinatio OCs, the ratio of ectopic to intrauterine pregnancies is no higher than in nonusers. There may be increased risk of ectopic after discontinuing. **12.** *Breast feeding:* OCs may decrease quantity and quality of milk. A small fraction of OC steroids appear in milk. Effects if any on child are unknown. If feasible defer OCs until after weaning. **13.** *Infertility* may occur after discontinuin OCs. While impairment diminishes with time, there is still an appreciable difference in nulliparas aged 25-29 after 30 months difference is negligible after 48 months. In nullips aged 30-34 impairment persists to 72 months and is more severe for 2 year users. In paras aged 25-34, difference is negligible after 30 months. **Precautions:** Conduct complete history and physica prior to OC use and annually thereafter. Include breasts, abdomen, and pelvis, with BP, Pap, and lab tests. Preexisting uterin leiomyomata may enlarge. Mental depression may occur; with positive history, observe carefully and discontinue if seriou recurrence. Fluid retention may occur; prescribe cautiously and monitor carefully in convulsive disorders, migraine, asthma or cardiac, hepatic, or renal dysfunction. History of jaundice in pregnancy increases risk of recurrence on OCs; observ carefully and discontinue if it occurs. Cholestatic jaundice occurred after OC and troleandomycin use. OC/cyclosporin combination may be hepatotoxic. Steroids may be poorly metabolized in liver dysfunction and should be given cautiously Tryptophan metabolism may be disturbed and result in pyridoxine deficiency; megaloblastic anemia has been reported. Serun folate may be depressed and may be exacerbated in post-OC pregnancy. Advise pathologist of OC use when submittin specimens. Endocrine and liver function tests may be altered; repeat abnormal tests after stopping for 2 months. These hav been observed: increased BSP retention and other abnormalities in liver function tests; increased prothrombin and coagulatio factors VII, VIII, IX, X; decreased antithrombin III, increased platelet aggregability; increased thyroid-binding globulin leadin to increased circulating total thyroid hormone (PBI, T_4); decreased T_3 uptake, unaltered free T_4, decreased pregnanedic excretion; reduced metyrapone response; increased blood transcortin, corticosteroid, triglyceride, phospholipid, reduced serun folate; impaired glucose tolerance; altered plasma trace minerals. Influence of prolonged OC use on pituitary, ovarian, adrenal hepatic, or uterine function, or on immune response not established. OCs may mask onset of climacteric. Cervical chlamydia prevalence is increased; do not assume OC protects against chlamydial PID. *Information for patients.* See patient labeling *Drug interactions.* OC effectiveness may be decreased and more BTB occur with rifampin, isoniazid, ampicillin, neomycin penicillin V, tetracycline, chloramphenicol, sulfonamides, nitrofurantoin, griseofulvin, barbiturates, phenytoin, carbamazepine primidone, phenylbutazone, analgesics, tranquilizers, antimigraines. OCs may alter effectiveness of oral anticoagulants anticonvulsants, tranquilizers, tricyclic antidepressants, antihypertensives, theophylline, caffeine, vitamins, hypoglycemics, clofibrate, glucocorticoids, acetaminophen. **Adverse reactions:** Increased risk of the following serious adverse reactions has been associated with OCs: thrombophlebitis and thrombosis; pulmonary embolism; arterial thromboembolism; Raynaud's disease, myocardial infarction; coronary thrombosis; cerebral thrombosis; cerebral hemorrhage; hypertension; gallbladder disease; Budd-Chiari syndrome; benign adenomas and other hepatic lesions, with or without intra-abdominal bleeding, liver cancer, congenital abnormalities. There is evidence of association between the following and OCs, though confirmatory studies have not been done: mesenteric thrombosis; neuro-ocular lesions, eg, retinal thrombosis and optic neuritis. The following are believed drug related: nausea, vomiting, gastrointestinal symptoms, BTB, spotting, change in menstrual flow, dysmenorrhea, amenorrhea during and after use, infertility post-OC, edema, chloasma or melasma, which may persist post-OC, breast changes (tenderness, enlargement, secretion), weight changes, change in cervical erosion and secretion, endocervical hyperplasia, lactation decrease, cholestatic jaundice, migraine, enlarged uterine leiomyomata, allergic rash, mental depression, reduced carbohydrate tolerance, vaginal candidiasis, change in corneal curvature (steepening), intolerance to contact lenses. Association not confirmed or refuted for: premenstrual-like syndrome, cataracts, libido changes, chorea, appetite changes, cystitis-like syndrome, headache, paresthesia, nervousness, dizziness, auditory disturbances, rhinitis, fatigue, backache, hirsutism, scalp hair loss, erythema multiforme or nodosum, hemorrhagic eruption, herpes gestationis, hemolytic uremia, malignant hypertension, itching, vaginitis, porphyria, impaired renal function, acute renal failure, sometimes irreversible, PVCs, abnormal EKG, pulmonary hypertension, TTP, anemia, pancreatitis, hepatitis, colitis, Crohn's disease, gingivitis, dry socket, lupus erythematosus, rheumatoid arthritis, pituitary tumors (eg, adenoma) with amenorrhea and/or galactorrhea after use, malignant melanoma, endometrial, cervical, breast, and bile duct carcinoma. **Acute overdosage:** Serious ill effects have not been reported after large acute doses of OCs in young children. Nausea and withdrawal bleeding might occur. **Important note: Complete prescribing information should be consulted prior to use and is available on request.**

9/21/87 • P87-A4830V

SEARLE

Searle & Co.
San Juan, PR 00936

Address medical inquiries to: G.D. Searle & Co.
Medical & Scientific Information Department
4901 Searle Parkway, Skokie, IL 60077

OB/GYN

Bay Shores Medical Group offers outstanding opportunities for board eligible/certified physicians to join rapidly expanding 80-physician multi-specialty group practice. Southern California community, close to ocean, easy access to city and recreational areas, excellent schools.

Please direct any inquiries and CV to:
Bradford B.Burnett, M.D.
Medical Director
Bay Shores Medical Group, Inc.
502 Torrance Boulevard
Redondo Beach, CA 90277
(213) 543-7247



Enhance your knowledge and clinical skills with . . .

Operative Gynecologic Endoscopy

edited by **Joseph S. Sanfilippo** and **Ronald L. Levine**

Operative Gynecologic Endoscopy is a clinically oriented text that covers both the theory and practice of basic to advanced endoscopic techniques. Noted authorities carefully detail procedures for operative endoscopy, laser laparoscopy, operative hysteroscopy, and laser hysteroscopy.

Only *Operative Gynecologic Endoscopy* covers the aspects of gynecologic endoscopy unique to practice in North America. Contributors to the volume are well-known clinicians and scientists from 24 leading North American institutions. They are the people who have pioneered the use of endoscopy to improve the healthcare of gynecologic patients.

Learn the best approach. The authors help you to decide which approach is best suited to a wide range of gynecologic pathologies and conditions, including ectopic pregnancy, pelvic inflammatory disease, ovarian cysts, infertility, and endometriosis.

Plus — An insightful history by Kurt Semm . . . How to choose the right equipment . . . Description of photographic equipment and techniques including video . . . Safety precautions and basic trouble-shooting methods . . . The role of operating room personnel . . . Medico-legal aspects . . . Economic impact . . . Credentialing and the future of gynecologic endoscopy.

Order your copy today to have access to the very best reference available on gynecologic endoscopy.
1989/325 pp., 156 figs./
0-387-96881-4/cloth $79.95
(A volume in the Clinical Perspectives in Obstetrics and Gynecology series)

Order Form
30-day return privilege guaranteed
Use this coupon, or call **1-800-SPRINGER**
(in NJ please call 201-348-4033)

Please send me **Operative Gynecologic Endoscopy,** Sanfilippo/Levine, #96881-4, $79.95
(Please include $2.50 for shipping, and sales tax if you reside in NY, NJ, or CA)
☐ Check enclosed
☐ Charge ☐ VISA ☐ AMEX ☐ MasterCard

Account #_____ Expires _____

Signature _____
☐ Bill me
Send to:

Name _____

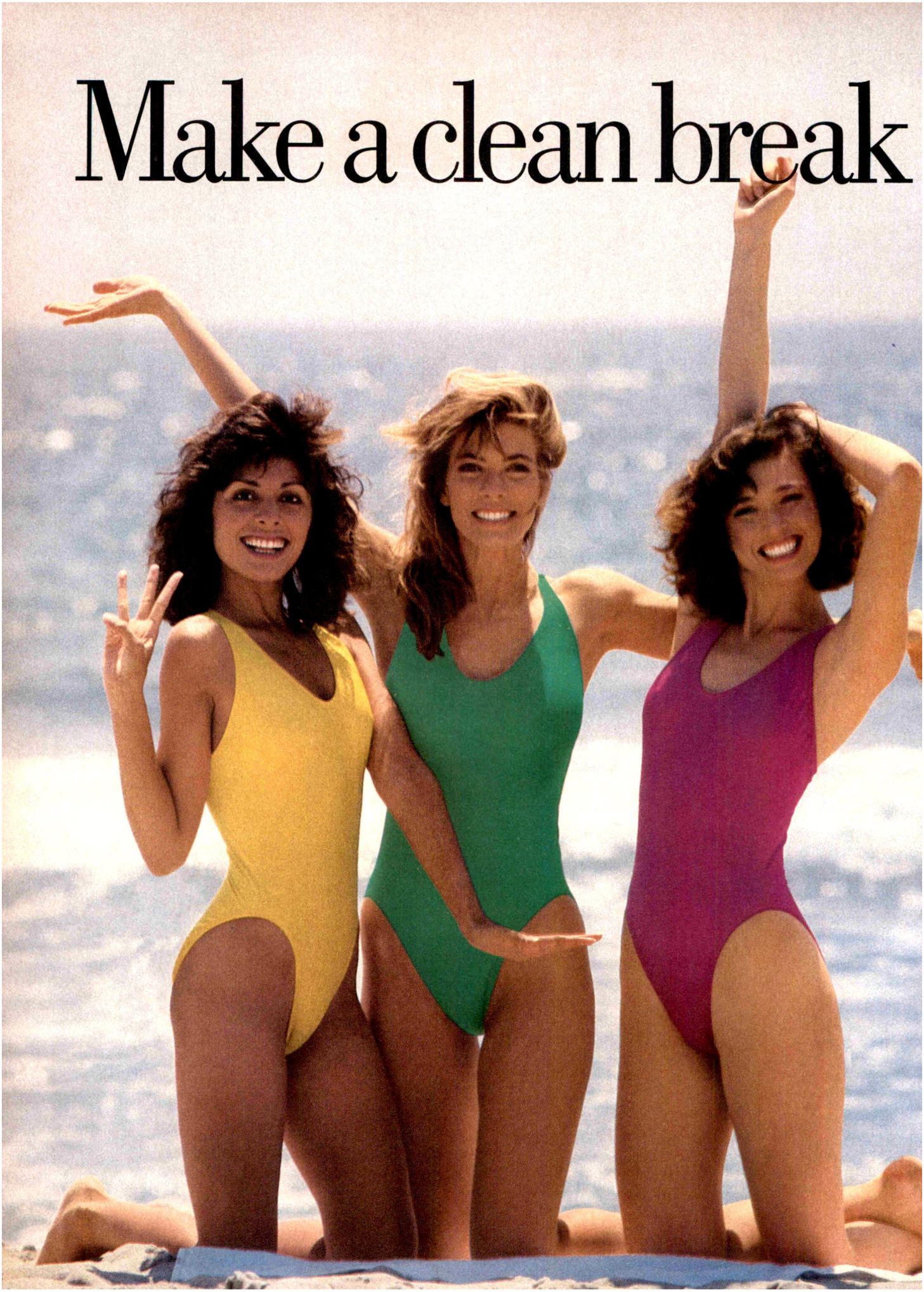
Address _____

City, State, Zip _____

Return to: Springer-Verlag New York, Inc.,
Attn. E. Teatsorth, 175 Fifth Ave., NY, NY 10010.
Please allow 4-6 weeks for delivery. Orders which cannot be filled within 90 days will be refunded.
Prices subject to change without notice.

Springer-Verlag
New York Berlin Heidelberg Vienna
London Paris Tokyo Hong Kong

Make a clean break



to 3-day therapy*

Cure vulvovaginal candidiasis in just three days*

Broad spectrum of activity

- Cures infections due to *Candida albicans* and *Candida tropicalis* within 3 days.

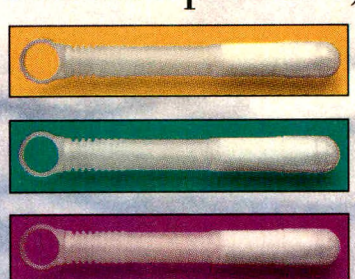
Documented 3-day efficacy

- Microbiological cure rates of 95% eight days following Femstat 3-day therapy.[1]
- Low relapse rates 30 days post therapy.

Femstat Prefill is:

- NOT a triazole/NO flu-like symptoms.
- Safe in second- and third-trimester pregnant patients.*

Patented prefilled, disposable applicators



- Disposable to reduce risk of reinfection.
- Prefilled for unit-dose accuracy.
- Designed for enhanced patient compliance.

*In pregnant patients (second and third trimesters only), therapy should be extended to six days.

vaginal cream 2%

Femstat® Prefill

(butoconazole nitrate)

™

Make it your choice for unsurpassed efficacy and convenience.

1. Droegemueller W, Adamson DG, Brown D, et al: Three-day treatment with butoconazole nitrate for vulvovaginal candidiasis. *Obstet Gynecol* 1984;64(4):530-534.

Please see brief summary of prescribing information on following page. © 1989 Syntex Laboratories, Inc.

SYNTEX

SYNTEX LABORATORIES, INC.
PALO ALTO, CA 94304

vaginal cream 2%

Femstat® Prefill

(butoconazole nitrate)



BRIEF SUMMARY

INDICATIONS: FEMSTAT® (butoconazole nitrate) vaginal cream 2.0% is indicated for local treatment of vulvovaginal mycotic infections caused by Candida species. Confirm the diagnosis by KOH smears and/or cultures. FEMSTAT can be used with oral contraceptives and antibiotics. It is effective in non-pregnant women and during the second and third trimesters of pregnancy.

CONTRAINDICATIONS: FEMSTAT is contraindicated in patients hypersensitive to any of the ingredients.

PRECAUTIONS: General: If clinical symptoms persist, repeat microbiological tests to rule out other pathogens and confirm the diagnosis. Discontinue drug if sensitization or irritation occurs.

Information for the Patient: Do not discontinue prematurely during menstruation or because of symptomatic relief.

Carcinogenesis: Animal studies have not been done.

Mutagenesis: Mutagenicity studies were negative.

Impairment of Fertility: Animal studies showed no impairment of fertility.

Pregnancy Category C: Adverse effects were noted in animals treated with high oral doses. No studies were done in women during first trimester. Patients in the second or third trimester have shown no adverse effects attributable to the drug.

Nursing Mothers: Use with caution.

Pediatric Use: Safety and efficacy have not been established.

ADVERSE REACTIONS: Vulvar/vaginal burning in 2.3% of patients, vulvar itching in 0.9%, discharge, soreness, swelling, itching of fingers each in 0.2%. Complaints caused 1.6% to discontinue drug.

DOSAGE AND ADMINISTRATION:
Non-pregnant Patients: The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for three days. Treatment can be extended for an additional three days if necessary.

Pregnant Patients (second and third trimesters only): The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for six days.

CAUTION:
Federal law prohibits dispensing without prescription.

SYNTEX

SYNTEX LABORATORIES, INC.
PALO ALTO, CA 94304

2V MEDTECH INTERNATIONAL CORP.

DIAGNOSIS



TREATMENT

PRESENTS

2V STAT Medical Decision Support Software Covering 69 Specialities – Algorithms and Text.

PLUS

COMPUTER 80286/10 MHz Turbo CPU, 80 MB HD, EGA Color Monitor, Color Graphic Card, 40 MB Back-up Drive, and Printer.

$5962
(for complete system)

ORDER NOW!
1•800•228•STAT
Technical Support Call 1•404•956•1855

Add volume to your practice and make an extra $500K with only a $5,962 one-time investment with 2V STAT including managerial support, brochures, +/- one-day seminar.

2480 Windy Hill Road, Suite 201 • Marietta, Georgia 30067
Fax 404•956•7068 Telex 551341 MedVideo Easy Link 622779660

KAISER PERMANENTE

OB/GYN

OB/GYN BOARD CERTIFIED OR ELIGIBLE to join large multi-specialty group in San Francisco. Large volume tertiary care obstetrical service; over 3400 deliveries per year; 18 staff OB/GYN; 4 year residency program in OB/GYN. Benefits include excellent vacation and educational leave, medical and dental coverages, malpractice and life insurance, disability, retirement program. Academic appointment and clinical research available.

Send inquiry and C.V. to:

Ira M. Golditch, M.D.
Chief, Dept. of Ob/Gyn
Kaiser Permanente Medical Center
2200 O'Farrell Street
San Francisco, CA 94115

or call
(415) 929-5604

TRANSACTIONS OF THE NINTH ANNUAL MEETING OF THE SOCIETY OF PERINATAL OBSTETRICIANS

Management of women with one abnormal oral glucose tolerance test value reduces adverse outcome in pregnancy

Oded Langer, MD, Akolisa Anyaegbunam, MD, Lois Brustman, MD, and Michael Divon, MD

Bronx, New York

In this study we sought to test the hypothesis that treatment of women with one abnormal oral glucose tolerance test value will result in reduction of adverse outcome. One hundred twenty-six women with one abnormal oral glucose tolerance test value and 146 women in the control group (normal oral glucose tolerance test values) participated in a prospective study during the third trimester of pregnancy. The subjects with one abnormal test result were randomized into treated (group 1) and untreated groups (group II). Group 1 subjects were treated with a strict diabetic protocol to maintain tight glycemic control by means of diet and insulin therapy. Group 2 subjects tested their capillary blood glucose for a baseline period. The study revealed that the level of glycemic control was similar before initiation of therapy (mean capillary blood glucose 118 ± 14 vs. 119 ± 15 mg/dl, p = NS) for groups 1 and 2, respectively. There was a significant difference in mean capillary blood glucose (95 ± 10 vs. 119 ± 15 mg/dl, $p < 0.0001$), preprandial, and postprandial determinations between the treated and untreated groups. The overall incidence of neonatal metabolic complications (4% vs. 14%, $p < 0.05$) and large infants (6% vs. 24%, $p < 0.03$) was significantly lower in the treated group. Comparison between the control (normal oral glucose tolerance test) and the untreated groups showed a significantly higher incidence of large infants and metabolic complications. No difference was found between the normal and treated groups. Thus we conclude that treatment of individuals with one abnormal oral glucose tolerance test value will result in significant reduction in adverse outcome in pregnancy. (Am J Obstet Gynecol 1989;161:593-9.)

Key words: Treatment, oral glucose tolerance test, one abnormal value, pregnancy outcome

Controversy exists with regard to the precise criteria for diagnosis of gestational diabetes mellitus. The most accepted forms are the World Health Organization criteria in Europe and the National Diabetes Data Group in the United States, which were recognized at two international gestational diabetes conference workshops in 1979 and 1984.[1, 2] At least two values on the oral glucose tolerance test (GTT) must be abnormal to diagnose gestational diabetes mellitus. Previously we tested the hypothesis that women with one abnormal oral GTT value are at higher risk for adverse outcome in pregnancy. It was demonstrated that these women are metabolically (glucose levels) similar to patients with gestational diabetes (NDDG). In addition, the group with one abnormal test result had a high incidence of neonatal morbidity (mainly macrosomia and metabolic complications).[3] Recently our finding of an increased risk of fetal macrosomia in patients with one abnormal value on their oral GTT results was confirmed by other investigators.[4] Thus these women are the closest "model" for untreated gestational diabetics diagnosed by the National Diabetes Data Group criteria. In this study we sought to test the hypothesis that treatment of women with one abnormal value (oral GTT) will result in reduction of adverse outcome.

Three objectives were identified to initiate investi-

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, The Albert Einstein College of Medicine.
Supported in part by an educational grant from Miles Laboratories.
Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.
Reprint requests: Oded Langer, MD, Department of Obstetrics and Gynecology, The University of Texas Health Science Center, 7703 Floyd Curl Dr., San Antonio, TX 78284-7836.
6/6/14089

Table I. Patient characteristics

	Group 1: Treated (n = 63)	*Group 2: Untreated (n = 63)*	*Group 3: Control (n = 146)*	*p*
Maternal age (yr)	31 ± 5*	28 ± 6	27 ± 5	<0.005
Nulliparous women (% and No.)	29 (18)	32 (20)	27 (39)	
Race (% and No.)				
Black	30 (19)	33 (21)	30 (44)	NS
Hispanic	33 (21)	33 (21)	34 (50)	NS
White	36 (23)	33 (21)	36 (53)	NS
Gestational age at screening (wk)	27 ± 3	27 ± 2	28 ± 5	NS
Screening <140 mg/dl (% and No.)	16 (10)	14 (9)	100 (146)	—
Gestational age at diagnosis (wk)	31 ± 3	31 ± 3	32 ± 4	NS
Obesity (% and No.)	38 (24)	41 (26)	30 (44)	NS
Family history of diabetes (% and No.)	25 (16)	22 (14)	28 (41)	NS
Previous LGA infant (% and No.)	29 (18)	29 (18)	9* (13)	<0.002
Previous stillbirth (% and No.)	5 (3)	5 (3)	2 (3)	NS

LGA, Large-for-gestational-age.
*Group 1 versus groups 2 and 3.

gation of this hypothesis: (1) to determine the glycemic profile in treated and untreated women with one abnormal value, (2) to ascertain the relationship between maternal and neonatal outcome in untreated and treated women with one abnormal value, and (3) to compare pregnancy outcome in normal women and women with one abnormal value.

Material and methods

Identification of study population. At our medical center all pregnant women are routinely screened for gestational diabetes between 24 and 28 weeks' gestation. Screening consists of a 50 gm oral glucose load. If the 1-hour plasma glucose value is ≥130 mg/dl, the patient is scheduled for a full 3-hour oral GTT. To assure consistency in testing procedure, all women are instructed to add 150 gm of carbohydrate to their usual meals for each of 3 days before the oral GTT. Interpretation of oral GTT results was made in accordance with the criteria established by the National Diabetes Data Group.[5] Patients are instructed to fast for 12 hours (overnight) before the test. The test is initiated between 8:30 and 9:00 AM.

Study design. Two hundred seventy-two women participated in this prospective study during the third trimester of pregnancy. The study group consisted of 126 patients with one abnormal value on their oral GTT. Written and informed consents were obtained from all subjects. They were randomized into treated (group 1) and untreated (group 2) groups. For the purpose of the study, women with screening results of <140 mg/dl (which are considered normal screening results according to the National Diabetes Data Group and the American College of Obstetricians and Gynecologists technical bulletin No. 92, May 1986) and normal oral GTT (all normal values) served as the control (group 3).

Blood glucose evaluation. All women with one abnormal value monitored their capillary blood glucose seven times a day with a memory-based reflectance meter to ascertain accurate and reliable data. Group 1 (treated subjects) was assigned to treatment modality in accordance with our diabetic management protocol. Briefly, diet treatment was determined by consideration of body mass index before pregnancy. For obese patients (defined as body mass index ≥27) 25 kcal/kg and for nonobese (body mass index <27) subjects, 30 kcal/kg was prescribed.

The insulin dose was calculated based on 0.7 U of insulin per kilogram of body weight measured in pregnancy. Human insulin was administered by multiple insulin injection regimen. The standard formula for the amount of insulin prescribed was two thirds of all insulin in the morning (2:1, intermediate-acting/regular insulin) and one third in the evening (1:1, regular/intermediate-acting). All subjects were treated with a strict diabetic protocol to maintain tight glycemic control (95 mg/dl). When diet therapy could not achieve this goal, insulin therapy was initiated.[6,7] Group 2 (untreated) subjects were instructed to continue their habitual eating patterns. They also were required to test their capillary blood glucose for a baseline period of 4 weeks.

Outcome evaluation criteria

Maternal. Selected variables (e.g., age, obesity, hypertensive disorder) associated with large infants were evaluated. Hypertensive disorders included pregnancy-induced hypertension and chronic hypertension. Pregnancy-induced hypertension was diagnosed by the presence of proteinuria (>0.5 gm/24 hr), blood pressure >140/90 mm Hg or mean arterial pressure >104 mm Hg. Chronic hypertension was defined as blood pressure >140/90 mm Hg on two separate occasions 6 hours apart, mean arterial pressure >104 mm Hg during the first trimester, or both. The cesarean section rate between the groups was compared.

Table II. Oral GTT result

	Group 1: Treated (n = 63)	*Untreated (n = 63)*	*Control (n = 146)*	*p*
Screening result (mean ± SD)	157 ± 21	150 ± 16	121 ± 15*	<0.0001
Oral GTT result (mg/dl)				
Fasting	93 ± 8	90 ± 11	86 ± 9	<0.0001
1 hr	185 ± 16	181 ± 28	148 ± 25	<0.0001
2 hr	156 ± 18	157 ± 18	123 ± 23	<0.0001
3 hr	116 ± 25	111 ± 26	107 ± 24	NS

Table III. Ambulatory glycemic profile before and after initiation of treatment

	Group 1: Treated (n = 63)		
	Baseline period only	*In treatment*	*Group 2: Untreated (n = 63) (baseline period only)*
Basline period (no. of days)	12 ± 6	73 ± 8	20 ± 6
Fasting (mg/dl)	100 ± 11	84 ± 9*	102 ± 12
Preprandial (mg/dl)	103 ± 11	86 ± 9*	105 ± 12
Postprandial (mg/dl)	130 ± 13	106 ± 10*	131 ± 13
Mean capillary blood glucose (mg/dl)	118 ± 14	95 ± 10*	119 ± 15

*$p < 0.0001$, baseline period versus in treatment.

Neonatal. Infants were considered large for gestational age if their birth weights were ≥ the 90th percentile and small for gestational age when their birth weights were ≤ the 10th percentile for a given gestational age.[8]

Hypoglycemia was defined as blood glucose <35 mg/dl,[9] and was evaluated in all study patients in the delivery room by Dextrostix. Low blood glucose levels were confirmed in the laboratory by the glucose oxidase method. Infants of women in the control group were tested only for symptoms of hypoglycemia. Other metabolic abnormalities included hyperbilirubinemia (bilirubin >12 mg/dl), hypocalcemia (calcium <7.5 mg/dl), and polycythemia (central venous hematocrit >65%). Admissions to the neonatal intensive care unit exceeding 24 hours and cases of respiratory distress syndrome were recorded.

Method of analysis. For purposes of analysis, data on the patients with one abnormal value were divided into two periods: period 1 (baseline before treatment) and period 2 (from initiation of treatment to delivery). Further analysis compared pregnancy outcome between the three groups. Categorical data were evaluated with χ^2 analysis and Fisher's exact test; continuous data were analyzed with Student's *t* test. Pearson's correlation coefficient was calculated for the relationships between level of glycemia and neonatal weight (birth percentile).

Results

Patient characteristics. During the 1-year study period, 3560 women underwent glucose screening; 2919 (82%) had normal results. Of the remaining 641 women with abnormal screening results, 93 had gestational diabetes, 126 had one abnormal value (groups 1 and 2), and 422 had normal oral GTT results. Of the latter group, 146 women had screening results <140 mg/dl (group 3, control). As shown in Table I, no significant difference in maternal age was found between the untreated and control groups. The treated patients were significantly older than the former two groups ($p < 0.005$). In this group, 65% (41 of 63) were diet-treated and 35% (22 of 63) required insulin therapy to achieve good glycemic control. A comparison of selected maternal characteristics of the three groups included race, parity, obesity, gestational age at screening and diagnosis, and revealed no statistically significant differences. In contrast, the subjects with one abnormal test result (groups 1 and 2) had an approximately fivefold greater history of previous macrosomia ($p < 0.009$) and a twofold higher incidence of previous stillbirth ($p < 0.01$).

Screening and oral GTT results. The screening and oral GTT results of group 3 women were significantly lower than those of groups 1 and 2. No difference was found between groups 1 and 2 (Table II). Within groups 1 and 2 the proportion of women with one abnormal value was 6% at fasting, 49% at 1 hour, 27% at 2 hours, and 10% at 3 hours.

Ambulatory glycemic profile

Period I. Table III shows the level of glycemia during the baseline period before initiation of treatment between groups 1 and 2. No significant difference was found in capillary mean blood glucose between the groups. In general, the postprandial values were higher

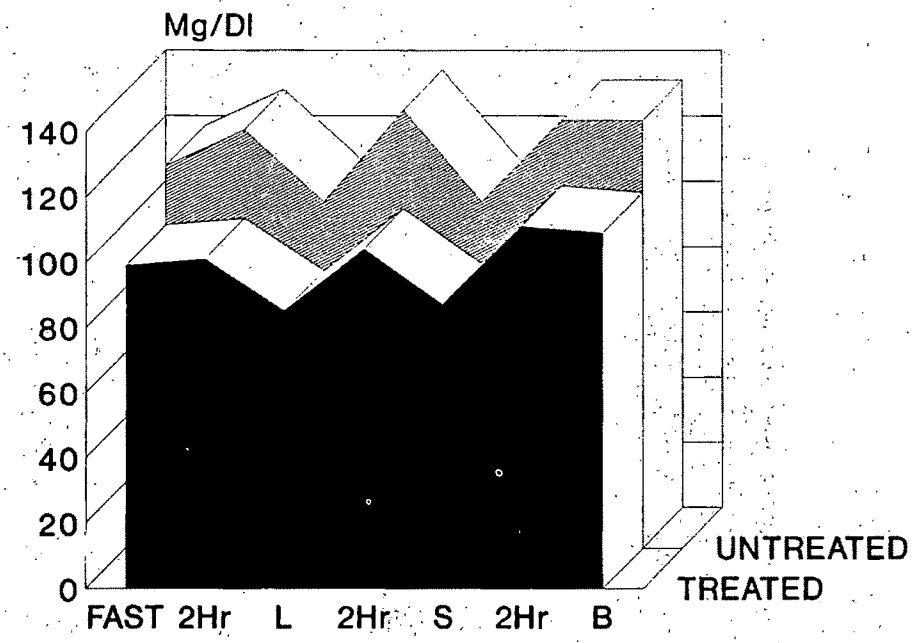


Fig. 1. Ambulatory level of glycemia after initiation of treatment. *L*, Lunch; *S*, supper; *B*, bedtime; *2Hr*, postprandial.

than the preprandial values, with an overall mean for the individuals with one abnormal test result of 119 ± 15 mg/dl.

Period II. Evaluation of ambulatory glycemic profile in group 2 before and after initiation of treatment is displayed in Fig. 1. The analysis revealed that the mean blood glucose level decreased significantly after treatment initiation. The fasting capillary blood glucose level was 102 ± 12 mg/dl before treatment and reduced significantly after treatment (84 ± 9 mg/dl).

Further evaluation comparing the mean blood glucose before and after treatment for the preprandial and postprandial determinations demonstrated significant reduction in blood glucose values (Table III).

Pregnancy outcome. A summary of maternal and neonatal pregnancy outcome appears in Table IV. Overall, 93% of the patients were delivered after 37 weeks, with no significant difference in the rate of prematurity between the groups. From all the selected maternal complications that were analyzed, the only significant difference was found in maternal weight gain between group 2 and the other two groups. In contrast, the weight change in the treated and the control individuals was similar. Infants were similar with respect to gestational age at delivery, with a mean of 39 weeks for all patients. The mean birth weights for the three groups were similar, but for group 1 a 161 gm difference in mean weight was found when compared with the untreated group. This difference was marginally significant ($p = 0.06$). Respiratory distress syndrome was documented for only 3.2% of all neonates, reflecting a range of 3% to 5% for the three groups. The overall incidence for metabolic complication was significantly higher in group 2 when compared with groups 1 and 2 (3% vs. 13%, $p < 0.04$). Hyperbilirubinemia was documented in 15% of group 2 and in approximately 5% of group 1 and 3 subjects. In group 2, polycythemia affected 14% of the neonates; hypoglycemia occurred in 12%. These findings were significantly higher in comparison to the other groups.

The incidence of large infants is displayed in Fig. 2. Significantly fewer appropriate-for-gestational-age infants were delivered of patients from group 2 compared with group 1 or 3. Of the total large-for-gestational-age infants, 82% were macrosomic infants with a similar proportion for each of the three groups.

Additional analysis (Mantel-Haenszel test) of the data was done to determine the relative risk between the groups. The relative risk for large-for-gestational-age infants between groups 1 and 2 was 4.61 (95% confidence interval, 1.44 to 14.81). Comparison between the untreated (group 2) and control (group 3) showed a relative risk of 2.22 (95% confidence interval, 1.04 to 4.76). There was no significant relative risk for large-for-gestational-age infants between the control and the treated groups.

The relationship between maternal obesity and large infants is displayed in Fig. 3. When obese and nonobese mothers were compared within each group, no significant difference was found in the incidence of large infants. However, obese women in the untreated group had a relative risk of 9.1 in comparison with obese

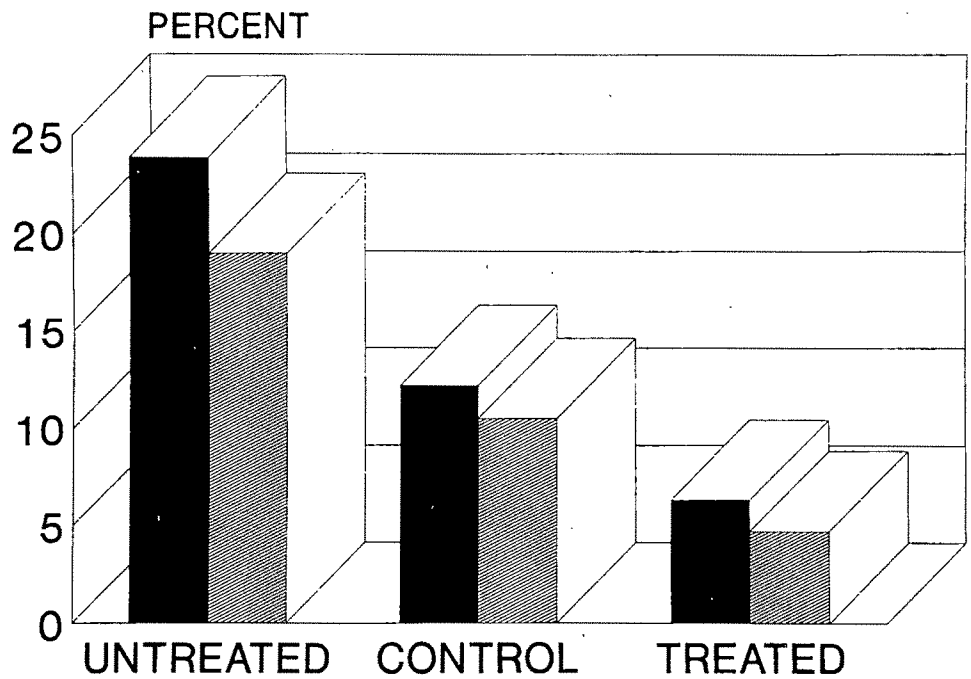


Fig. 2. Association between overall incidence of LGA infants *(darkened area)* and macrosomic *(shaded area)* infants among three groups.

Table IV. Pregnancy outcome

	Group 1: Treated (n = 63)	*Group 2: Untreated (n = 63)*	*Group 3: Control (n = 146)*	*p*
Maternal				
Gestational age at delivery (wk)	39 ± 2	39 ± 1	40 ± 2	NS
Weight gain (kg)	13 ± 4	15 ± 6*	13 ± 6	<0.03
Primary cesarean section (% and No.)	10 (6)	11 (7)	12 (18)	NS
Repeat cesarean section (% and No.)	5 (3)	6 (4)	6 (9)	NS
Hypertensive disorder (% and No.)	3 (2)	5 (3)	7 (10)	NS
Neonatal				
Birth weight (gm) (mean ± SD)	3261 ± 496	3422 ± 584	3390 ± 540	NS
SGA (<10th percentile)	10 (6)	6 (4)	10 (15)	NS
LGA (>90th percentile)	6 (4)	24* (15)	12 (18)	<0.03
Prematurity (<37 wk) (% and No.)	6 (4)	6 (4)	8 (12)	NS
Hypoglycemia (<35 mg/dl) (% and No.)	2 (1)	13 (8)	5 (8)	<0.02
Hyperbilirubinemia (>12 mg/dl) (% and No.)	6 (4)	14 (9)	4 (6)	NS
Polycythemia (>65%) (% and No.)	2 (1)	14 (9)	3 (4)	<0.02
Neonatal intensive care admission (% and No.)	6 (4)	11 (7)	5 (7)	NS

*Group 2 versus group 1 and group 3.

women in the treated group (95% confidence interval, 1.04 to 80.9) for large-for-gestational-age infants.

Finally, the association between level of glycemia and neonatal size was evaluated. For groups 1 and 2, positive correlations were found between birth percentile and overall mean capillary blood glucose, preprandial and postprandial determinations ($r = 0.30$, $p < 0.01$; $r = 0.40, p < 0.005$; $r = 0.36, p < 0.004$, respectively).

Comment

The results of this randomized study provide insight into several key issues central to the continuing controversy surrounding the precise definition of gestational diabetes. For example, the glycemic profile of the treated and untreated patients with one abnormal oral GTT value were significantly different, suggesting that a single glycemic abnormality may herald a pattern similar to the well-established profile for an accepted case of gestational diabetes.[3, 7] The significant differences between neonatal outcome variables and the overall effect reflected in the significant relative risks for large infants in treated compared with untreated and control patients further underscores the importance of a single abnormal oral GTT result.

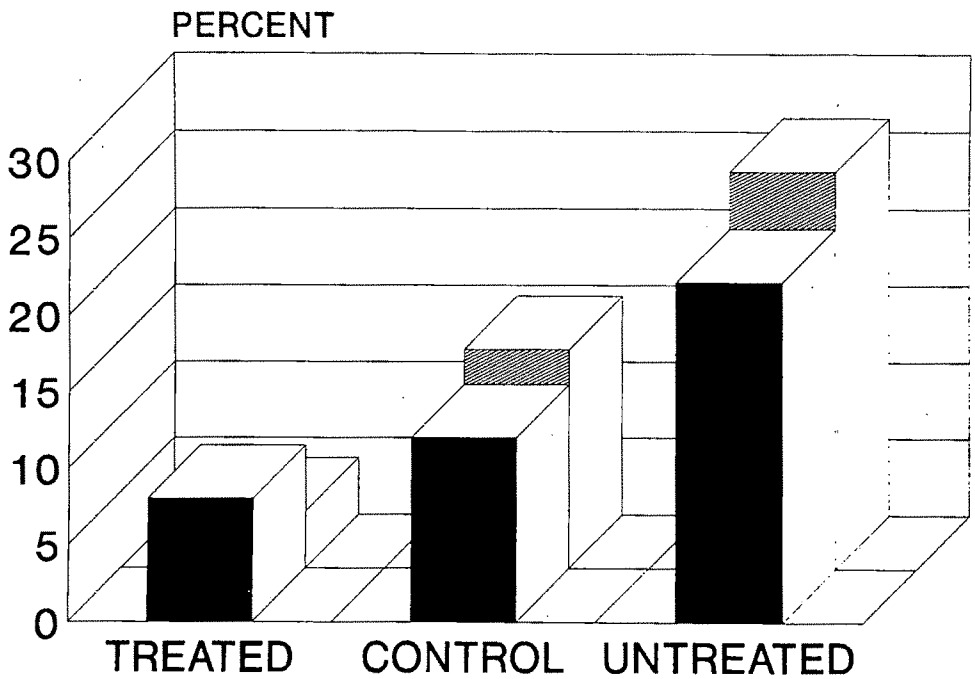


Fig. 3. Incidence of large infants in obese *(shaded area)* and nonobese *(darkened area)* women among three groups.

Previous studies of treated patients with gestational diabetes report a relatively high incidence of large infants. Furthermore, in a recent study comparing the World Health Organization and National Diabetes Data Group criteria, in which the latter served as the control (untreated), it was found that the incidence of large infants in the "control" was 28%.[10] In this investigation, although the overall mean birth weight was not significantly different between the groups, the incidence of large infants in the untreated group with one abnormal result parallels that expected from gestational diabetes. Thus it again suggests the congruence between the patients with one abnormal oral GTT with those patients with gestational diabetes mellitus who meet the National Diabetes Data Group criteria.

The inconsistent findings reported with regard to the association between level of glycemia and perinatal mortality and morbidity are the result of lack of uniformity in research methodology. The majority of studies were retrospective. The outcome was subject to the bias of clinical intervention (patient advised to modify her diet) in response to "borderline" oral GTT.

Few studies have concurrently included a nondiabetic control group. When a prospective design was used, randomization was not performed to measure the effect of intervention. There also was inconsistency in the definition of large infants. Some researchers used the 90th percentile standard growth curve, whereas others defined large infants as >4000 to 4500 gm.[11-16] These diverse factors have resulted in a wide variation in the reported prevalence of large infants.

The findings between the treated and untreated groups were significantly different. Therefore the high prevalence of unrecognized mild maternal hyperglycemia in the "normal" population (one abnormal result) may represent a group of patients with false-negative results or undiagnosed diabetes as suggested by the higher than expected incidence of large infants.

No association was discovered between the maternal variables evaluated and large infants. Although unexplained, the significantly older mean age of the treated group should have only contributed to more adverse outcome, which was not observed. Although advanced maternal age can be a factor contributing to macrosomia, it did not bias our result.

Contrary to the findings of other investigators[4, 12] who found an increased risk of preeclampsia, the incidence of hypertensive disorder was similar in the control and study groups. Thus it casts doubt on the relationship between level of glycemia and preeclampsia and women with a comparatively mild degree of hyperglycemia.

The data did not show any difference in maternal morbidity between the three study groups. Maternal weight gain was the single variable that showed a significant difference in the untreated group. This finding can be explained by the uncontrolled level of hyperglycemia.

There was a comparable rate of cesarean section

among the groups. Cesarean section as an outcome variable can reflect bias introduced by the liberal policy in many institutions with regard to method of delivery of the macrosomic infant. Future studies with a larger data base are needed to determine whether women with one abnormal oral GTT value are at greater risk for cesarean section.

The data we have presented strongly support the need to continue to refine the criteria for gestational diabetes. These findings conclusively demonstrate that neonatal morbidity can be substantially improved if treatment is provided the patient with just a single abnormal oral GTT value.

REFERENCES

1. Summary and recommendations of the Second International Workshop-Conference on Gestational Diabetes Mellitus. Diabetes 1985;34;123-6.
2. World Health Organization Expert Committee on Diabetes Mellitus. Geneva: World Health Organization, 1980; WHO Technical Report Series No. 646.
3. Langer O, Brustman L, Anyaegbunam A. The significance of one abnormal glucose tolerance test value on adverse outcome in pregnancy. AM J OBSTET GYNECOL 1987;157:758-63.
4. Lindsay MK, Graves W, Klein L. The relationship of one abnormal glucose tolerance test value and pregnancy complications. Obstet Gynecol 1989;73:103-6.
5. National Diabetes Data Group. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 1979;28:1039-57.
6. Langer O. Scientific rationale for management of diabetes in pregnancy: recent approaches with innovative computer-based technology. Diabetes Care 1988;11(suppl 1):67-72.
7. Langer O, Anyaegbunam A, Brustman L, Guidetti D, Mazze R. Gestational diabetes: insulin requirements in pregnancy. AM J OBSTET GYNECOL 1987;157:669-75.
8. Brenner WE, Edelman DA, Hendricks CH. A standard of fetal growth for the United States of America. AM J OBSTET GYNECOL 1976;126:555-64.
9. Cornblath M, Schwartz R. Disorders of carbohydrate in infancy. In: Schaffer A, Markowitz M, eds. Major problems in clinical pediatrics. Philadelphia: WB Saunders, 1976.
10. Li DFH, Wong VCW, O'Hoy KM, Ma HK. Evaluation of the WHO criteria for 75 g oral glucose tolerance test in pregnancy. Br J Obstet Gynaecol 1987;94:847-50.
11. Leikin EL, Jenkins JH, Pomerantz GA, Klein L. Abnormal glucose screening tests in pregnancy: a risk factor for fetal macrosomia. Obstet Gynecol 1987;69:570-3.
12. Tallarigo L, Giampietro O, Penno G, Miccoli R, Gregori G, Navalesi R. Relation of glucose tolerance to complications of pregnancy in nondiabetic women. N Engl J Med 1986;315:989-92.
13. Weiner CP. Effect of varying degrees of "normal" glucose metabolism on maternal and perinatal outcome. AM J OBSTET GYNECOL 1988;159:862-70.
14. Spellacy WN, Miller S, Winegar A, Peterson PQ. Macrosomia—maternal characteristics and infant complications. Obstet Gynecol 1985;66:158-61.
15. Herman G, Raimondi B. Glucose tolerance, fetal growth, and pregnancy complications in normal women. Am J Perinatol 1988;5:168-71.
16. Johar R, Rayburn W, Weir D, Eggert L. Birth weights in term infants: a 50-year perspective. J Reprod Med 1988;33:813-6.

Routine umbilical cord blood gas determinations?

James A. Thorp, MD, Jone E. Sampson, MD, Valerie M. Parisi, MD, MPH, and Robert K. Creasy, MD

Houston, Texas

Between 1986 and 1988, 1924 term nulliparous patients with spontaneous onset of labor were studied to assess the importance of obtaining umbilical cord blood gas levels on all deliveries. The umbilical cord arterial and venous pH values (expressed as mean ± 2 SD) were 7.24 ± 0.14 (n = 1694) and 7.32 ± 0.12 (n = 1820), respectively. The incidence of newborn depression (1- or 5-minute Apgar score <7) was 14.1%; of these depressed newborns, the incidence of normal umbilical cord arterial pH values (≥ −2 SD) was 77.8%. Of the vigorous newborns, there was a 2.1% incidence of umbilical cord arterial blood acidemia. Umbilical cord arterial blood acidemia in vigorous newborns was not highly predictive of specific morbidity in the immediate newborn period. Regression analysis demonstrated the umbilical cord arterial pH to correlate best with the Apgar scores when compared with all other arterial or venous blood gas meaurements. We reached the following conclusions: (1) that obtaining cord arterial pH values in vigorous newborns should be considered since the values will provide objective documentation or normal fetal acid base balance in 98% of infants. (2) Only a cord *arterial* pH determination is recommended since it reflects fetal or newborn status more accurately than all other measurements. Additional measurements increase the likelihood of abnormal results and do not contribute to neonatal management. (3) An umbilical cord blood pH value is extremely useful in ruling out the diagnosis of birth asphyxia in the depressed newborn. (Am J Obstet Gynecol 1989;161:600-5.)

Key words: Umbilical cord blood pH, umbilical cord blood gas, vigorous newborn

Birth asphyxia is a relatively rare cause of major neurologic morbidity in children, despite the fact that it is the most commonly alleged cause in the courtroom. Recent studies estimate that less than 8% of cerebral palsy in children is actually related to birth asphyxia.[1, 2] Newborn depression is not a specific indicator of birth asphyxia. In fact, newborn depression has many diverse causes, including maternal drug ingestion, trauma, neonatal manipulation, congenital anomalies, prenatal fetal insults, prematurity, and finally, birth asphyxia, which is a relatively minor contributor. It is generally agreed that umbilical cord blood acidemia is the most sensitive indicator of birth asphyxia at the time of delivery. Most authorities agree that it is extremely important to obtain umbilical cord blood pH samples in the presence of newborn depression since it will rule out birth asphyxia in approximately 80% of cases.[3] However, the value of umbilical cord blood pH and gas measurement in the vigorous newborn is not clearly established. The purpose of this study was to assess the value of umbilical cord pH and gas measurements in the vigorous newborn.

From the Division of Maternal-Fetal Medicine, Department of Obstetrics, Gynecology, and Reproductive Sciences and Department of Pediatrics, University of Texas Medical School–Houston.

Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.

Reprint requests: James A. Thorp, MD, St. Luke's Perinatal Center, 4400 Wornall Rd., Kansas City, MO 64111.

6/6/13890

Material and methods

Between 1986 and 1988, 1924 term nulliparous patients with spontaneous onset of labor, cephalic presentations, and singleton gestations were included in this study. We selected these patients to minimize the confounding variables of gestational age, abnormal presentations, multiple gestations, and parity. In addition, we chose to study only nulliparous patients since it is known that they have different mean cord blood pH values than multiparous patients.[4, 5] All of these patients' labors were managed according to the principles of active management of labor.[6] Labor was diagnosed by regular painful uterine contractions and cervical effacement, and was usually accompanied by either bloody show or rupture of membranes. Patients who were seen with fetal distress were not included in this study. After the diagnosis of labor was established, amniotomy was performed. If 2 cm of cervical dilatation did not occur within 2 hours of amniotomy, oxytocin augmentation was initiated. Oxytocin was administered by controlled intravenous infusion (model 960, IMED Corp., San Diego, Calif.) of 20 units of oxytocin diluted in 1 L of lactated Ringer's solution. Infusion was begun at 4 mU/min, increased by 4 mU/min every 15 minutes, and did not exceed a maximum dose of 36 mU/min. If uterine activity exceeded a frequency of seven contractions in 15 minutes, the oxytocin infusion rate was not increased, and possibly decreased. Continuous electronic fetal monitoring was performed in all patients.

Arterial and venous umbilical cord blood samples were obtained immediately after delivery and were placed on ice. Prepackaged, preheparinized 3 ml syringes (Marquest Medical products, Englewood, Colo.) were used to obtain a consistent preparation and concentration of heparin. Umbilical cord arterial and venous pH and gas measurements were performed routinely on all deliveries if possible. The blood gas analyzer (IL-1312, Instrumentation Laboratory, Lexington, Mass.) measured pH, P_{CO_2}, and P_{O_2} and calculated bicarbonate and base deficit. Cord blood gas analysis was routinely performed in a satellite laboratory, within 15 minutes of delivery under the supervision and direction of the Department of Pathology. In this study the 705 patients from 1986 and the 500 patients from 1988 had only umbilical cord pH values recorded in our investigational data base, whereas the 719 consecutive patients from 1987 also had the remainder of the gas measurements recorded. A "vigorous" newborn was defined as a newborn with normal Apgar scores (≥7 at 1 and 5 minutes). A depressed newborn was defined as a newborn with a 1- or 5-minute Apgar score of <7. Acidemic arterial and venous cord pH values were defined statistically as those being ≤2 SD below the mean for our population. Analysis of variance for the regression was performed with the Apgar score as the dependent variable, and each of the umbilical cord blood gas measurements, separately, as the independent variable. The 1-minute, 5-minute, and a combined 1- and 5-minute Apgar scores were used as the dependent variable in separate analyses. The umbilical arterial-venous difference of each of the gas measurements were also assessed as separate independent variables. Isolated respiratory acidemia in the umbilical cord arterial blood was defined by the following measurements: pH ≤ −2 SD, P_{CO_2} ≥ +2 SD, and a base deficit ≤ mean. Isolated metabolic acidemia in umbilical cord arterial blood was defined by the following measurements: pH ≤ −2 SD, base deficit ≥ +2 SD, and a P_{CO_2} ≤ mean. Although these definitions are arbitrary, they represent an accepted statistical standard for evaluation of a population with a normal Gaussian distribution. A microcomputer and appropriate software were used for assistance with the data base and statistical analyses (IBM PC XT, Symphony, Lotus Development Corp., Cambridge, Mass.; Abstat, Anderson-Bell, Parker, Colo.; SPSS, SPSS Inc., Chicago).

Results

Of the 1924 term nulliparous patients, 1919 had infants with 1- and 5-minute Apgar scores recorded on their medical charts. The 1-minute Apgar score was <7 in 268 newborns (14.0%), and the 5-minute Apgar score was <7 in 27 newborns (1.4%). The 1- or 5-minute Apgar score was <7 in 270 (14.1%), and the 1- and 5-minute Apgar score was <7 in 25 of 1919 newborns (1.3%). The umbilical cord arterial pH (mean ± SD) was 7.24 ± 0.07 (n = 1694), and the venous pH (mean ± SD) was 7.32 ± 0.06 (n = 1820). The remainder of the umbilical cord arterial and venous blood gas measurements are listed in Table I. In Table I, the n for the gas measurements other than the pH values is smaller since these values were only recorded on the 1987 portion of the investigational data base.

Table I. Umbilical cord blood gas measurements*

Umbilical cord artery	
pH	7.24 ± 0.07 (n = 1694)
P_{CO_2} (mm Hg)	56.3 ± 8.6 (n = 652)
P_{O_2} (mm Hg)	17.9 ± 6.9 (n = 651)
Bicarbonate (mEq/L)	24.1 ± 2.2 (n = 652)
Base deficit (mEq/L)	3.6 ± 2.7 (n = 652)
Umbilical cord vein	
pH	7.32 ± 0.06 (n = 1820)
P_{CO_2} (mm Hg)	43.8 ± 6.7 (n = 680)
P_{O_2} (mm Hg)	28.7 ± 7.3 (n = 680)
Bicarbonate (mEq/L)	22.6 ± 2.1 (n = 680)
Base deficit (mEq/L)	2.9 ± 2.4 (n = 680)

*All values listed as mean ± SD.

The frequencies of normal pH values with various combinations of abnormal Apgar scores are listed in Table II. The majority of depressed newborns (as previously defined) had normal umbilical cord blood pH values. Normal umbilical cord arterial pH values occurred in 77.8% (210 of 270) of depressed newborns as defined by a 1- or 5-minute Apgar score of <7. Table III demonstrates frequencies of umbilical cord and blood acidemia (variously defined) in vigorous newborns. There was a 2.1% incidence of cord arterial acidemia (≤7.10), 3.0% incidence of cord venous acidemia (≤7.20), and 4.0% incidence of cord arterial or venous acidemia. There was a twofold greater risk ($p < 0.005$) of finding umbilical cord blood acidemia in the vigorous newborn if both arterial and venous samples were considered (Table III).

Analysis of variance of the regression demonstrated cord arterial pH to correlate best (albeit poorly) with the Apgar scores, regardless of whether the 1-minute, the 5-minute, or a combination of both scores was used as the dependent variable. There was no combination of other arterial or venous pH/gas measurements, including the arterial-venous differences, that could approach the correlation of the umbilical cord arterial pH to the Apgar scores. Table IV demonstrates the correlation coefficient (r values), the F test for the analysis of variance for the regression, and the p values for each respective cord blood gas parameter in correlating with the 1-minute Apgar score. In Table IV, the umbilical cord gas measurements are listed in order of decreasing correlation with the 1-minute Apgar score. Similar re-

Table II. Frequency of normal umbilical cord pH values in newborns with abnormal Apgar scores*

	1 min Apgar score <7	*5 min Apgar score <7*	*1 or 5 min Apgar score <7*	*1 and 5 min Apgar score <7*
Normal umbilical cord arterial pH	208/244 (85.2%)	14/22 (63.6%)	210/270 (77.8%)	12/20 (60.0%)
Normal umbilical cord venous pH	221/256 (86.3%)	15/24 (62.5%)	223/258 (86.4%)	13/22 (59.1%)
Normal cord arterial pH and venous pH	198/240 (82.5%)	13/21 (61.9%)	200/242 (82.6%)	11/19 (57.9%)

*Normal umbilical cord pH values are defined as those values > −2 SD.

Table III. Frequency of umbilical cord blood acidemia in newborns with normal Apgar scores*

	No. affected/ total No.	%
Abnormal umbilical arterial pH (≤7.10)†	35/1690	2.1
Abnormal umbilical venous pH (≤7.20)	54/1816	3.0
Abnormal arterial or venous pH†	68/1682	4.0
Abnormal arterial and venous pH	21/1682	1.2

*Normal Apgar scores are defined as 1- and 5-minute scores of ≥7. Abnormal pH values are defined as a value that is ≤ 2 SD below the mean.

†There is a significant difference ($p < 0.005$) between these frequencies by χ^2 analysis.

lationships were observed with the 5-minute, or a combination of the 1- and 5-minute Apgar score when used as the dependent variable. Use of the combined 1- and 5-minute Apgar score yielded the best correlation coefficient (increase of 5%). The umbilical cord arterial pH, although correlating the best, still had a poor correlation (correlation coefficient = 0.31).

The majority of newborns with umbilical cord acidemia had a combination of metabolic and respiratory components. Isolated metabolic acidemia occurred in only 1 of 652 newborns. There were no cases of isolated respiratory acidemia among 652 newborns who had no missing arterial gas values. When the definition of isolated respiratory acidemia was broadened (pH ≤ −2 SD; P_{CO_2} ≥ 2 SD; base deficit ≤ +1 SD), it remained an unusual event (1 of 652).

Charts of all 35 of the 1690 vigorous newborns with umbilical cord arterial acidemia were reviewed. The mean umbilical cord arterial pH was 7.07 ± 0.03 (mean ± SD, $n = 35$). None of the 35 acidemic vigorous newborns or the 1655 nonacidemic vigorous newborns required admission to the neonatal intensive care unit. There was a significantly greater proportion ($p = 0.01$) of the acidemic vigorous newborns who required admission to the special care nursery (3 of 35, 8.6%), compared with the nonacidemic vigorous newborns (21 of 1655, 1.3%). One of the newborns was subsequently diagnosed as having a cardiac malformation (transposition), and the arterial cord blood bases values were as follows: pH, 7.10; P_{CO_2}, 68; P_{O_2}, 13; bicarbonate, 22; and base deficit, 9. Another infant had a neonatal seizure at 8 hours of life, and a cerebral hemorrhage, consistent with the diagnosis of birth asphyxia. The cord arterial blood gas values of this infant were as follows: pH, 7.08; P_{CO_2}, 74; P_{O_2}, 15; bicarbonate, 22; and base deficit, 9. The third infant had meconium aspiration syndrome; the cord arterial gas values were as follows: pH, 7.07; P_{CO_2}, 63; P_{O_2}, 13; bicarbonate, 19; and base deficit, 12. The duration of hospitalization for all 35 infants was 2.8 ± 1.9 (mean ± SD). There was no culture-documented infectious morbidity.

Comment

Umbilical cord blood pH is generally considered to be the most sensitive indicator of birth asphyxia.[3, 7, 8] As demonstrated in previous studies,[3, 7] the majority of depressed newborns (1- or 5-minute Apgar score <7) in our study also had normal cord blood pH values (Table II), thus ruling out the diagnosis of birth asphyxia, both in the delivery room and in the courtroom. The tremendous importance of obtaining cord blood pH measurement in the depressed newborn is generally recognized[3, 7-9]; however, the benefit of cord blood pH and gas values in the vigorous newborn is not well documented.

Our data are consistent with previous studies and demonstrate a small percentage of vigorous newborns with significant umbilical cord arterial acidemia. The cause of this acidemia in vigorous newborns is controversial. Some authors speculate that it may be of maternal origin.[10, 11] However, others provide convincing evidence that it may result from intrapartum asphyxia.[3, 8, 9] Previous studies suggest that neurologic impairment caused by birth asphyxia may develop in children with normal Apgar scores.[8] Brain damage by birth asphyxia in the monkey may be quite extensive in brainstem centers, and yet is hardly perceptible clinically.[12] The degree and duration of asphyxial insult necessary to produce brain damage probably varies, depending on several factors including fetal reserves, previous

Table IV. Correlation of umbilical cord gas measurements and the 1-minute Apgar score (listed in order of decreasing correlation)

Umbilical cord blood gas parameter	*n*	*Correlation coefficient*	*f ratio*	*p value**
Arterial pH	1690	0.31	189.0	<0.001
Venous pH	1816	0.29	169.5	<0.001
Arterial P_{CO_2}	652	0.27	51.3	<0.001
Venous P_{CO_2}	680	0.23	38.3	<0.001
Arterial P_{O_2}	651	0.18	22.6	<0.001
Arterial base deficit	652	0.18	22.1	<0.001
Venous base deficit	680	0.15	16.2	<0.001
Venous P_{O_2}	680	0.10	7.3	<0.001
Arterial bicarbonate	652	0.04	0.9	NS
Venous bicarbonate	680	0.03	0.5	NS

**p* values and *f* ratios were obtained by analysis of variance of the regression with Apgar score at 1 minute used as the dependent variable and the umbilical cord gas parameter as the independent variable. The 5-minute Apgar score, or addition of the Apgar scores, yielded similar relationships; the addition of the Apgar scores improved the correlation coefficient by approximately 5%.

aphyxial insults, glucose loading or starvation, the metabolic status at the time of the insult, and the perfusion of the brain.[13] Studies in the fetal monkey have suggested that total anoxia in excess of 10 to 12 minutes will result in a neuropathologic state.[14] However, partial hypoxia with acidemia must be present for at least 2 hours before neuropathologic damage can be anticipated.[15] The finding that one of our acidemic vigorous newborns (Apgar scores of 7 and 9 at 1 and 5 minutes, respectively) had birth asphyxia is also consistent with these observations (i.e., birth asphyxia may occur in a newborn vigorous in the first minutes of life).

Umbilical cord blood acidemia in the vigorous newborn was not highly predictive of a specific morbidity in the early newborn period. Contrary to popular opinion, cord blood acidemia in the vigorous newborn did not predict newborn sepsis in our study. However, three of 35 acidemic vigorous newborns had significant morbidity in the early newborn period. Although acidemia was poorly predictive of nonspecific morbidity and provided supporting evidence for the diagnosis of birth asphyxia in one vigorous newborn, the clinical benefit derived from umbilical cord blood acidemia in the vigorous newborn was limited. It may be speculated that of the three acidemic vigorous newborns described, the acidemic cord arterial pH could be harmful to the obstetrician in court in the cases of the infant with meconium aspiration and the infant with early neonatal seizures, but probably not in the infant with the cardiac anomaly. Similarly, one could speculate that all the acidemic vigorous newborns (2%) could potentially be labeled as asphyxiated in court. It is important to consider that the majority of children with neurologic deficits are vigorous at birth, and that cord blood acidemia is a poor predictor of neurologic deficit at 1 year of life.[16] Unfortunately, a plaintiff's attorney may allege birth asphyxia as the cause of neurologic deficit regardless of whether there is an absent cord pH or an acidemic cord pH on the medical record. The vulnerability of the obstetrician would depend not only on the acidemic cord arterial pH, since that information was obtained after delivery, but also on the intrapartum management that was based on the fetal heart rate tracing. It should be emphasized, however, that obtaining umbilical cord arterial pH values in the vigorous newborn will provide objective documentation that rules out the diagnosis of birth asphyxia in approximately 98% of vigorous newborns.

Experts in litigation involving potential birth asphyxia suggest that the Apgar score is a very subjective evaluation, and may be biased by delivery room staff; these opinions are also shared by many physicians.[3, 7, 8] Indeed, the only Apgar parameter with an objective end point is the pulse; the others (respiratory effort, muscle tone, reflex irritability, and color) are subjective. The Apgar scores are assigned by delivery room staff, who may give high scores because of fear of criticism from colleagues. In addition, the Apgar scores are often assigned retrospectively. In contrast, the cord arterial pH is determined by staff outside the department by means of a machine that is required to be maintained and reviewed by rigorous quality assurance policies. For these reasons, an objective measurement of umbilical cord pH in the vigorous newborn may be a judicious medicolegal practice if performance is impaired in postnatal life.

Previous data in the literature suggest that cord arterial pH is the most accurate reflection of fetal and newborn status when compared with all other cord arterial and venous blood gas measurements.[3, 7, 8, 11, 17] This observation is based primarily on correlation of blood gas measurements with Apgar scores, in addition to anatomic relationships of fetal circulation. Umbilical arterial blood, in general, reflects fetal tissue status, and umbilical venous blood reflects placental function and fetal status. Our study is consistent with the previous

literature. There was no combination of, or arterial-venous differences between, the pH, P_{CO_2}, P_{O_2}, bicarbonate, or base deficit values, which correlated with the Apgar scores, as well as the cord arterial pH. Also consistent with the observations of others[8, 11] is the very poor correlation between the cord blood P_{O_2} and the fetal status or the Apgar scores.

Some authorities[18] suggest that the P_{CO_2} and base deficit may be important in differentiation of respiratory acidemia and metabolic acidemia. Adult respiratory physiology would support the concept that respiratory acidemia caused by carbon dioxide retention will precede a metabolic acidemia caused by hypoxia. Unfortunately, our data and that of others[8] suggest that an isolated respiratory acidemia in umbilical cord blood is extremely rare, and that acidemia is almost exclusively of mixed respiratory and metabolic origins. We arbitrarily chose to define isolated respiratory acidemia ($pH \leq -2$ SD; $P_{CO_2} \geq +2$ SD; base deficit $\leq$ mean) and isolated metabolic acidemia ($pH \leq -2$ SD; $P_{CO_2} \leq$ mean; base deficit $\geq +2$ SD) statistically. Even when these definitions were broadened, similar findings were noted (i.e., the majority of cord blood acidemia was mixed, and isolated respiratory acidemia was an absent, or unusual finding). Thus it is unlikely that obtaining the other gas parameters will demonstrate an isolated respiratory acidemia. Therefore, although of academic interest, the value of obtaining measurements other than the umbilical cord arterial pH may be limited.

We believe that although our statistical definitions of cord blood gas parameters are accurate, they should be evaluated for clinical usefulness in large populations. Definitions of abnormal fetal cord blood gas parameters derived clinically rather than statistically may vary widely among institutions. These differences in definition subsequently do not allow for standardized comparisons of cord gas values among various populations, whereas values expressed in SD above or below the mean are universally applicable. In addition, we believe that the review of both umbilical arterial and venous cord blood gases may be clinically useful in certain patients by providing insight into the evaluation of intrapartum events and the resultant response to neonatal resuscitation.

If an institution chooses to obtain cord pH values in all deliveries, the best single parameter is cord arterial pH. It is the most accurate reflection of fetal and newborn status. Obtaining additional measurements, such as a cord venous pH, in the vigorous newborn results in a twofold greater chance ($p < 0.005$) of finding an acidemic result (Table III). This rise in frequency of acidemia in the vigorous newborn would increase anxiety without providing any additional clinically useful information for neonatal management.

The Apgar score has only a limited correlation with long-term neurologic outcome. An infant with an Apgar score of 0 to 3 at 5 minutes and ≥ 4 at 10 minutes has a 99% chance of not having cerebral palsy at 7 years of age.[19] One study found a 12.7% incidence of abnormal neurologic findings at 1 year of age if the 5-minute Apgar score was <7, and 1.7% incidence if the Apgar scores were normal.[20] There is a paucity of data with regard to the long-term outcome of newborns with cord blood acidemia. Initial studies[9, 16] suggest that the majority of newborns with cord blood acidemia have normal neurologic development. One study of term infants with metabolic acidemia at birth demonstrated no increased risk of neurologic or developmental problems when compared with newborns who had normal acid-base values.[9] Another study found that cord blood acidemia in newborns had a poor positive predictive value (only 8%) when used to assess adverse outcome at 1 year of life.[16] These observations are further supported by a recent study demonstrating that only 8% of cerebral palsy in children was possibly related to intrapartum asphyxia.[1, 2] The majority of neurologic lesions may occur before labor or be unrelated to birth asphyxia. Therefore a poor correlation between neurologic impairment and low Apgar scores or cord acidemia should be expected. Ultrasonography has clearly demonstrated significant neurologic lesions in the fetus well before the onset of labor. When neurologic deficit occurs, lay jurors are easily persuaded to believe that minor deviations from the standard of care at birth caused the deficit and often support excessive and unwarranted awards. Objective cord blood pH measurements reduce this possibility.

Cost consideration is certainly a relevant factor. Expenditure reductions of approximately 86% will occur if an institution chooses to selectively obtain cord blood studies only when deliveries result in depressed newborns (i.e., only approximately 14% of newborns will be depressed). Unfortunately, selective use will inevitably result in failure to obtain umbilical cord blood pH measurement in at least some depressed newborns as a result of the anxiety and confusion of emergency situations and the lack of an established routine. In our institution umbilical cord arterial and venous pH and gas measurement have been obtained on all deliveries since 1986. We have chosen to charge for this by adding a cost of $20 to all deliveries and therefore we do not bill separately for this service. This cost might be significantly reduced if a pH analyzer rather than a blood gas analyzer were used.

We derived the following conclusions from this study. (1) Obtaining umbilical cord arterial pH values in the vigorous newborn should be considered since it will provide objective documentation of normal fetal and newborn acid-base balance and by inference, metabolic status, in 98% of infants. (2) Only a cord *arterial* pH

determination is recommended since it reflects fetal and newborn status more accurately than all other measurements, and additional measurements increase the likelihood of abnormal results, without contributing to neonatal management. (3) As others have shown, an umbilical cord blood pH is also useful in ruling out the diagnosis of birth asphyxia in the depressed newborn.

REFERENCES

1. Blair E, Stanley FJ. Intrapartum asphyxia: a rare cause of cerebral palsy. J Pediatr 1988;112:515-19.
2. Nelson KB. What proportion of cerebral palsy is related to birth asphyxia? J Pediatr 1988;112:572-4.
3. Sykes GS, Johnson P, Ashworth F, et al. Do Apgar scores indicate asphyxia? Lancet 1982;1:494-6.
4. Yudkin PL, Johnson P, Redman CWG. Obstetric factors associated with cord blood gas values at birth. Eur J Obstet Gynecol Reprod Biol 1987;24:167-76.
5. Martinsen K, Peltosa J, Tervila L, Virtanen A. Umbilical cord cortisol and arterial pH levels in spontaneous and induced labors. Obstet Gynecol 1982;59:171-5.
6. O'Driscoll K, Foley M, MacDonald D. Active management of labor as an alternative to cesarean section for dystocia. Obstet Gynecol 1984;63:485-490.
7. Silverman F, Suidan J, Wasserman J, Antoine C, Young BK. The Apgar score: is it good enough? Obstet Gynecol 1985;66:331-336.
8. Gordon A, Johnson JWC. Value of umbilical blood acid-base studies in fetal assessment. J Reprod Med 1985; 30:329-336.
9. Low JA, Galbraith RS, Muir DW, Killen HL, Pater EA, Karchmar EJ. Intrapartum fetal hypoxia: a study of long-term morbidity. Am J Obstet Gynecol 1983;145:129-34.
10. Roversi GD, Canussio V, Spennacchio M. Recognition and significance of maternogenic fetal acidosis during intensive monitoring of labor. J Perinat Med 1973;3:53.
11. Seeds AE. Maternal-fetal acid-base relationships and fetal scalp-blood analysis. Clin Obstet Gynecol 1978;21:579-91.
12. Dawes GS, Hibbard E, Windle WF. The effect of alkali and glucose infusion on permanent brain damage in rhesus monkeys asphyxiated at birth. J Pediatr 1964;65: 801-6.
13. Myers RE, Adamsons K. Obstetric considerations of perinatal brain injury. In: Scarpelli EM, Cosmi EV, eds. Reviews in perinatal medicine. New York: Raven Press, 1981 vol 4:222-45.
14. Ranck JB, Windle WF. Brain damage in the monkey, *Macaca mulatta* by asphyxia neonatorum. Exp Neurol 1959; 1:130.
15. Myers RE. Fetal asphyxia and perinatal brain damage affecting human development. Publication no 85. Washington, DC: Pan American Health Organization, 1969:205-14.
16. Ruth VJ, Raivio KO. Perinatal brain damage: predictive value of metabolic acidosis and the Apgar score. Br Med J 1988;297:24-7.
17. Marrin M, Bosco AP. Birth asphyxia: does the Apgar score have diagnostic value? Obstet Gynecol 1988;72:120-3.
18. Wible JL, Petrie RH, Koons A, Perez A. The clinical use of umbilical cord acid-base determinations in perinatal surveillance and management. In: Petrie RH, ed. Clinics in perinatology. Philadelphia: WB Saunders, 1982 vol 9:387-97.
19. American Academy of Pediatrics: Committee on Fetus and Newborn. Use and abuse of the Apgar score. Pediatrics 1986;78:1148-9.
20. Drage JS, Kennedy C, Berendes H, Schwarz BK, Weiss W. The Apgar score as an index of infant morbidity. Dev Med Child Neurol 1966;8:141-8.

Comparison of six different ultrasonographic methods for predicting lethal fetal pulmonary hypoplasia

Anthony M. Vintzileos, MD, Winston A. Campbell, MD, John F. Rodis, MD, David J. Nochimson, MD, Michael G. Pinette, MD, and Boris M. Petrikovsky, MD
Farmington, Connecticut

Nomograms of six different ultrasonographic fetal parameters were established by studying uncomplicated, singleton pregnancies, with well-established dates, between 16 and 40 weeks of gestation. The studied parameters could reflect fetal lung mass and included the following: chest circumference (CC), chest area (CA), chest area minus heart area (CA − HA), chest circumference/abdominal circumference ratio (CC × 100/AC), chest area/heart area ratio (CA/HA), and chest area minus heart area divided by chest area ratio [(CA − HA) × 100/CA]. The effect of ruptured membranes on these six ultrasonographic parameters was assessed by studying patients who had premature rupture of the membranes of <1 week's duration. It was found that premature rupture of the membranes is associated with increased frequency of CC, CA, and CA − HA measurements at or below the 5th percentile. However, in patients with premature rupture of the membranes all measurements were within the normal range for the three ratios. The efficacy of each of the six parameters was determined by studying 13 fetuses at high risk for development of lethal pulmonary hypoplasia. The (CA − HA) × 100/CA parameter had the best diagnostic accuracy (sensitivity 85%, specificity 85%, positive predictive value 83%, and negative predictive value 85%). (Am J Obstet Gynecol 1989;161:606-12.)

Key words: Ultrasonography, fetal pulmonary hypoplasia

Fetal pulmonary hypoplasia is commonly associated with both renal and nonrenal origins of decreased amniotic fluid.[1-4] There is a wide spectrum of clinical manifestations of pulmonary hypoplasia that ranges from acute respiratory failure leading to early neonatal death (lethal form) to respiratory insufficiency including pulmonary hemorrhage, severe bronchopulmonary dysplasia, subacute lung disease, or transient respiratory disease (nonlethal form).[5] From the clinician's standpoint the antepartum prediction of lethal pulmonary hypoplasia would be very beneficial with regard to both obstetric management and parental counseling.

In the past, two main ultrasonographic techniques have been used to diagnose pulmonary hypoplasia: chest circumference measurements and chest circumference/abdominal circumference (CC/AC) ratio.[6-10] Nomograms for chest circumferences throughout gestation have been developed by previous investigators.[6-8] The use of chest circumference nomograms, however, requires accurate knowledge of the length of gestation. Therefore these nomograms cannot be used in fetuses with unknown gestational age or fetuses with suspected intrauterine growth retardation, which is frequently present. To circumvent these drawbacks some investigators have proposed the use of a gestational age–independent ratio (CC/AC)[9,10] or nomograms that allow for comparison of the chest circumference with other ultrasonographic parameters (e.g., biparietal diameter, head circumference, abdominal circumference, femur length), which hopefully are not affected when growth or structural abnormalities are present.[11] These quantitative interthoracic relationships, however, may be misleading in cases where growth or structural abnormalities do not compromise the various fetal parameters to the same degree. An additional limitation of the previously established chest circumference nomograms is that measurements were derived from outer edge to outer edge of the fetal thorax, and therefore their use in fetuses with excessive subcutaneous tissue and skin may give erroneous results. For instance, normal chest circumference measurements have been described in a fetus with wrinkled skin (Pena-Shokeir syndrome) and lethal pulmonary hypoplasia.[8]

The major advantage of the CC/AC ratio is that it is gestational age independent and could be reliably used without accurate knowledge of the gestational age. The major limitation is that it cannot be used in fetuses with large chest circumferences caused by excessive skin and subcutaneous tissue or in conditions that affect or distort abdominal circumference measurements (e.g., fetuses with polycystic kidneys, obstructive uropathy, om-

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of Connecticut Health Center.
Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.
Reprint requests: Anthony M. Vintzileos, MD, Department of Obstetrics and Gynecology, Division of Maternal-Fetal Medicine, University of Connecticut Health Center, Farmington, CT 06032.
6/6/13714

phalocele). It is known that many cases of pulmonary hypoplasia are due to prolonged premature rupture of the membranes (PROM); in these cases, however, the use of the two commonly used parameters (chest circumference measurements and AC/CC ratio) is questionable since the effect of PROM on these measurements has not been investigated.

The antenatal diagnosis of pulmonary hypoplasia is always suspected by the subjective impression of cardiothoracic disproportion noted during an ultrasonographic examination. Since it is important to quantitatively assess the intrathoracic structures when abnormal lung development is suspected, the present study was undertaken with the following objectives: (1) to establish nomograms of six different ultrasonographic parameters that could reflect fetal lung mass, (2) to assess the effect of PROM on these parameters, and (3) to determine the usefulness of these parameters in predicting lethal pulmonary hypoplasia in high-risk fetuses.

Material and methods

In the present study the first step was to establish the nomograms of the six fetal parameters throughout gestations. One hundred eighty-one uncomplicated pregnancies between 16 and 40 weeks of gestation (dates confirmed by early ultrasonography) were used for single ultrasonographic measurement to generate the nomograms in a cross-sectional manner. All patients had intact membranes and singleton pregnancies. Delivery occurred within 2 weeks of the predicted date of delivery either by spontaneous labor or by elective repeat cesarean section. Patients with an abnormal fetal outcome (e.g., congenital anomalies, intrauterine growth retardation, fetal macrosomia) were excluded. The ultrasonographic examinations were performed with a linear-array real-time ultrasonographic instrument (GE 3000 or Acuson 128) with a 3.5 or 5.0 MHz transducer. Freeze-frame capabilities were available, and on-screen calipers were used for the measurements. The ultrasound velocity was 1540 m/sec. In addition to routine biometry, the bony chest circumference (CC), chest area (CA), heart circumference (HtC), and heart area (HA) were determined from a cross section of the fetal chest at right angles to the fetal spine at the level of the atrioventricular valves with the heart in ventricular diastole (Fig. 1). From this view, the circumferences were directly measured and the areas were automatically calculated. For the purpose of this study the relationship between gestational age and each of the following six parameters was defined: CC, CA, (CA − HA), CC × 100/AC, CA/HA, and (CA − HA) × 100/CA. Scattergrams with increasing gestational age were developed and regression analyses were used to establish the nomograms including the 5th, 50th, and 95th percentile lines. The statistical analysis of the data was performed by computer (Macintosh II) with the StatView 512+ and Cricket Graph statistical packages.

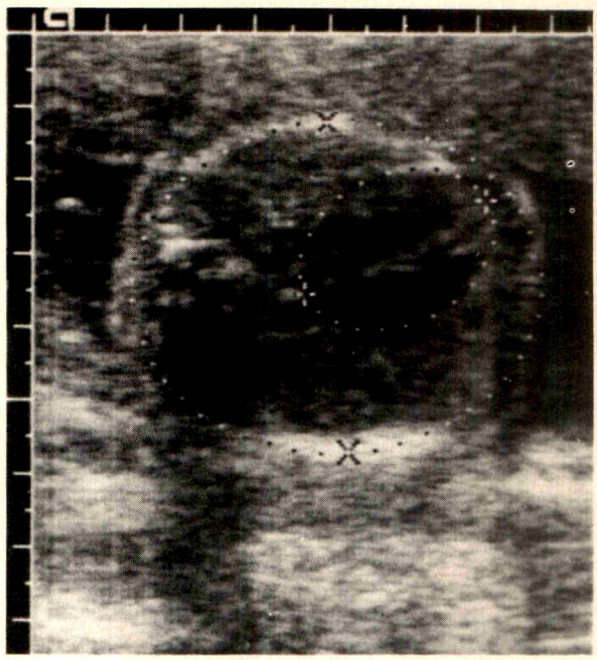


Fig. 1. Ultrasonographic photograph showing cross section of fetal chest at level of four-chamber view, illustrating placement of calipers for measurements of bony chest circumference and heart circumference. Areas are automatically calculated.

After the nomograms were established, the second step was to assess the effect of PROM by studying 18 patients with ruptured membranes not at risk for pulmonary hypoplasia (duration of PROM <1 week). All 18 patients had a single measurement within 1 week of delivery and none of the infants developed any signs of pulmonary hypoplasia. The measurements of the groups with intact versus ruptured membranes were compared by Fisher's exact and two-tailed t tests. A value of $p < 0.05$ was considered to be statistically significant.

The efficacy of each of the six parameters to predict lethal pulmonary hypoplasia (third step) was determined by studying 13 fetuses at high risk. Four of these fetuses had renal anomalies (three obstructive uropathy and one bilateral polycystic kidney disease), four had PROM at <26 weeks' gestation and >5 weeks' duration, and five had idiopathic oligohydramnios. All 13 fetuses had severe oligohydramnios, as defined by largest vertical amniotic fluid pocket <1 cm, of at least 5 weeks' duration. The last examination within 1 week of delivery was used for analysis. Of the 13 high-risk fetuses, six developed lethal pulmonary hypoplasia, three developed respiratory insufficiency compatible with clinical pulmonary hypoplasia but survived, and the re-

Table I. Effect of PROM on gestational age–independent ratios

	CC × 100/AC			*CA/HA*			*(CA − HA) × 100/CA*		
	5th	*50th*	*95th*	*5th*	*50th*	*95th*	*5th*	*50th*	*95th*
Intact membranes (n = 181)	75	85	95	2.65	3.74*	5.46	62	74†	82
PROM (n = 18)	72	84	93	2.67	3.10*	4.36	62	68†	77

CC, Chest circumference; *AC*, abdominal circumference; *CA*, chest area; *HA*, heart area.
*$p < 0.05$.
†$p < 0.05$.

maining four did not show any signs of pulmonary hypoplasia. Since the prediction of lethal pulmonary hypoplasia may alter obstetric management, the efficacy (sensitivity, specificity, and positive and negative predictive values) of each of the six parameters to predict lethal pulmonary hypoplasia only was addressed. Measurements at or below the 5th percentile were considered abnormal. All six neonates with lethal pulmonary hypoplasia died in the first 48 hours after birth, and the diagnosis was confirmed at autopsy by lung weight/body weight ratios and radial alveolar counts.[12]

Results

The best-fit curves were second- and third-degree polynomials for chest circumference and chest area, respectively. The regression equation for chest circumference versus gestational age was: $y = -10.315 + 1.35x - 0.009x^2$($R^2 = 0.902$) and for chest area versus gestational age was: $y = 36.36 - 5.176x + 0.253x^2 - 0.003x^3$ ($R^2 = 0.848$). The relationship between CA − HA and gestational age was linear ($y = -24.954 + 1.7142x$, $R^2 = 0.740$). Regression analyses of the relationships among the three ratios [CC × 100/AC, CA/HA, and (CA − HA) × 100/CA] and gestational age revealed these ratios to be relatively constant. The mean value for CC/AC × 100 was 85 (5th to 95th percentile range, 75 to 95). The regression of this ratio versus gestational age revealed that this ratio decreases only at a rate of 0.125 units per week ($R = 0.091$, $R^2 = 0.008$, SE = 0.055, $F = 1.492$, intercept = 0.877, and slope = −0.001). The regression of CA/HA versus gestational age revealed that this ratio decreases only at a rate of 0.031 units per week of gestation ($R = 0.179$, $R^2 = 0.032$, SE = 0.836, $F = 5.951$, intercept = 4.629, and slope = −0.027). The regression of (CA − HA) × 100/CA revealed that this ratio decreases only at a rate of 0.312 units per week ($R = 0.22$, $R^2 = 0.049$, SE = 0.068, $F = 9.13$ intercept = 0.814, and slope = −0.003). Regression analyses were also used to define the relationships between CC versus AC, CA versus HA, and (CA − HA) versus CA. All three plots were linear with high R^2 values (0.920, 0.86, and 0.957), which further substantiates the hypothesis that all three ratios are relatively constant between 16 and 40 weeks of gestation. The intraobserver and interobserver variabilities were ≤8% for all parameters, the lowest being 2.6% for CC (intraobserver) and the highest 8% for HA (interobserver).

After having established the nomograms, the effect of PROM was assessed by studying 18 patients with PROM (gestational ages between 22 and 36 weeks). The measurements of these patients were compared to the nomograms (including the mean and the 5th and 95th percentile lines) as established from the patients with intact membranes. Chest circumference measurements were at or below the 5th percentile in 6 of 18 patients (33.3%) with PROM versus 15 of 181 (8.2%) with intact membranes ($p < 0.05$); 4 of 18 (22.2%) with PROM versus 9 of 181 (4.9%) with intact membranes had CA measurements at or below the 5th percentile ($p < 0.05$); and 4 of 18 (22.2%) with PROM versus 6 of 181 (3.3%) with intact membranes had CA − HA measurements at or below the 5th percentile ($p < 0.05$). The frequency of measurements at or below the 5th percentile for patients with PROM was the highest for gestational ages <28 weeks. Table I compares the three ratios (5th, mean, and 95th percentiles) between patients with intact and ruptured membranes. Although patients with PROM had lower mean values for CA/HA and (CA − HA) × 100/CA (3.74 versus 3.10 and 74 versus 68, respectively; $p < 0.05$), there were no differences in the 5th percentiles for any of the three ratios between patients with intact and ruptured membranes, and all patients with PROM had measurements within the normal range (between the 5th and 95th percentile).

As a third step in the study, the efficacy of the six ultrasonographic parameters to predict lethal pulmonary hypoplasia in 13 fetuses at high risk was determined. A total of six fetuses developed lethal pulmonary hypoplasia. There were five fetuses with CC measurements at or below the 5th percentile for gestational age, only two of whom developed lethal pulmonary hypoplasia. From four fetuses with CA measurements at or below the 5th percentile only two developed lethal

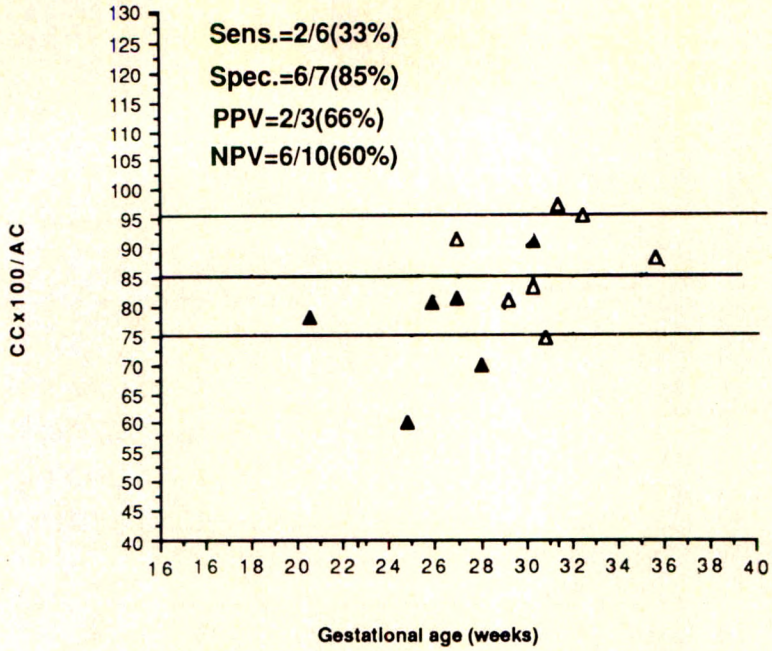


Fig. 2. Graph illustrating distribution of CC/AC ratios of 13 fetuses at risk for pulmonary hypoplasia (*solid triangles*, lethal pulmonary hypoplasia; *open triangles*, no lethal pulmonary hypoplasia). Values at or below 5th percentile were considered abnormal.

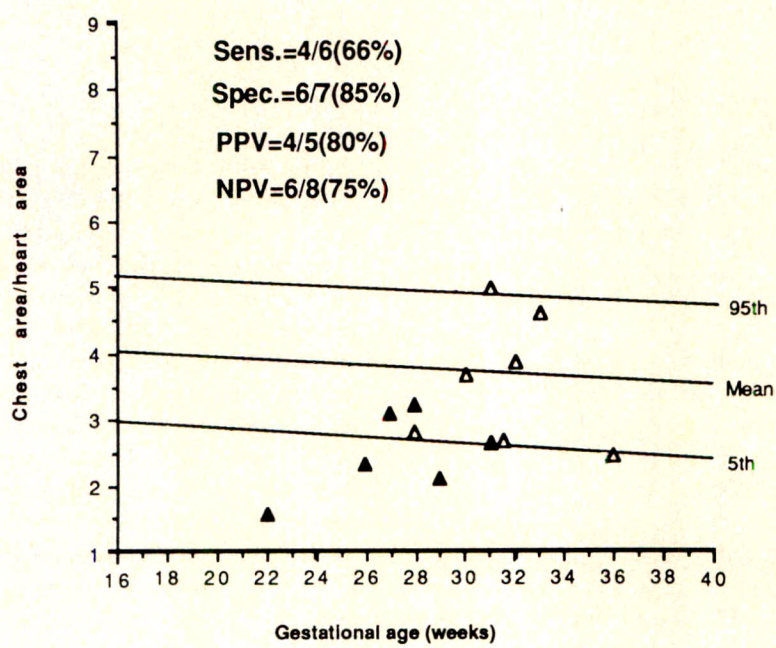


Fig. 3. Graph illustrating distribution of chest area/heart area ratios of 13 fetuses at risk for pulmonary hypoplasia (*solid triangles*, lethal pulmonary hypoplasia; *open triangle*, no lethal pulmonary hypoplasia). Values at or below 5th percentile were considered abnormal.

pulmonary hypoplasia, and from five fetuses with CA − HA measurements at or below the 5th percentile only three developed lethal pulmonary hypoplasia. Figs. 2, 3, and 4 illustrate the distribution of the three ratios of the 13 fetuses at risk. The (CA − HA) × 100/CA ratio had the best diagnostic accuracy (sensitivity 85%, specificity 85%, positive predictive value 83%, negative predictive value 85%, and overall efficiency 85%). Table II summarizes the efficacy of the six fetal parameters to predict lethal pulmonary

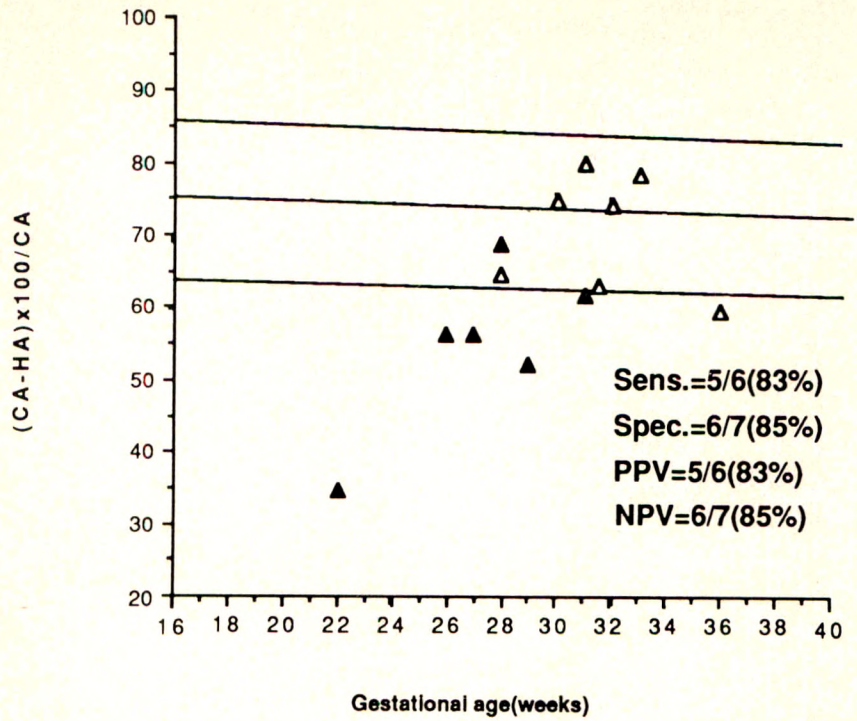


Fig. 4. Graph illustrating distribution of (CA − HA) × 100/CA ratios of 13 fetuses at risk for pulmonary hypoplasia (*solid triangles*, lethal pulmonary hypoplasia; *open triangles*, no lethal pulmonary hypoplasia). Values at or below 5th percentile were considered abnormal.

hypoplasia. Other parameters, such as CC − HtC, CC/HtC, and (CC − HtC)/CC, as well as interthoracic relationship nomograms (e.g., femur length vs. CC, CA, and CA − HA), were also established, but these did not improve diagnostic accuracy in predicting lethal pulmonary hypoplasia.

Comment

The presence of pulmonary hypoplasia has been well documented in fetuses with long-standing oligohydramnios, which could be the result of chronic amniotic fluid leakage or fetal renal disease or it could be idiopathic. The extent of fetal pulmonary hypoplasia depends on the degree of oligohydramnios, its duration, and the gestational age and stage of lung development when it occurs.[4] A spectrum of pulmonary hypoplasia can therefore be expected, ranging from mild transient respiratory disease to severe respiratory insufficiency leading to death in the immediate neonatal period.[5] From the clinician's standpoint it would be desirable to consider hypoplasia as either lethal or nonlethal. Although prediction of the nonlethal forms of pulmonary hypoplasia may or may not change obstetric management, prediction of the lethal form during the antepartum period may drastically alter obstetric management. It was for this reason, as well as the controversies surrounding the clinical diagnosis of pulmonary hypoplasia in surviving infants, that the present study was undertaken with the prediction of the lethal form of pulmonary hypoplasia used as an endpoint.

Previous investigators have attempted to make the antenatal diagnosis of lethal and nonlethal forms of pulmonary hypoplasia by using nomograms of chest circumference measurements throughout gestation,[6-8] interthoracic relationship nomograms (chest circumference measurements versus biparietal diameter, head circumference, abdominal circumference, or femur length),[11] or the CC/AC ratio, which has the advantage of being constant throughout gestation.[9, 10] Since the clinical setting of pulmonary hypoplasia is frequently accompanied by structural and growth abnormalities, the use of interthoracic nomograms is appropriate but only in cases where the different fetal organs are compromised to the same degree as the chest circumference. In many cases, however, the various fetal parameters are not equally compromised. Therefore, the idea of quantitatively defining intrathoracic growth relationships (e.g., heart or bony chest measurements) is attractive, and it was pursued in this study without including the skin and subcutaneous tissue in the thoracic measurements. In fetuses with obstructive uropathy or polycystic kidney disease who are at substantial risk for pulmonary hypoplasia, the use of the CC/AC ratio is inappropriate because of the frequently observed abdominal enlargement, which is secondary to the distended urinary tract. The use of this ratio is also in-

Table II. Efficacy to predict lethal pulmonary hypoplasia

Ultrasonographic parameter	*Sensitivity (%)*	*Specificity (%)*	*Positive predictive value (%)*	*Negative predictive value (%)*	*Test efficiency (overall) (%)*
CC	33	57	40	50	46
CA	33	71	50	55	54
CA-HA	50	71	60	62	62
CC × 100/AC	33	85	66	60	62
CA/HA	66	85	80	75	77
(CA-HA) × 100/CA	85	85	83	85	85

CC, Chest circumference; *CA*, chest area; *HA*, heart area; *AC*, abdominal circumference.

appropriate in the presence of fetal congenital anomalies such as omphalocele, ascites, diaphragmatic hernia, etc., which may distort or alter the abdominal circumference measurements. With the above considerations in mind the present cross-sectional study was undertaken with one of the major objectives to be the establishment of nomograms of ultrasonographic parameters that possibly could reflect fetal lung mass by studying 181 uncomplicated pregnancies with intact membranes at the time of the examination. Two of the six studied parameters were chest circumference measurements and CC/AC ratio. The relationship between gestational age and bony chest circumference measurements was curvilinear and the best fit was a second-degree polynomial equation. In some previous studies[6, 7] the relationship between gestational age and chest circumference measurements has been found to be linear. The mean, 5th, and 95th percentile values established in the present study were somewhat less than the corresponding values of the previously published nomograms.[6, 8] These differences can be explained by the different method that we used; specifically, we measured the bony chest circumference instead of measuring from outer edge to outer edge of the fetal thorax as done previously. For the same reason the CC/AC ratio was found to be somewhat less than previously reported norms (mean values 0.91 to 0.94 in previous studies[8, 10] versus 0.85 at the present study). In the present study the relationship between gestational age and chest area was found to be curvilinear with the best fit being a third-degree polynominal equation, whereas the relationship between gestational age and CH − HA was linear. One of the interesting findings of the present study was that the remaining two intrathoracic ratios (CA/HA) and [(CA − HA) × 100/CA] remained relatively constant throughout gestation and therefore could be used in patients with unknown or uncertain dates.

Although previous investigators have reported on the ability of chest circumference measurements and CC/AC ratio to predict pulmonary hypoplasia, there are significant differences between those studies and the present study in regards to methods, design, and prediction of nonlethal versus lethal forms of pulmonary hypoplasia. In the majority of previous studies fetuses at risk for pulmonary hypoplasia because of prolonged rupture of the membranes were evaluated by use of nomograms established from patients with intact membranes. However, rupture of the membranes by itself is known to cause fetal compression and therefore may be inherently associated with alterations in fetal biometry. The present study addressed the latter point by evaluating fetuses with ruptured membranes who were very unlikely to develop pulmonary hypoplasia since the duration of PROM was <1 week. It was found that PROM itself is associated with decreased thoracic measurements. Fetuses with PROM had a significantly higher frequency of measurements at or below the 5th percentile for CC (33.3%), CA (22.2%) and CA − HA (22.2%). This effect of PROM on these measurements was most pronounced at <28 weeks' gestation. The finding of the same mean value, as well as range, for CC/AC ratio would suggest that the same degree of thoracic and abdominal compression exists in the presence of PROM. In all cases with PROM, however, the measurements were within the normal range for all three gestational age–independent ratios. The fact that the 5th percentiles of the three ratios are not altered by the presence of PROM makes them potentially valuable tools for evaluating fetuses with prolonged PROM and fetuses of uncertain gestational ages.

As noted in Table II, most parameters had low positive and negative predictive values to be clinically useful. Other parameters, such as CC − HtC, CC/HtC, and (CC − HtC)/CC, were also studied as were interthoracic relationship nomograms (femur length vs. CC, CA, and CA − HA). However, these did not improve diagnostic accuracy. Most parameters had disappointingly low sensitivities. The diagnostic accuracies of the studied parameters were much lower than those of previous reports.[7, 10] This discrepancy may be due to: (1) differences in the populations studied; (2) inclusion of fetuses at particularly high risk for development of pulmonary hypoplasia in this study (all had documented oligohydramnios of >5 weeks' duration); and (3) dif-

ferences regarding the end point of prediction, which in our study was the lethal form of the disease. In the present study the (CA − HA) × 100/CA ratio had the best diagnostic accuracy (85%). This ratio is gestational age independent and it is not altered by PROM; in addition it can be used in fetuses with large or distorted AC (e.g., obstructive uropathy, omphalocele, etc.) where the use of CC/AC is inappropriate. We are continuing to evaluate the use of this ratio in our institution by collecting data from a larger number of fetuses.

REFERENCES

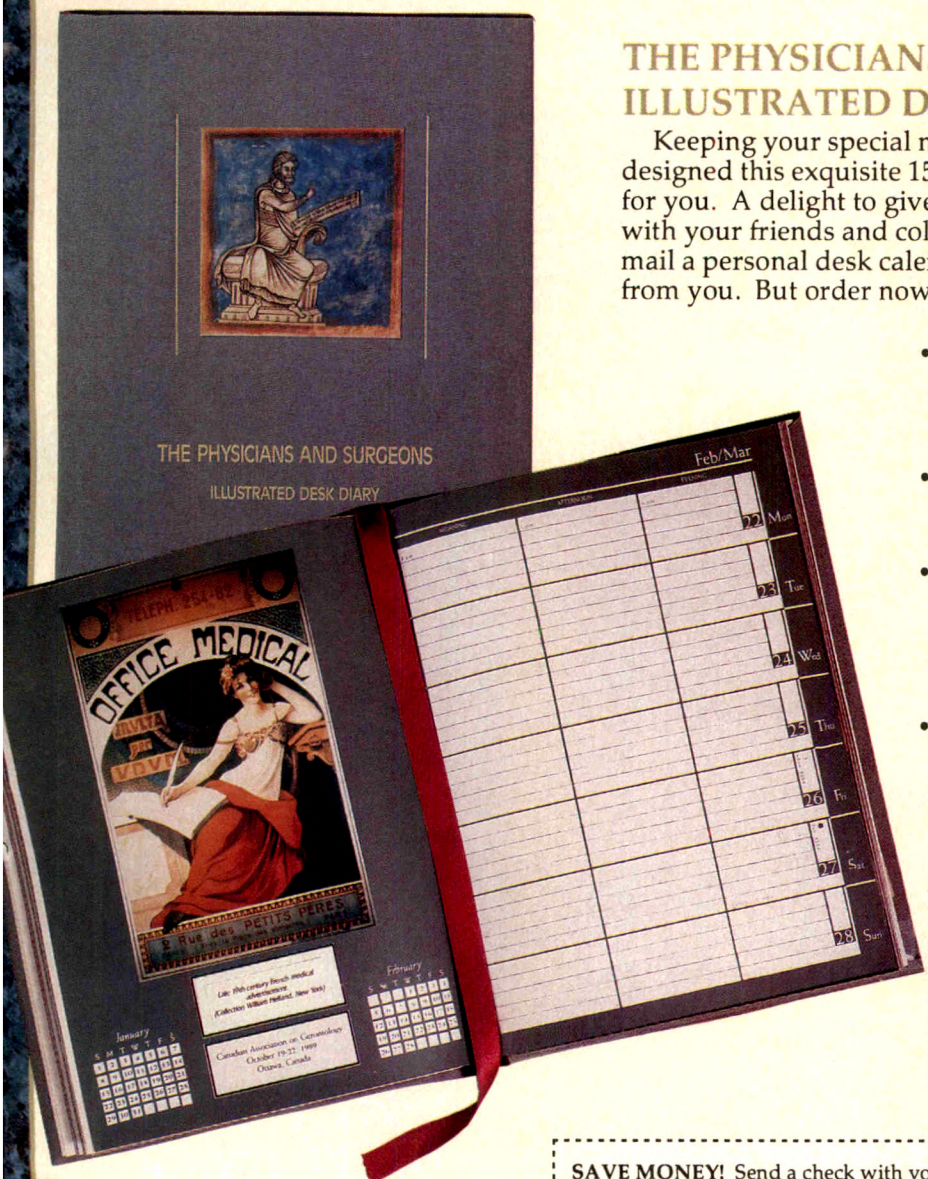
1. Hislop A, Hey E, Reid L. The lung in congenital bilateral renal agenesis and dysplasia. Arch Dis Child 1979; 54:32-8.
2. Potter EL. Bilateral renal agenesis. J Pediatr 1946;29:68-73.
3. Perlman M, Williams J, Hirsch M. Neonatal pulmonary hypoplasia after prolonged leakage of amniotic fluid. Arch Dis Child 1976;51:349-53.
4. Nimrod C, Varela-Gittings F, Machin G, Campbell D, Wesenberg R. The effect of very prolonged membrane rupture on fetal development. AM J OBSTET GYNECOL 1984;148:540-3.
5. Bhutani VK, Abbasi S, Weiner S. Neonatal pulmonary manifestations due to prolonged amniotic leak. Am J Perinatol 1986;3:225-30.
6. Nimrod C, Davies D, Iwanicki S, Harder J, Persaud D, Nicholson S. Ultrasound prediction of pulmonary hypoplasia. Obstet Gynecol 1986;68:495-8.
7. Nimrod C, Nicholson S, Davies D, Harder J, Dodd G, Sauve R. Pulmonary hypoplasia testing in clinical obstetrics. AM J OBSTET GYNECOL 1988;158:277-80.
8. Fong K, Ohlsson A, Zalev A. Fetal thoracic circumference: a prospective cross-sectional study with real-time ultrasound. AM J OBSTET GYNECOL 1988;158:1154-60.
9. Callan NA, Colmorgen GHC, Weiner S. Lung hypoplasia and prolonged preterm ruptured membranes: a case report with implications for possible prenatal ultrasonic diagnosis. AM J OBSTET GYNECOL 1985;151:756-7.
10. Johnson A, Callan N, Bhutani VK, Colmorgen GHC, Weiner S, Bolognese RJ. Ultrasonic ratio of fetal thoracic to abdominal circumference: an association with fetal pulmonary hypoplasia. AM J OBSTET GYNECOL 1987;157: 764-9.
11. VeDore GR, Horenstein J, Platt LD. Fetal echocardiography. VI. Assessment of cardiothoracic disproportion. A new technique for the diagnosis of thoracic dysplasia. AM J OBSTET GYNECOL 1986;155:1066-71.
12. Askenazi SS, Perlman M: Pulmonary hypoplasia: lung weight and radial count as criteria of diagnosis. Arch Dis Child 1979;54:614-8.

Bound volumes available to subscribers

Bound volumes of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY are available to subscribers (only) for the 1989 issues from the Publisher, at a cost of $56.00 ($79.00 international) for Vol. 160 (January-June) and Vol. 161 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue in the volume. The binding is durable buckram with the JOURNAL name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact The C.V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318, USA; phone (800) 325-4177, ext. 351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular JOURNAL subscription.

Presenting the Only Desk Calendar Designed Exclusively for the Busy Physician and Surgeon!



THE PHYSICIANS AND SURGEONS ILLUSTRATED DESK DIARY 1989-1990

Keeping your special needs and demands in mind, we've designed this exquisite 15-month desk calendar especially for you. A delight to give or receive--you'll want to share it with your friends and colleagues. At your request, we'll mail a personal desk calendar directly to them with a card from you. But order now--the supply is limited.

- *66 captivating reproductions of works by such well-known artists as Michelangelo, Rockwell, Hannah, and Metsu*
- *dates and locations of the major medical and surgical meetings in 1990*
- *all you need to plan your convention schedule --major airports and airlines, hotels and restaurants, including locations and telephone numbers*
- *high quality paper with gilt edges, classic ribbon marker and luxurious leather-bound cover (elegant cloth-bound cover also available)*

Act fast--
there will be only one printing, so supply is limited!
September, 1989. Approx. 160 pages, 66 illustrations including 50 in full color! Leather (Book Code: **06206**); Cloth (Book Code: **06205**)

To order, call toll-free: 800-221-7700, ext. 15A
24 hours a day, 7 days a week.
In Canada, call collect: 416-298-2465
FAX orders: 314-432-1380, Attn: Direct Marketing
Please mention this number when calling:
FLR-494-999
For Customer Service: 800-633-6699
Monday-Friday, 7AM-6PM, Central Time

Mosby

The C.V. Mosby Company
11830 Westline Industrial Drive
St. Louis, MO 63146
800-325-4177

The C.V. Mosby Company, Ltd.
5240 Finch Avenue East
Scarborough, Ontario
Canada, M1S 5A2
416-298-2465

SAVE MONEY! Send a check with your order or charge it to your credit card. Mosby pays the shipping and handling on all prepaid orders.
___Payment enclosed Charge my: ___MasterCard ___VISA ___AMEX

Card # _____ Exp. date_____

Signature _____

Business Phone (_____) _____

___Bill me, plus shipping and handling
Please send the number of Physicians Desk Calendars I've indicated to the address below:

___leather-bound **(06206)** pre-pub **$34.95** (U.S.); $45.50 (Can.); after 10/31/89 **$42.95** (U.S.); $56.00 (Can.)
___cloth-bound **(06205)** pre-pub **$24.95** (U.S.); $32.50 (Can.); after 10/31/89 **$32.95** (U.S.); $43.00 (Can.)

Name _____

Address _____

City _____

State _____ Zip _____

(Please attach a separate sheet for each additional address, indicating your choice of leather- or cloth-bound.)
If you're using a hospital Purchase Order, please attach this coupon.
Outside the U.S. and Canada, please pay by check or money order in dollar equivalent. 30-day approval good in U.S. and Canada. Prices subject to change without notice. Add applicable sales tax.

FLR-494-999-03



A 21-year-old female estimated at 42-weeks gestation was admitted for induction of labor. An excellent labor pattern was established within two hours. The cervix was completely effaced and dilated to 5 cms, with the vertex at zero station. Mild to moderate fetal heart rate decelerations on external monitors prompted amniotomy and placement of internal monitors.*

Clinical Status	Monitored Variables	
Admission to labor room—Fetal distress, variable decelerations, and thick meconium were noted at amniotomy. Oxygen administered via nasal cannula at 5 L/min and mother placed in left lateral decubitus position.	BP HR RR Temp	117/72 86/min 14/min 38° C
At 60 minutes—Controlled delivery of a 4100 gram meconium-stained infant. Apgar 7 and 9.		
Immediately after delivery—Mother gasped and became unresponsive.		
Within seconds—ECG, pulse oximetry and automatic blood pressure recordings instituted.	BP HR RR **SpO_2****	73/53 70/min 28/min (shallow) **88%**
At 65 minutes—Mother was intubated and ventilated with 100% oxygen and 10 cm PEEP. 500 ml of crystalloid were infused over 10 minutes.	BP HR RR **SpO_2**	73/51 60/min 0 - Spontaneous 12/min via Ambu bag **91%**
At 85 minutes—Central monitoring begun. PAP = 42/15, CVP = 10, PCWP = 14, CO = 9.4 ABG's obtained: PaO_2 = 76 mmHg, $PaCO_2$ = 47 mmHg, pH = 7.20, SaO_2 (Calc) = 91%.	BP HR **SpO_2**	110/69 90/min **90%**
180 minutes postpartum—Mother transferred to the intensive care unit and remained intubated. FiO_2 = 0.5, PAP = 32/16, CVP = 7, PCWP = 11, CO = 7.7, SaO_2 (Calc) = 89%.	BP HR **SpO_2**	93/57 84/min **91%**
Mother sustained significant physiologic insult as a result of acute amniotic fluid embolization.		
Over the ensuing 96 hours pulmonary function progressively improved and the patient was successfully weaned and extubated.		

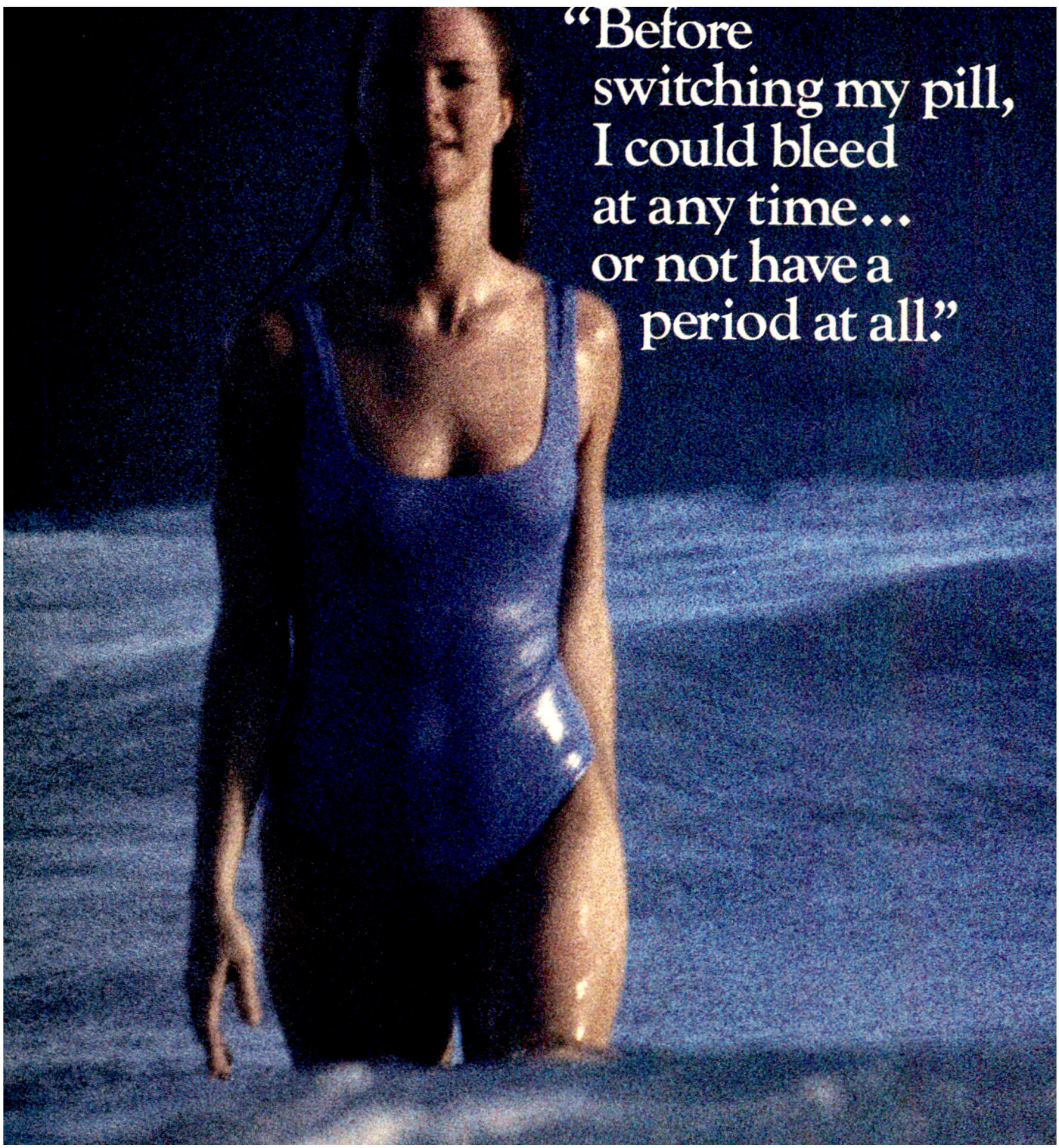
*Actual Case Study on file at Nellcor Incorporated. **SpO_2 is the measurement of arterial oxygen saturation by pulse oximetry.

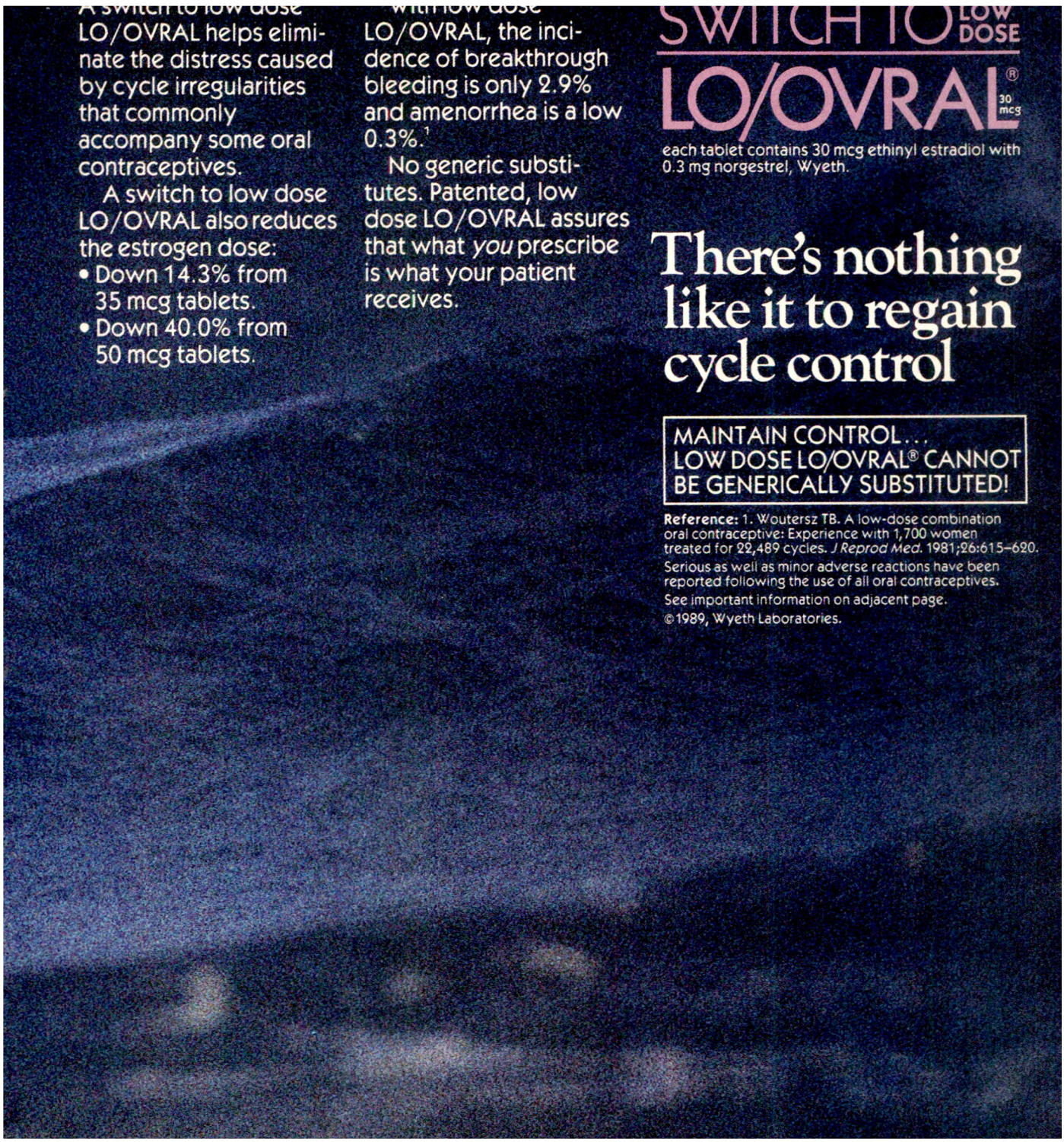
Discussion

This previously healthy labor patient developed a suddenly compromised cardiopulmonary system resulting in hypoxemia that was detected and managed through the early use of a pulse oximeter.

Routine use of a *NELLCOR*® N-200 pulse oximeter to continuously monitor patients during delivery provides for improved patient safety through early warning of respiratory complications.

NELLCOR®
Nellcor Incorporated
25495 Whitesell Street
Hayward, CA 94545 USA
Telephone 415 887-5858
Toll free 1-800-NELLCOR


"Before
switching my pill,
I could bleed
at any time...
or not have a
period at all."


A switch to low dose
LO/OVRAL helps eliminate the distress caused by cycle irregularities that commonly accompany some oral contraceptives.
A switch to low dose LO/OVRAL also reduces the estrogen dose:
• Down 14.3% from 35 mcg tablets.
• Down 40.0% from 50 mcg tablets.
With low dose LO/OVRAL, the incidence of breakthrough bleeding is only 2.9% and amenorrhea is a low 0.3%.[1]
No generic substitutes. Patented, low dose LO/OVRAL assures that what *you* prescribe is what your patient receives.
SWITCH TO LOW DOSE
LO/OVRAL® 30 mcg
each tablet contains 30 mcg ethinyl estradiol with 0.3 mg norgestrel, Wyeth.
There's nothing like it to regain cycle control
MAINTAIN CONTROL...
LOW DOSE LO/OVRAL® CANNOT BE GENERICALLY SUBSTITUTED!
Reference: 1. Woutersz TB. A low-dose combination oral contraceptive: Experience with 1,700 women treated for 22,489 cycles. *J Reprod Med.* 1981;26:615–620.
Serious as well as minor adverse reactions have been reported following the use of all oral contraceptives.
See important information on adjacent page.
©1989, Wyeth Laboratories.

IN BRIEF:

Indications and Usage—LO/OVRAL® is indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OC's) as a method of contraception.

Contraindications—OC's should not be used in women with any of the following conditions:

1. Thrombophlebitis or thromboembolic disorders.
2. A past history of deep-vein thrombophlebitis or thromboembolic disorders.
3. Cerebral-vascular or coronary-artery disease.
4. Known or suspected carcinoma of the breast.
5. Known or suspected estrogen-dependent neoplasia.
6. Undiagnosed abnormal genital bleeding.
7. Known or suspected pregnancy (see Warning No. 5).
8. Benign or malignant liver tumor which developed during use of OC's or other estrogen-containing products.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems*—An increased risk of thromboembolic and thrombotic disease associated with use of OC's is well established. Three principal studies in Great Britain and 3 in the U.S. have demonstrated increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OC's are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS—In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION (MI)—An increased risk of MI associated with use of OC's has been reported, confirming a previously suspected association. These studies, conducted in the UK, found, as expected, that the greater the number of underlying risk factors for coronary artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclamptic toxemia) the higher the risk of developing MI, regardless of whether the patient was an OC user or not. OC's, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to MI) are about twice as likely to have a fatal MI as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, amount of smoking is also an important factor. In determining importance of these relative risks, however, baseline rates for various age groups must be given serious consideration. Importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; quite likely the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE—In an analysis of data derived from several national adverse-reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to dose of estrogen in OC's. Preparations containing 100 mcg or more of estrogen were associated with higher risk of thromboembolism than those containing 50-80 mcg. Their analysis did suggest, however, that quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the U.S.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES—A large prospective study carried out in the UK estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OC's according to age, smoking habits, and duration of use. Overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34—5/100,000; ages 35-44—33/100,000; ages 45-49—140/100,000), risk being concentrated in older women, in those with long duration of use, and in cigarette smokers. It was not possible, however, to examine interrelationships of age, smoking, and duration of use, nor to compare effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess magnitude of relative risk for this younger group. Available data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates of risk of death for each method include combined risk of contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OC's) plus risk attributable to pregnancy or abortion in event of method failure. This latter risk varies with effectiveness of method. The study concluded that mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OC's in women over 40 who smoke. Lowest mortality is associated with condom or diaphragm backed up by early abortion. Risk of thromboembolic and thrombotic disease associated with OC's increases with age after about 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of pre-eclamptic toxemia, and especially cigarette smoking. Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A 4- to 6-fold increased risk of postsurgery thromboembolic complications has been reported in OC users.

If feasible, OC's should be discontinued at least 4 weeks before surgery of a type associated with increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS—Findings from one study in Britain involving cerebrovascular disease and another in the U.S. concerning MI suggest an increased risk of these conditions in users of OC's persists after discontinuation of the OC's. In the British study, risk of cerebrovascular disease remained elevated in former OC users for at least 6 years after discontinuation. In the U.S. study, increased risk of MI persisted for at least 9 years in women 40- to 49-years-old who had used OC's for 5 or more years. Findings in both studies require confirmation since they are inconsistent with other published information.

2. *Ocular Lesions*—There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with use of OC's. Discontinue OC's if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. *Carcinoma*—Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in OC's, have been noted to increase incidence of mammary nodules, benign and malignant, in dogs. In humans, 3 case-control studies have reported an increased risk of endometrial carcinoma associated with prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OC's. Of cases found in women without predisposing risk factors (e.g., irregular bleeding at the time OC's were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These are no longer marketed. No evidence has been reported suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only OC's. Several studies have found no increase in breast cancer in women taking OC's or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women on OC's, found an excess risk in subgroups of OC users with documented benign breast disease. Reduced occurrence of benign breast tumors in users of OC's has been well documented. In summary, there is at present no confirmed evidence from human studies of increased risk of cancer associated with OC's. Close clinical surveillance of all women on OC's is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or abnormal mammograms should be monitored with particular care if they elect to use OC's.

4. *Hepatic Tumors*—Benign hepatic adenomas have been found to be associated with use of OC's. One study showed that OC's with high hormonal potency were associated with higher risk than lower potency OC's. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users. Two studies relate risk with duration of use of OC's, the risk being much greater after 4 or more years' use. While hepatic adenoma is rare, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women on OC's. Relationship of these drugs to this type of malignancy is not known.

5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring*—Use of female sex hormones—both estrogenic and progestational agents—during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have increased risk of developing in later life a form of vaginal or cervical cancer ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence now that OC's further enhance risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OC's. Furthermore, 30 to 90% of such exposed women have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with use of other estrogens, it cannot be presumed they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with use of sex hormones, including OC's, in pregnancy. One case control study estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OC's, hormonal withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some exposures involved only a few days. Data suggest that risk of limb-reduction defects in exposed fetuses is somewhat less than 1 in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OC's. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OC's is unknown. It is recommended that, for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing OC's. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at time of first missed period, and further use of OC's should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and advisability of continuation of the pregnancy should be discussed. It is also recommended that women who discontinue OC's with intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this. The administration of progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease*—Studies report increased risk of surgically confirmed gallbladder disease in users of OC's and estrogens. In one study, increased risk appeared after 2 years' use and doubled after 4 or 5 years' use. In one of the other studies, increased risk was apparent between 6 and 12 months' use.

7. *Carbohydrate and Lipid Metabolic Effects*—Decrease in glucose tolerance has been observed in a significant percentage of patients on OC's. For this reason, prediabetic and diabetic patients should be carefully observed while on OC's. Increase in triglycerides and total phospholipids has been observed in patients on OC's; clinical significance of this finding remains to be defined.

8. *Elevated Blood Pressure*—Increase in blood pressure has been reported in patients on OC's. In some women, hypertension may occur within a few months of beginning OC's. In the 1st year of use, prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. Prevalence in users increases, however, with longer exposure, and in the 5th year of use is 2½ to 3 times the reported prevalence in the 1st year. Age is also strongly correlated with development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure on OC's. Hypertension that develops as a result of taking OC's usually returns to normal after discontinuing the drug.

9. *Headache*—Onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of OC's and evaluation of the cause.

10. *Bleeding Irregularities*—Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing OC's. In breakthrough bleeding, as in all cases of irregular vaginal bleeding, nonfunctional causes should be borne in mind.

In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another OC may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase risk of thromboembolic disease. Women with past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuing OC's. Women with these pre-existing problems should be advised of this possibility and encouraged to use other methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. *Ectopic Pregnancy*—Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. *Breast-feeding*—OC's given in the postpartum period may interfere with lactation and decrease quantity and quality of breast milk. Furthermore, a small fraction of the hormones in OC's has been identified in the milk of mothers on OC's; effects, if any, on the breast-fed child have not been determined. If feasible, defer OC's until infant has been weaned.

Precautions—GENERAL—1. A complete medical and family history should be taken prior to initiation of OC's. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Pap smear and relevant laboratory tests.

As a general rule OC's should not be prescribed for longer than 1 year without another physical examination.

2. Under influence of estrogen-progestogen preparations, pre-existing uterine leiomyomata may increase in size.

3. Patients with history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while on OC's should stop OC's and use an alternate method to try to determine whether the symptom is drug-related.

4. OC's may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency.

5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while on OC's. If jaundice develops, OC's should be discontinued.

6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution.

7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. Clinical significance is undetermined.

8. Serum folate levels may be depressed by OC's. Since the pregnant woman is predisposed to development of folate deficiency and incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OC's, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency.

9. The pathologist should be advised of OC therapy when revelant specimens are submitted.

10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OC's: a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test.

Information for the Patient—See Patient Package Labeling.

Drug Interactions—Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Carcinogenesis—See Warnings on carcinogenic potential of OC's.

Pregnancy—Category X. See Contraindications, Warnings.

Nursing Mothers—See Warnings.

Adverse Reactions—An increased risk of these serious adverse reactions has been associated with use of OC's (see Warnings): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies.

There is evidence of an association between the following conditions and use of OC's although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients on OC's and are believed to be drug-related: nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea, amenorrhea during and after treatment, temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement, and secretion; change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening), intolerance to contact lenses.

The following adverse reactions have been reported in users of OC's, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, hemolytic uremic syndrome.

Acute Overdose—Serious ill effects have not been reported following acute ingestion of large doses of OC's by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

2/27/85 3209-3 **54541**

LO/OVRAL® each tablet contains 0.3 mg norgestrel with 0.03 mg ethinyl estradiol.

WYETH AYERST™

Prophylactic amnioinfusion during labor complicated by meconium: A preliminary report

Yoel Sadovsky, MD, Erol Amon, MD, Mary E. Bade, BSMT, and Roy H. Petrie, MD, ScD
St. Louis, Missouri

The purpose of this prospective and randomized study is to evaluate the safety and efficacy of prophylactic transcervical amnioinfusion in the management of labor complicated by meconium. A preexisting intrauterine pressure catheter was used for amnioinfusion. The incidence of thick meconium was significantly lower after amnioinfusion, compared with standard management (5% versus 62% $p < 0.0005$). This was also demonstrated with spectrophotometry. Significant differences between the amnioinfusion group and the control group were found for the following: arterial cord pH <7.20 (16% versus 38%, $p < 0.05$); meconium more than trace below the vocal cords at delivery (0% versus 29%, $p < 0.05$) and need for positive pressure ventilation at birth (16% versus 48%, $p < 0.05$). No complications related to amnioinfusion were observed. We conclude that (1) transcervical amnioinfusion during labor complicated by meconium is a simple and apparently safe procedure, and that (2) amnioinfusion effectively decreases the frequency of thick meconium, the frequency of neonatal acidemia, the frequency of more than trace meconium below the vocal cords, and the need for positive pressure ventilation. (AM J OBSTET GYNECOL 1989;161:613-7.)

Key words: Amnioinfusion, meconium, meconium aspiration syndrome, labor

Passage of meconium in utero occurs in 8% to 16% of all deliveries.[1-3] Meconium aspiration syndrome occurs in 1% to 3% of all cases of meconium-stained amniotic fluid.[4] The presence of meconium in the amniotic fluid is associated with increased neonatal morbidity and mortality. The mechanism of this association and a cause-effect relationship is still unclear. Although meconium aspiration syndrome is a relatively uncommon complication, this syndrome is a major cause of unexpected neonatal mortality and morbidity. The neonatal mortality associated with this syndrome is as high as 28%.[1,5] Meconium aspiration may occur before labor, during labor, or at delivery. Current methods to prevent meconium aspiration syndrome include the combined obstetric and pediatric approach as described by Carson.[5] This technique includes suctioning of the nasopharynx, mouth, and hypopharynx with a DeLee catheter (ARGYLE, Division of Sherwood Medical Co., St. Louis, Mo.) as soon as the head is born. Immediately after the delivery is completed the pediatrician suctions the oropharynx with a bulb and inspects the vocal cords by direct laryngoscopy. The trachea is directly suctioned if meconium is present at the cords. This technique, which was reported to decrease the frequency of meconium aspiration syndrome, is based on the assumption that meconium aspiration occurs mainly during the delivery process.

The clinical application of transcervical amnioinfusion was first described by Miyazaki and coworkers[6,7] as an intrapartum procedure for the relief of variable decelerations. Later Nageotte and coworkers[8] applied this prophylactic measure to prevent variable decelerations in patients with preterm membrane rupture.

Our hypothesis is that intrapartum dilution of meconium by transcervical amnioinfusion may reduce the risk of meconium aspiration if it occurs during labor or at delivery. It may also improve neonatal outcome. The purpose of this study is to evaluate the safety and efficacy of transcervical amnioinfusion during labor complicated by meconium.

Material and methods

This prospective and randomized study was performed at the three University hospitals of the Department of Obstetrics and Gynecology at Washington University Medical Center. The Human Studies Committee of the medical school approved the study. The patients included women in labor who were found to have more than trace meconium on membrane rupture. Additional inclusion criteria were singleton pregnancy, vertex presentation, ≥34 weeks' gestation, and anticipated time to delivery >1 hour. The exclusion criteria were fetal malpresentation, multiple gestation, fetal malformation, polyhydramnios, chorioamnionitis, cord prolapse, any situation that necessitated urgent

From the Division of Maternal Fetal Medicine, Department of Obstetrics and Gynecology, Washington University School of Medicine.
Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.
Reprint requests: Erol Amon, MD, Washington University School of Medicine, 4911 Barnes Hospital Plaza, St. Louis, MO 63110.
6/6/13670

Table I. Patient characteristics

	Total $n = 40$	*Control* $n = 21$	*Amnioinfusion* $n = 19$
Maternal age (yr)	24.0 ± 6.2	24.4 ± 5.9	23.4 ± 6.6
Primigravidous patients	19 (48%)	11 (52%)	8 (42%)
Nulliparous patients	24 (60%)	13 (62%)	11 (58%)
Gestational age (wk)	40.1 ± 1.5	40.3 ± 1.5	39.9 ± 1.5
Birth weight (gm)	3378 ± 427	3368 ± 432	3390 ± 432
Neonatal gender (male)	20 (50%)	11 (52%)	9 (47%)

p = NS.

Table II. Labor characteristics

	Total $n = 40$	*Control* $n = 21$	*Amnioinfusion* $n = 19$
Initiation			
Spontaneous	29 (73%)	16 (76%)	13 (68%)
Induced	4 (10%)	1 (5%)	3 (16%)
Augmented	7 (18%)	4 (19%)	3 (16%)
Membrane rupture			
Spontaneous	21 (53%)	11 (52%)	10 (53%)
Artificial	19 (47%)	10 (48%)	9 (47%)
Narcotic use	15 (38%)	8 (38%)	7 (37%)
Dilatation (cm)*	3.8 ± 1.5 (0.5-8)	3.8 ± 1.6 (0.5-8)	3.8 ± 1.4 (1.5-6.5)
Labor duration (hr)*	15.9 ± 8.0 (4-35.5)	14.7 ± 8.2 (6-35.5)	17.3 ± 7.7 (4-32)
Admission to delivery interval (hr)*	11.6 ± 6.7 (2-27)	11.2 ± 7.1 (2-27)	12.0 ± 6.4 (3.5-27)
Meconium diagnosis to delivery interval (hr)*	9.9 ± 6.1 (2-25)	9.5 ± 7.0 (2-25)	10.3 ± 5.0 (3.5-19.5)

p = NS.
*Values are mean ± SD.

intervention, and maternal heart disease classified as New York Heart Association's class II or greater.

Informed consent was obtained before each patient was randomly assigned to an amnioinfusion or a control group. Randomization was carried out with sealed envelopes. The control group received standard labor management without amnioinfusion. In the amnioinfusion group a normal saline solution was connected through a Y-set to an existing intrauterine pressure catheter. The infused saline solution was continuously warmed to 37° C. The initial infusion was 600 ml during the first hour, then 180 ml/hr. The amnioinfusion was stopped before delivery. All patients had continuous internal fetal monitoring. The combined obstetric-pediatric suctioning approach to prevent meconium aspiration was used in all deliveries.

Samples of the meconium-stained fluid were aspirated through the pressure catheter, whenever possible, before and after the amnioinfusion. Meconium concentration, as measured by spectrophotometry, was used as an index of meconium thickness. The spectrophotometric determination was a modification of the method of Molcho et al.[9] Samples were frozen at −20° C. For meconium thickness determination the samples were brought to room temperature, vortexed for 60 seconds, and centrifuged at 1000 rpm for 5 minutes. Any sample that contained thick meconium was diluted with distilled water. Absorption readings were recorded by spectrophotometry, and compared with a distilled water control at 340 to 500 nm. The absorbance measurements were then plotted on linear graph paper, and the "background" absorbance line of nonstained amniotic fluid was determined. The "expected" optical density (OD) is the point on the drawn line that intersects with the 410 nm wavelength. The Δ OD 410 was calculated as the difference between the

Table III. Mode of delivery

	Total $n = 40$		*Control* $n = 21$		*Amnioinfusion* $n = 19$	
	n	%	*n*	%	*n*	%
Vaginal						
Spontaneous	22	55	9	43	13	69
Instrumental	6	15	5	24	1	5
Cesarean section	12	30	7	33	5	26
Dystocia	10	25	5	24	5	26
Fetal distress	2	5	2	9	0	0

p = NS.

measured OD and the expected OD at this wavelength. The Δ OD 410 value is the absorbance contributed by meconium.

Neonatal outcome was assessed by pediatricians who were unaware of the group to which each patient was allocated. The independent two-tailed Student t test, χ^2 test, and Fisher's exact test, when appropriate, were used for statistical analysis. Significance was set at $p < 0.05$.

Results

These data were collected between December 1987 and September 1988. A total of 40 patients were enrolled in the study; 19 were randomly assigned to the amnioinfusion group, and 21 were assigned to the control group. Table I compares demographic characteristics for the two groups. Antepartum risk factors were present in 12 patients. This included four patients with chronic hypertension, two in each group; three patients with mild preeclampsia, two in the control group and one in the amnioinfusion group; five patients with post dates, three in the control group and two in the amnioinfusion group. One patient in each group underwent a trial of labor after a previous cesarean section. Various clinical characteristics of labor are compared in Table II. No significant differences were found.

In the group that underwent amnioinfusion, a mean volume of 1357 ml (range, 700 to 2750 ml) of fluids was infused over a mean period of 6½ hours (range, 2 to 12½ hours). There were no procedure-related complications during this study.

Outcome data were compared between the group that underwent amnioinfusion and the control group. The frequency of thick meconium at initial evaluation was 62% in the control group and 79% in the amnioinfusion group (p = NS). After amnioinfusion the frequency of thick meconium remained at 62% for the control group, whereas it decreased to 5% ($p < 0.0005$) in the amnioinfusion group. Fig. 1 demonstrates the sepectrophotometric measurements for 11 of the 19 patients who underwent amnioinfusion from whom samples were obtained before and after the amnioinfusion. The mean Δ OD 410 value was significantly reduced from 0.526 ± 0.29 to 0.201 ± 0.17 after amnioinfusion in these 11 patients ($p < 0.005$). A total of 95% of the patients in both groups had a reactive fetal heart rate on admission. Before the second stage of labor 95% of the patients who underwent amnioinfusion had a reactive tracing versus 81% of the control group (p = NS). The percentage of patients with variable decelerations increased from 24% to 57% as labor progressed in the control group, whereas the percentage of patients with variable decelerations remained at 37% in the group that underwent amnioinfusion (p = NS).

Table III gives the mode of delivery of both groups. Of note is the observation that two cesarean sections were performed because of fetal distress, both in the control group. Postpartum fever occurred in two patients in the group that underwent amnioinfusion, both after cesarean section. In the control group five patients had postpartum fever, and of these, three were delivered of infants by cesarean section. Endometritis occurred in three patients. In each case endometritis followed cesarean section, two in the group subjected to amnioinfusion and one in the control group. The incidence of 1-minute Apgar scores <4 did not differ significantly between the amnioinfusion and the control groups (5% versus 9%, respectively). Similarly, no difference was found for the incidence of 5-minute Apgar scores <7 (5% in both groups).

Umbilical arterial cord blood analyses are shown in Fig. 2. A significantly lower rate of neonatal acidemia is noted in the group that underwent amnioinfusion ($p < 0.05$). Meconium, in any degree, was found below the vocal cords in 20% of all newborns. Fig. 3 compares the incidence of meconium below the cords, classified by pediatricians as greater than "trace." This occurred in 29% of newborns in the control group, and 0% in the group subjected to amnioinfusion ($p = 0.01$). Every newborn underwent suctioning with the DeLee catheter and direct laryngoscopy for the management of

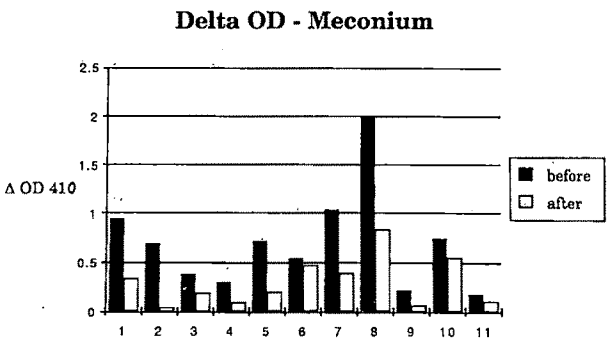


Fig. 1. Spectrophotometric measurements of meconium concentration in amniotic fluid in 11 patients before and after amnioinfusion. *OD* = optical density.

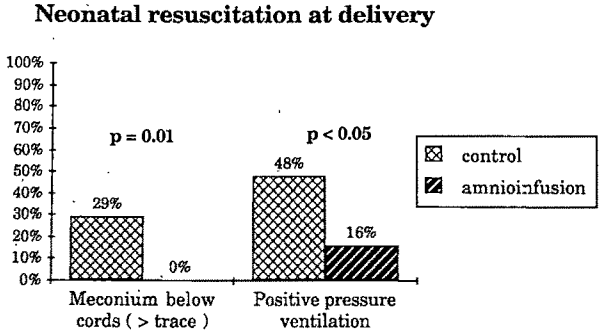


Fig. 3. Comparison of meconium below the vocal cords and need for resuscitation at birth between control and amnioinfusion groups.

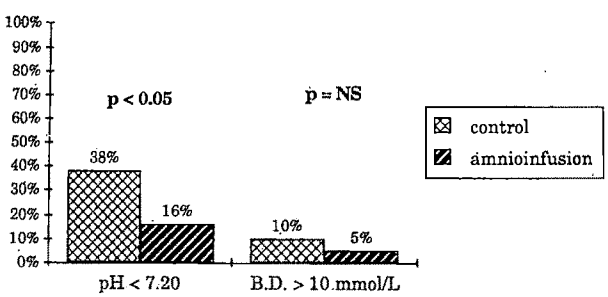


Fig. 2. Comparison of relative neonatal acidemia at birth between control and amnioinfusion groups. *B.D.* = base deficit; *NS* = not significant.

delivery complicated by meconium. As shown in Fig. 3, 48% of newborns in the control group also needed positive pressure ventilation. This was significantly greater than the 16% in the group that underwent amnioinfusion ($p < 0.05$). No newborn required cardiac or pharmacologic resuscitation, and none left the delivery room intubated. No newborn had meconium aspiration syndrome. One infant in the control group had transient respiratory distress of unclear cause and required oxygen support for 24 hours.

Comment

Several investigators have emphasized the risk of meconium aspiration occurring during the newborn's first few breaths, and the benefit from the combined obstetric-pediatric resuscitation approach.[3, 5, 10] Davis et al.[2] and later Falciglia[11] have shown that meconium aspiration syndrome may still occur despite appropriate use of the combined approach described by Carson. This occurrence may represent intrauterine meconium aspiration, which may have occurred before labor or during labor. Several studies have provided evidence that suggests the occurrence of fetal meconium aspiration.[4, 12-14] We adopted the technique of amnioinfusion as described by Miyazaki and coworkers[7] and modified by Nageotte[8] in an attempt to improve the outcome for fetuses exposed to meconium during labor.

The following conclusions can be drawn from our preliminary findings. First, transcervical amnioinfusion during labor complicated by meconium is a simple procedure that carries no apparent increase in maternal and neonatal morbidity. Second, amnioinfusion was significantly effective in the dilution of meconium-stained amniotic fluid. Third, amnioinfusion during labor complicated by meconium improved neonatal outcome as demonstrated by: (1) a significant decrease in the frequency of neonatal acidemia, (2) a significant reduction in the frequency of greater than "trace" meconium below the newborn's vocal cords, and (3) the reduction in need for positive pressure ventilation during resuscitation at birth.

We propose that the observed improvement in neonatal outcome is achieved by mechanical dilution of meconium during labor, which decreases the risk of intrapartum aspiration of thick and tenacious meconium. The increased risk of meconium aspiration syndrome when thick meconium is present early in labor has been previously suggested by Meis.[15] Furthermore, a decrease in the incidence of fetal acidemia during labor may decrease the frequency of fetal gasping,[13, 14] and thus the absence of acidemia may prevent the passage of meconium into the deeper airways.[12, 16] In the fetal lamb, asphyxia induced by temporary occlusion of the umbilical cord caused gasping and a change in tracheal pressure to −75 mm Hg for more than 1 second. In these circumstances, >10 ml of viscous amniotic fluid contaminated with meconium can be inhaled into the tracheobronchial tree.[16] Obviously, transcervical amnioinfusion will not prevent meconium aspiration that may have occurred before the onset of labor.

Although meconium aspiration syndrome is a leading cause of unanticipated neonatal mortality and mor-

bidity among term infants, this syndrome is a relatively uncommon complication. More patients will be needed to definitively study the effects of amnioinfusion on the syndrome. Power calculations reveal that >750 patients in each group will be needed to detect a reduction in the frequency of meconium aspiration syndrome from an incidence of 3% to 1% ($\alpha = 0.05$ and $\beta = 0.20$). Despite the small number of patients in this study we were able to demonstrate beneficial effects of amnioinfusion. The observed reduction in relative fetal-neonatal acidemia and the quantity of meconium below the vocal cords are two factors thought to be closely related to the development of meconium aspiration syndrome.

It is unclear whether the observed reduction of relative acidemia in the group that underwent amnioinfusion is due to mechanical dilution of the meconium or some other mechanism. It is possible that the relative sparing of umbilical cord compression by amnioinfusion lessens the frequency of variable decelerations as labor progresses, and thus contributes to an improved acid-base status. Perhaps amnioinfusion ameliorates yet unknown factors involved in the pathogenesis of meconium-related complications.

Collection of further data with this technique may assist investigators in the evaluation of the risk of meconium aspiration before labor. The association between meconium thickness and the incidence and severity of meconium aspiration may be studied in greater detail.

REFERENCES

1. Rosenberg AA. Neonatal adaptation. In: Gabbe SG, Niebyl JR, Simpson JL, eds. Obstetrics, normal and problem pregnancies. New York: Churchill Livingstone, 1986.
2. Davis RO, Philips JB, Harris BA, Wilson ER, Huddleston JF. Fetal meconium aspiration syndrome occurring despite airway management considered appropriate. AM J OBSTET GYNECOL 1985;151:731-6.
3. Gregory GA, Gooding CA, Phibbs RH, Tooley WH. Meconium aspiration in infants—a prospective study. J Pediatr 1974;85:848-52.
4. Brown BL, Gleicher N. Intrauterine meconium aspiration. Obstet Gynecol 1981;57:26-9.
5. Carson BS, Losey RW, Bowes WA, Simmons MA. Combined obstetric and pediatric approach to prevent meconium aspiration syndrome. AM J OBSTET GYNECOL 1976;126:712-5.
6. Miyazaki FS, Taylor NA. Saline amnioinfusion for relief of variable or prolonged decelerations. AM J OBSTET GYNECOL 1983;146:670-8.
7. Miyazaki FS, Nevarez F. Saline amnioinfusion for relief of repetitive variable decelerations: a prospective randomized study. AM J OBSTET GYNECOL 1985;153:301-6.
8. Nageotte MP, Freeman RK, Garite TJ, Dorchester W. Prophylactic intrapartum amnioinfusion in patients with preterm premature rupture of membranes. AM J OBSTET GYNECOL 1985;153:557-62.
9. Molcho J, Lieberman JR, Hagay Z, Hagay Y. Spectrophotometric determination of meconium concentration in amniotic fluid. J Biomed Eng 1986;8:162-5.
10. Benny PS, Malani S, Hoby MA, Hutton JD. Meconium aspiration—role of obstetric factors and suction. Aust NZ J Obstet Gynaecol 1987;27:36-9.
11. Falciglia HS. Failure to prevent meconium aspiration syndrome. Obstet Gynecol 1988;71:349-53.
12. Block MF, Kallenberger DA, Kern JD, Nepveux RD. In utero meconium aspiration by the baboon fetus. Obstet Gynecol 1981;57:37-40.
13. Byrne DL, Gau G. In utero meconium aspiration: an unpreventable cause of neonatal death. Br J Obstet Gynaecol 1987;94:813-4.
14. Manning FA, Schreiber J, Turkel SB. Fatal meconium aspiration "in utero": a case report. AM J OBSTET GYNECOL 1978;132:111-3.
15. Meis PJ, Hall M, Marshall JR, Hobel CJ. Meconium passage: a new classification for risk assessment during labor. AM J OBSTET GYNECOL 1978;131:509-13.
16. Dawes GS, Fox HE, Leduc BM, Liggins GC, Richards RT. Respiratory movements and rapid eye movement sleep in the fetal lamb. J Physiol 1972;220:119-43.

Lack of evidence of a vasodepressor role for relaxin in spontaneously hypertensive and normotensive pregnant rats

Robert A. Ahokas, PhD, Baha M. Sibai, MD, and Garland D. Anderson, MD
Memphis, Tennessee

To test the possibility that the ovarian polypeptide hormone relaxin mediates the vasodepressor effect of pregnancy, we measured changes in blood pressure in three groups of spontaneously hypertensive and Wistar-Kyoto normotensive rats: (1) pregnant, bilaterally oophorectomized rats on postmating day 13, (2) pregnant, sham-oophorectomized rats on postmating day 13, and (3) nonpregnant, sham-oophorectomized rats after 13 days. The day before parturition we also measured the pressor responsiveness to angiotensin II and norepinephrine. Systolic blood pressure fell significantly during the last week of gestation in both strains, reaching normotensive levels in the hypertensive rats by term. The pressor responsiveness to angiotensin II, but not norepinephrine, of the pregnant rats was reduced compared with that of the nonpregnant rats in both strains. Oophorectomy did not prevent the fall in blood pressure or the decrease in vascular reactivity to angiotensin II. Therefore, although ovarian relaxin secretion increases during pregnancy, it apparently does not decrease vascular sensitivity to angiotensin II or cause vasodilation. (AM J OBSTET GYNECOL 1989;161:618-22.)

Key words: Blood pressure, relaxin, pregnancy, angiotensin II, spontaneously hypertensive rat

Maternal cardiac output increases and total peripheral vascular resistance decreases during normal pregnancy in both humans and laboratory rats.[1] Pregnancy has a profound antihypertensive effect in hypertensive animals such as the spontaneously hypertensive rat. During the last third of gestation, blood pressure falls progressively and usually reaches normotensive levels by parturition.[2-4] The exact mechanism that causes these decreases is not known, but it is widely believed that a relative insensitivity to the pressor effects of angiotensin,[5] perhaps mediated by prostanoids,[6] may be involved. Recently a long-term vasodepressor action of relaxin, an ovarian polypeptide hormone produced in large amounts during pregnancy, has been described.[7] In addition, the pregnancy-associated decrease in blood pressure in humans[8] and in rats[9] coincides with increased circulating levels of relaxin. Long-term relaxin infusion blunts the in vitro vasoconstrictor responses of various vascular beds to arginine vasopressin and norepinephrine in spontaneously hypertensive rats,[10] similar to the responses observed in near-term pregnant spontaneously hypertensive rats.[11] Together these observations suggest that relaxin may play a role in the attenuation of vascular responsiveness to pressor agents and the reduction in blood pressure during normal pregnancy. This study was undertaken to determine if the removal of the source of endogenous relaxin (bilateral oophorectomy) would prevent the normal fall in blood pressure and the decrease in pressor responsiveness to vasoconstrictors during the last week of pregnancy in spontaneously hypertensive rats and normotensive Wistar-Kyoto rats.

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of Tennessee, Memphis.
Supported by Tennessee affiliate of the American Heart Association, grant No. TN-87-G-9.
Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.
Reprint requests: Robert A. Ahokas, PhD, Department of Obstetrics and Gynecology, University of Tennessee, Memphis, Memphis, TN 38163.
6/6/13713

Material and methods

Female spontaneously hypertensive rats and Wistar-Kyoto rats, 15 to 20 weeks old (Harlan Laboratories, Inc., Indianapolis) were housed at 23 ± 1° C and a 12:12 hour light-dark cycle (with lights on from 7 AM to 7 PM), and fed Purina rodent chow (Ralston Purina, St. Louis) as desired with tap water to drink. For breeding, the females were caged with mature male breeders (1:1) of the same strain, and vaginal smears were checked daily in the morning for the presence of spermatozoa. Timed-pregnant females were then housed one per cage throughout pregnancy. Systolic blood pressure was measured weekly through the first 2 weeks of pregnancy and semidaily during the last week of pregnancy by means of tail-cuff plethysmography (IITC, Inc., Woodland Hills, Calif.). Since the corpus luteum is the exclusive source of relaxin in the rat,[9] bilateral oophorectomy was used to prevent the increased endogenous relaxin secretion. On day 13 of gestation the rats were anesthetized with methoxyflurane and a midline laparotomy was performed. Both

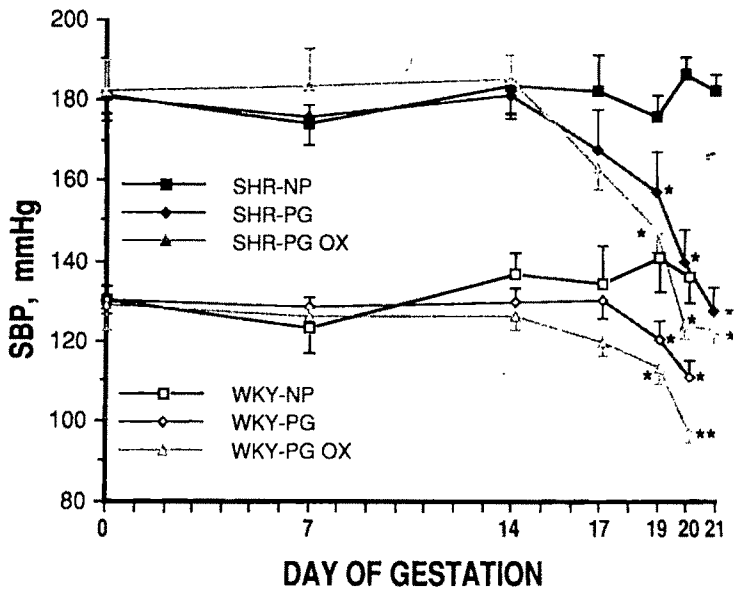


Fig. 1. Changes in systolic blood pressure *(SBP)* in nonpregnant and pregnant, spontaneously hypertensive *(SHR)* and normotensive Wistar-Kyoto *(WKY)* rats. Data plotted as mean ± SEM. *NP*, Nonpregnant, sham-oophorectomized; *PG*, pregnant sham-oophorectomized; *PG OX*, pregnant, bilaterally oophorectomized. *Asterisk* indicates significant difference from *NP* of same strain ($p \leq 0.05$); *double asterisk* indicates significant difference between *PG* and *PG OX* of same strain ($p \leq 0.005$).

ovaries were removed after ligation of the ovarian vessels ($n = 9$ spontaneously hypertensive rats; $n = 7$ Wistar-Kyoto rats). In other rats the ovaries were only visualized (sham-oophorectomized) ($n = 8$ spontaneously hypertensive rats; $n = 8$ Wistar-Kyoto rats). Nonpregnant females of each strain ($n = 8$ spontaneously hypertensive rats; $n = 7$ Wistar-Kyoto rats) were also sham oophorectomized after 13 days. Pregnancy was maintained in the oophorectomized rats by daily subcutaneous injection of 200 ng 17β-estradiol and 3 mg progesterone in 0.1 ml sesame oil. Both pregnant and nonpregnant sham-oophorectomized rats received 0.1 ml sesame oil only.

The day before expected delivery (postmating day 21 for spontaneously hypertensive rats; postmating day 20 for Wistar-Kyoto rats) whole-body pressor responsiveness to angiotensin II and norepinephrine were measured. Catheters had been placed in the left carotid artery (for blood pressure measurement) and the right jugular vein (for vasoconstrictor infusion) the previous day. For these measurements, the rat was placed in a Plexiglas restrainer cage. The arterial catheter was connected to a Statham P23Gb strain-gauge pressure transducer, and blood pressure was recorded continuously on a Gould 2200S physiologic recorder (Gould, Inc., Cleveland). After blood pressure stabilization (approximately 30 minutes), mean blood pressure responses to bolus injections of angiotensin II (5 to 2000 ng/kg, intravenously) and norepinephrine (10 to 4000 ng/kg, intravenously) were measured. Each dose was contained in a volume of 50 μl and was flushed into the catheter with 0.2 ml of normal saline solution. Blood pressure was allowed to return to baseline between each dose; a 1-hour recovery period was allowed between pressor substances. Dose-response curves for each pressor substance were generated from the maximum change in mean arterial pressure at each dose. Maximum effect was estimated as the intercept of the linear regression line of the double reciprocal plot of dose and maximum blood pressure responses. The 50% effective dose of each pressor substance was determined by log dose-probit analysis.[12]

The data were analyzed statistically by means of two-factor analysis of variance. Significant differences between means were determined by the least-squares means method. Values of $p \leq 0.05$ were considered statistically significant for all analyses.

Results

At conception systolic blood pressure of the spontaneously hypertensive rats was significantly higher than that of the Wistar-Kyoto rats (Fig. 1). During the last week of gestation, systolic blood pressure of both the sham-oophorectomized and the bilaterally oophorectomized spontaneously hypertensive rats decreased progressively and was significantly lower than that of the nonpregnant spontaneously hypertensive rats by day 19. Furthermore, the systolic blood pres-

Table I. Baseline values of mean arterial pressure, and angiotensin II, and norepinephrine maximum blood pressure effects and doses to induce 50% maximal effects in three groups of rats

Group	N	MAP (mm Hg)	Angiotensin II		Norepinephrine	
			E_{max} (mm Hg)	ED_{50} (ng/kg)	E_{max} (mm Hg)	ED_{50} (ng/kg)
Spontaneously hypertensive rats						
NP control	8	187 ± 3	37.6 ± 2.8	17.4 ± 4.8	45.5 ± 4.5	508 ± 170
PG control	8	137 ± 12*	63.8 ± 4.1*	77.5 ± 8.7*	53.0 ± 4.0	698 ± 118
PG ovariec.	9	124 ± 6*	66.9 ± 3.1*	74.8 ± 9.5*	51.3 ± 2.3	681 ± 81
Wistar-Kyoto rats						
NP control	7	133 ± 4†	45.7 ± 2.4	59.2 ± 7.1†	41.4 ± 1.4	493 ± 98
PG control	8	101 ± 3*,†	52.6 ± 1.9†	120.5 ± 15.8*,†	41.1 ± 2.6†	673 ± 99
PG ovariec.	7	78 ± 2*,†,‡	53.4 ± 2.7†	113.1 ± 27.9*	40.0 ± 5.9†	688 ± 88

MAP, Mean arterial pressure; E_{max}, maximum effect; ED_{50}, 50% effective dose; *N-P control*, nonpregnant, sham-oophorectomized; *PG control*, term-pregnant, sham-oophorectomized; *PG ovariec.*, pregnant, oophorectomized.

Values are mean ± SEM.

*Significantly different from NP control ($p \leq 0.05$) of same strain.

†Significantly different from spontaneously hypertensive rats ($p \leq 0.05$) of same group.

‡Significantly different from PG control ($p \leq 0.05$) of same strain.

sure of both the oophorectomized and the sham-oophorectomized pregnant spontaneously hypertensive rats was not significantly higher than that of the nonpregnant normotensive Wistar-Kyoto rats by day 19. There were no significant differences in systolic blood pressure between oophorectomized and sham-oophorectomized spontaneously hypertensive rats at any time during gestation. The systolic blood pressure of pregnant normotensive Wistar-Kyoto rats also fell, although not as much as in the spontaneously hypertensive rats, during the last week of gestation and was significantly lower than that of the nonpregnant rats by day 19. Systolic blood pressure of the oophorectomized pregnant Wistar-Kyoto rats, unlike spontaneously hypertensive rats, was significantly lower than that of the sham-oophorectomized pregnant Wistar-Kyoto rats on day 20.

The angiotensin II dose–pressor response curves for both the oophorectomized and sham-oophorectomized pregnant rats were shifted to the right of the curves for the nonpregnant rats, in both the spontaneously hypertensive rats (Fig. 2, *A*) and Wistar-Kyoto rats (Fig. 2, *B*). The angiotensin II 50% effective dose and maximum effect were significantly higher in both groups of pregnant spontaneously hypertensive rats compared with the nonpregnant spontaneously hypertensive rats (Table I). In the normotensive Wistar-Kyoto rats, only the angiotensin II 50% effective dose was significantly increased in the pregnant rats in comparison with the nonpregnant rats. In addition, the angiotensin II dose-response curves for the normotensive Wistar-Kyoto rats were shifted to the right of those of the spontaneously hypertensive rats with a significantly higher angiotensin II 50% effective dose.

The norepinephrine dose-response curves of the pregnant spontaneously hypertensive rats (Fig. 3, *A*) and Wistar-Kyoto rats (Fig. 3, *B*) were similar to those of the nonpregnant rats of each strain. There were no significant differences in the norepinephrine 50% effective dose or maximum effect (Table I).

Comment

In the rat, as in humans, pregnancy is accompanied by a decrease in total peripheral vascular resistance and blood pressure. Although the mechanism causing vasodilation is not entirely understood, it is widely believed that attenuation of resistance vascular sensitivity to endogenous pressor substances such as angiotensin II plays a central role. Results of our study confirm those of Massicotte et al.[13] that whole-body pressor responsiveness to angiotensin II is reduced in term-pregnant spontaneously hypertensive rats and Wistar-Kyoto rats in relation to nonpregnant rats (Fig. 2, Table I). However, we cannot state with certainty that the refractoriness of the vascular response to angiotensin II is the cause of the decrease in blood pressure. Whole-body pressor responsiveness to norepinephrine in term-pregnant rats was not significantly different from that of nonpregnant rats in either strain (Fig. 3, Table I).

Increased plasma levels of relaxin are also associated with the decrease in blood pressure during pregnancy in the rat[9] and in humans.[8] The primary function of relaxin is apparently to relax the uterus and pubic symphysis and to soften the cervix.[14] Recently, however, relaxin has been observed to also have a vasodepressor action in the rat. Long-term intravenous infusions of homologous relaxin in cycling nonpregnant spontaneously hypertensive rats markedly lowered systolic

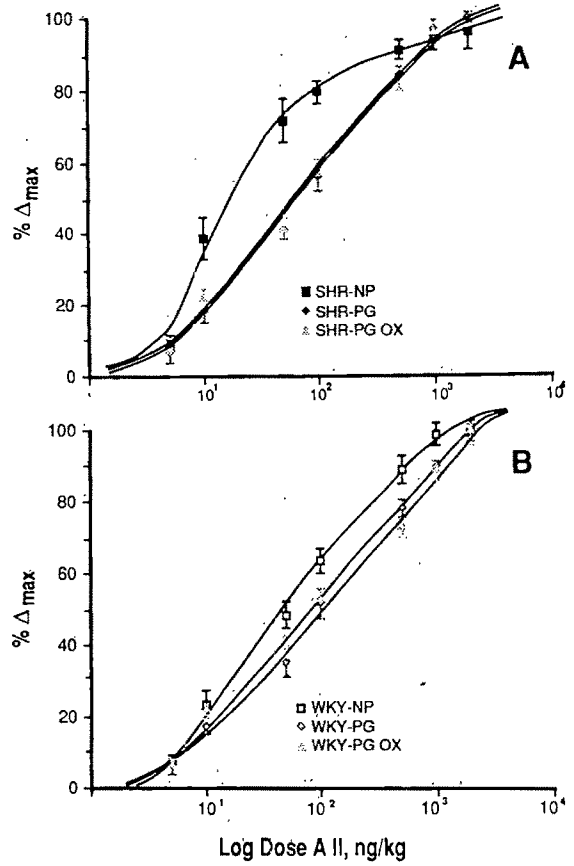


Fig. 2. Angiotensin II dose–pressor response curves for nonpregnant and term-pregnant spontaneously hypertensive *(SHR)* rats **(A)** and Wistar-Kyoto *(WKY)* rats **(B).** Data plotted as mean ± SEM percentage of maximum change in mean arterial pressure *(% Δ_{max})* at each dose against log of dose (nanograms per kilogram). *NP,* Nonpregnant, sham-oophorectomized; *PG,* pregnant, sham-oophorectomized; *PG OX,* pregnant, bilaterally oophorectomized.

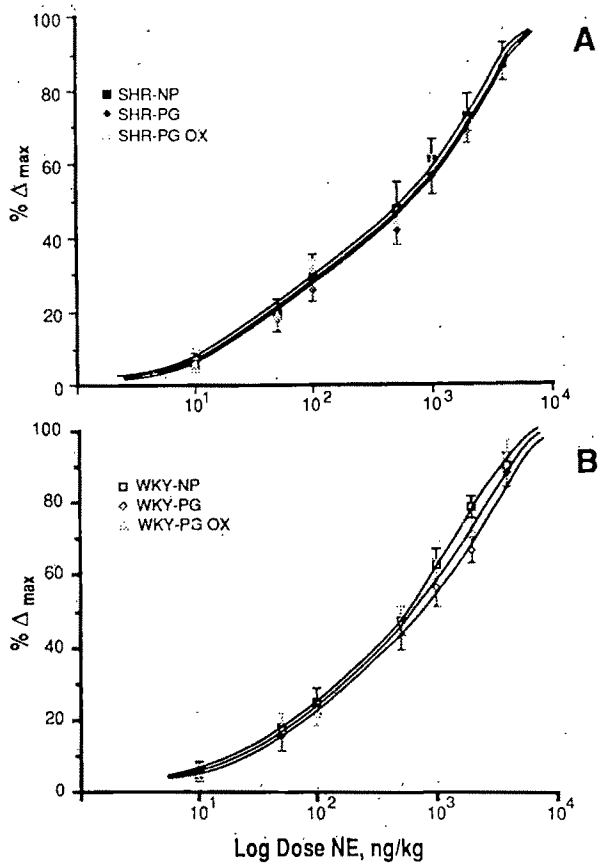


Fig. 3. Norepinephrine dose–pressor response curves for nonpregnant and term-pregnant spontaneously hypertensive *(SHR)* rats **(A)** and Wistar-Kyoto *(WKY)* rats **(B).** Data plotted as mean ± SEM percentage of maximum change in mean arterial pressure *(% Δ_{max})* at each dose against log of dose (nanograms per kilogram). *NP,* Nonpregnant, sham-oophorectomized; *PG,* pregnant, sham-oophorectomized; *PG OX,* pregnant, bilaterally oophorectomized.

blood pressure,[7] and blunted the constrictor response to arginine vasopressin and norepinephrine in the perfused mesenteric vasculature.[10] The dose-response curves were nearly identical to those obtained from the perfused mesenteric vascular beds of 20- to 21-day-pregnant spontaneously hypertensive rats.[11] These results suggested that relaxin may be one of the circulating factors responsible for the decrease in vascular sensitivity to circulating vasoconstrictor hormones and blood pressure during pregnancy. However, when we prevented the pregnancy-associated increase in endogenous relaxin in both spontaneously hypertensive rats and normotensive Wistar-Kyoto rats by removing the ovaries on day 13 of gestation, systolic blood pressure fell normally during the last week of gestation (Fig. 1), and the in vivo vascular reactivity to exogenous angiotensin II was attenuated to the same degree as in pregnant sham-oophorectomized rats at term (Fig. 2, Table I), suggesting that relaxin does not have a vasodepressor effect. An alternative explanation for these results is that other tissues may produce and secrete relaxin during pregnancy (relaxin is produced by the placenta in rabbits and horses, and by the endometrium in guinea pigs.[14]). Although we did not measure plasma relaxin concentration at the end of the experiment because of our lack of a homologous radioimmunoassay for rat relaxin, this explanation is not likely. It has been conclusively shown that the corpus luteum is the only tissue that produces relaxin in the rat; and plasma relaxin immunoactivity falls rapidly to prepregnant levels with a mean clearance half-life of approximately 1 hour after bilateral ovariectomy on day 21 of pregnancy.[9] Another alternative is that the ovaries may not have been entirely removed in our study. Some corpus luteum tissue may have remained and continued to produce and secrete relaxin. This also is unlikely because

great care was taken to ligate the ovarian vessels proximal to the ovaries, and both the ovaries and surrounding fat were excised. Careful inspection at the end of the experiment revealed no trace of ovarian tissue.

Although removal of the ovaries did not prevent the normal decrease in vascular reactivity and blood pressure associated with pregnancy, the factor(s) involved in these cardiovascular adaptations may nevertheless be of ovarian origin. Plasma levels of 17β-estradiol and progesterone increase throughout pregnancy, and recent evidence has shown that 17β-estradiol infusion (although not progesterone), produced significant systemic vasodilation[15] and suppressed the pressor responses to angiotensin II in nonpregnant oophorectomized ewes.[15, 16] Our results in rats indirectly support this observation. We maintained pregnancy in the oophorectomized rats by daily injection of pharmacologic doses of 17β-estradiol and progesterone. Blood pressure in the oophorectomized pregnant Wistar-Kyoto rats decreased even more than that of sham-oophorectomized pregnant rats and reached statistical significance by the day before delivery. This suggests that one or both steroids may induce vasodilation in a dose-dependent manner, but additional work is necessary to substantiate this possibility.

We thank Ed B. Cannon for technical assistance and Kristopher Arheart for assistance in statistical analysis of the data.

REFERENCES

1. deSwiet M. The physiology of normal pregnancy. In: Rubin PC, ed. Handbook of hypertension. Hypertension in pregnancy. Amsterdam: Elsevier Science, 1988, vol 10:1.
2. Aoi W, Gable D, Cleary RE, Young PCM, Weinberger MH. The antihypertensive effect of pregnancy in spontaneously hypertensive rats. Proc Soc Exp Biol Med 1976; 153:13-5.
3. Lorenz RP, Picchio LP, Weisz J, Lloyd T. The relationship between reproductive performance and blood pressure in the spontaneously hypertensive rat. Am J Obstet Gynecol 1984;150:519-23.
4. Ahokas RA, Reynolds SL, Wang Y-F, Anderson GD, Lipshitz J. The effect of carbohydrate overfeeding on blood pressure in the pregnant, spontaneously hypertensive rat. Am J Obstet Gynecol 1986;155:1113-8.
5. Gant NF, Daly GL, Chand S, Whalley PJ, McDonald PC. A study of angiotensin II pressor response throughout primigravid pregnancy. J Clin Invest 1973;52:2682-9.
6. Broughton-Pipkin F, Hunter JC, Turner SR, O'Brien PMS. The effect of prostaglandin E_2 upon the biochemical response to infused angiotensin II in human pregnancy. Clin Sci 1984;66:399-406.
6. St-Louis J, Massicotte G. Chronic decrease of blood pressure by rat relaxin in spontaneously hypertensive rats. Life Sci 1985;37:1351-7.
8. O'Byrne EM, Corriere BT, Sorensen L, Segaloff A, Schwabe C, Stinetz BG. Plasma immunoreactive relaxin levels in pregnant and nonpregnant women. J Clin Endocrinol Metab 1978;47:1106-10.
9. Sherwood OD, Crnekovic VE, Gordon WL, Rutherford JE. Radioimmunoassay for relaxin throughout pregnancy and during parturition in the rat. Endocrinology 1980; 107:691-8.
10. Massicotte G, Parent A, St-Louis J. Blunted responses to vasoconstrictors in mesenteric vasculature but not in portal vein of spontaneously hypertensive rats treated with relaxin. Proc Soc Exp Biol Med 1989;190:254-9.
11. Massicotte G, St-Louis J, Parent A, Schiffrin EL. Decreased in vitro responses to vasoconstrictors during gestation in normotensive and spontaneously hypertensive rats. Can J Physiol Pharmacol 1987;24:2466-71.
12. Tallarida RJ, Murray RB. Manual of pharmacologic calculations with computer programs. New York: Springer-Verlag, 1987:26.
13. Massicotte G, St-Louis J, Schiffrin EL. Blood pressure effects of angiotensin II and vasopressin in conscious pregnant normotensive and spontaneously hypertensive rats. Clin Exp Hypertens [B] 1986,B5:135-58.
14. Weiss G. Relaxin. Annu Rev Physiol 1984;46:43-52.
15. Naden RP, Rosenfold CR. Systemic and uterine responsiveness to angiotensin II and norepinephrine in estrogen-treated nonpregnant sheep. Am J Obstet Gynecol 1985;153:417-25.
16. Tamai T, Matsuura S, Tatsumi N, Takaaki N, Sagawa N. Role of sex steroid hormones in relative refractoriness to angiotensin II during pregnancy. Am J Obstet Gynecol 1984;149:177-83.

Perinatal factors influencing atrial natriuretic peptide levels in umbilical arterial plasma at the time of delivery

Lony C. Castro, MD, Chander P. Arora, PhD, Kay E. Roll, RN, Deborah A. Sassoon, MD, and Calvin J. Hobel, MD

Los Angeles, California

Little is known about atrial natriuretic peptide metabolism or secretion in the human fetus. The purpose of this study was to determine if both the placenta and umbilical vessels are possible sites of atrial natriuretic peptide metabolism and to evaluate the effects that labor, route of delivery, prolonged pregnancy, preeclampsia, and fetal distress have on umbilical arterial atrial natriuretic peptide levels. We found that plasma atrial natriuretic peptide levels in the umbilical artery are significantly greater than those in the vein ($p < 0.001$). Umbilical arterial and umbilical venous atrial natriuretic peptide levels were higher in plasma samples collected immediately at delivery when compared with those obtained 10 minutes later ($p < 0.001$). Umbilical arterial atrial natriuretic peptide levels were elevated in pregnancies complicated by preeclampsia and fetal distress ($p < 0.01$). Labor, route of delivery, and prolonged pregnancy had no effect on umbilical arterial atrial natriuretic peptide levels. We propose that both the placenta and umbilical vessels contain atrial natriuretic peptide receptors that are involved in the clearance or metabolism of atrial natriuretic peptide. The increased umbilical arterial atrial natriuretic peptide levels present in preeclampsia and fetal distress may reflect an attempt by the fetus to regulate blood flow. (AM J OBSTET GYNECOL 1989;161:623-7.)

Key words: Atrial natriuretic peptide, umbilical artery, fetal distress

Atrial natriuretic peptide (ANP) refers to a family of peptides secreted by the heart that are involved in the regulation of blood pressure and blood volume through a variety of effects on the cardiovascular, renal, and endocrine systems.[1] The major stimulus for ANP release appears to be atrial distension, but neural and humoral factors,[2, 3] as well as alterations in myocardial work,[4] may also modulate ANP secretion. ANP is cleared rapidly from the circulation of adults probably by binding to specific receptors located in many vascular beds.[5, 6] There is also evidence for a much slower degradation of ANP, possibly by a circulating peptidase.[7]

ANP has been identified in the fetal heart and circulation of a number of mammalian species. The ovine fetus has been the most intensively studied and has been shown to respond to infused ANP with an increase in urine output, as well as a decrease in blood pressure and blood volume.[8] Two seemingly disparate stimuli, intravascular volume expansion and acute hypoxia with accompanying volume depletion, have been shown to stimulate ANP release in fetal sheep.[9, 10] Under basal conditions, ovine fetal plasma ANP levels have been shown to be higher than maternal levels. Experiments demonstrating that fetal plasma ANP clearance rates exceed maternal rates suggest that the high baseline fetal levels are due to an increase in the secretion of ANP by the fetus.[11]

Little is known about the metabolism or secretion of ANP in the human fetus. The possibility that the placenta may be a site of ANP clearance or metabolism is suggested by a recent report documenting the presence of ANP receptors on the fetal side of the placenta.[12] An umbilical arterial-venous difference in ANP levels has also been reported by one group but this has not been confirmed by others.[13, 14] Information about possible stimuli for ANP secretion in the human fetus is also limited. However, one can postulate that relatively common conditions such as advanced gestational age, preeclampsia, and fetal distress, which may be associated with hypoxia or alterations in the fetal cardiovascular system and hormonal environment, could affect fetal ANP secretion.

The purpose of this study was to determine if both the placenta and umbilical vessels are possible sites of ANP clearance or metabolism and to determine if a circulating peptidase with the capability of degrading ANP is present in the umbilical placental circulation. We also attempted to evaluate the effects that prolonged pregnancy, preeclampsia, and fetal distress have

From the Department of Obstetrics and Gynecology, Cedars-Sinai Medical Center.

Supported in part by a grant from the Helping Hand of Los Angeles.
Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.

Reprint requests: Lony C. Castro, MD, Department of OB/GYN, Room 3210, Cedars-Sinai Medical Center, 8700 Beverly Blvd., Los Angeles, CA 90048.

6/6/14702

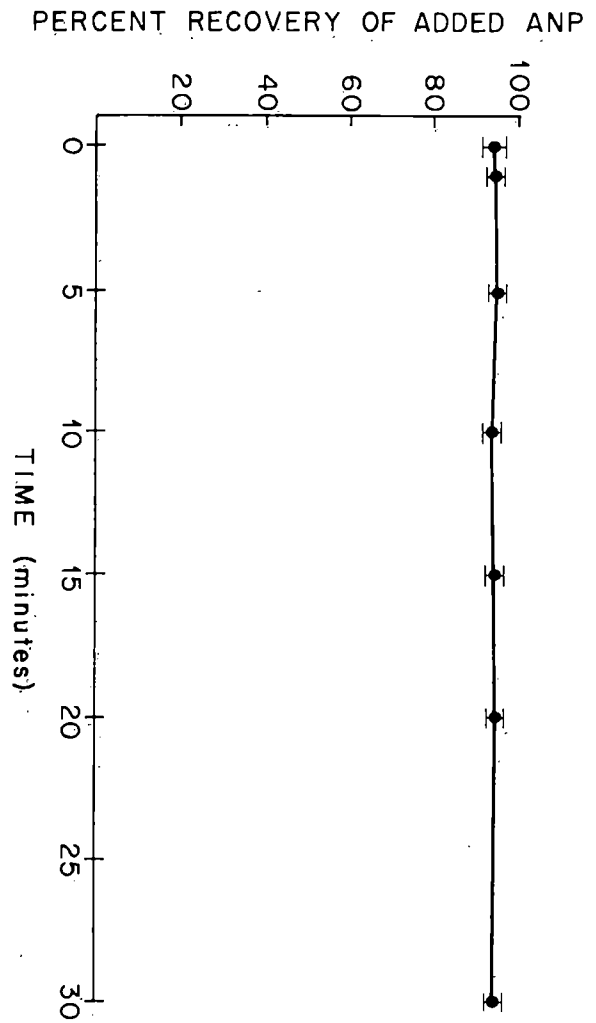


Fig. 1. Amount of ANP (expressed as percent of recovery of added ANP) in umbilical plasma incubated at 37° C for up to 30 minutes.

on umbilical arterial ANP levels at the time of delivery. Because labor and route of delivery could have confounding effects on the above variables, their influence on umbilical arterial ANP levels was also examined.

Material and methods

ANP metabolism by the placenta and umbilical vessels. To evaluate placental ANP metabolism, 165 paired umbilical arterial and umbilical venous blood samples were collected from double-clamped umbilical cords immediately at the time of delivery. To evaluate the possibility of ANP metabolism in the umbilical vessels, paired umbilical arterial (n = 65) or umbilical venous (n = 61) blood samples were collected. The first sample was obtained from a double-clamped umbilical cord immediately at delivery and the second was obtained approximately 10 minutes after delivery (range 9 to 12 minutes).

ANP degradation by a circulating peptidase. Umbilical arterial and venous blood was collected at the time of delivery in tubes containing ethylenediaminetetraacetic acid (1.0 mg/ml), pooled, and centrifuged at 2000 g for 10 minutes at 4° C. ANP (260 pg/ml) was added to the plasma and the plasma was incubated at 37° C. Aliquots of plasma were then removed, extracted, and assayed for ANP after 0, 1, 5, 10, 15, 20, and 30 minutes of incubation. Each incubation study used the pooled umbilical plasma samples from four patients, and 16 patients were studied in a total of four separate incubations.

Table I. ANP levels at delivery and 10 minutes after delivery

Samples	*ANP (pg/ml) (mean ± SEM)*	*p Value*
At delivery (n = 165)		<0.001
Umbilical artery	98 ± 15	
Umbilical vein	56 ± 9	
Umbilical artery (n = 65)		<0.001
At delivery	80 ± 14	
10 min after delivery	45 ± 9	
Umbilical vein (n = 62)		<0.001
At delivery	45 ± 8	
10 min after delivery	31 ± 4	

Selection of patient groups. Umbilical arterial cord blood was collected for ANP from a double-clamped cord within 1 minute of delivery in five groups of patients.

Group 1: Normal controls. Nineteen patients with gestational ages from 37 to 40 weeks had spontaneous vaginal deliveries. None of the pregnancies had any antenatal, intrapartum, postpartum, or neonatal complications. Birth weights ranged from 2880 to 3729 gm and all 1- and 5-minute Apgar scores were ≥8. The only medications administered during labor or delivery were epidural or local anesthetics.

Group 2: Elective cesarean section. Ten patients between 37 and 40 weeks' gestation were delivered by elective cesarean section without labor. Eight were repeat cesarean sections and two were done because of breech presentations. As in the normal control group, this group had no complications. Birth weights ranged from 3345 to 4000 gm. All 1- and 5-minute Apgar scores were ≥8. All patients had epidural anesthesia for delivery.

Group 3: Prolonged pregnancies. Twenty-one patients were delivered between 41 and 43 weeks' gestation. Medications administered in labor included fentanyl and Pitocin, as well as epidural anesthesia. Patients in this group had either vaginal or cesarean section deliveries. All had normal fetal heart rate tracings during labor with the exception of three patients with fetal heart rate tracings showing either severe variable or repetitive late decelerations. Each of these patients had umbilical arterial pH values at delivery that were >7.25.

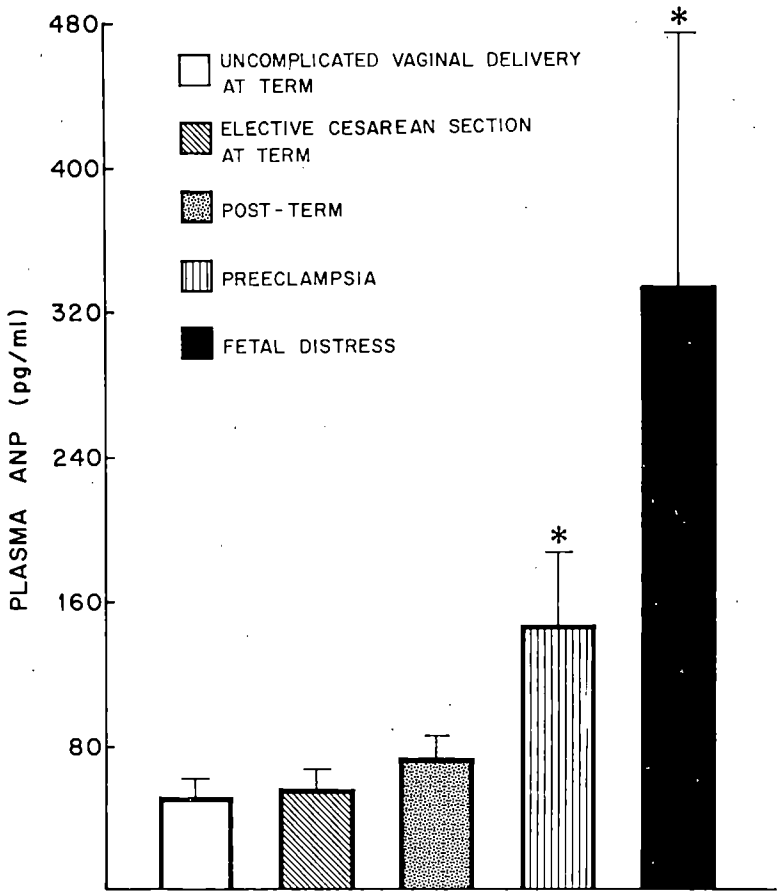


Fig. 2. Umbilical arterial ANP levels in different groups studied. *Asterisk,* $p < 0.01$ versus uncomplicated vaginal delivery at term.

All 1- and 5-minute Apgar scores were ≥7. Birth weights ranged from 2800 to 4200 gm and there were no neonatal complications.

Group 4: Preeclampsia. Eight patients between 27 and 42 weeks' gestation were delivered with a diagnosis of preeclampsia according to the criteria of the American College of Obstetricians and Gynecologists.[15] Three had mild preeclampsia and five had severe preeclampsia. Blood pressures before delivery ranged from 140/85 to 160/110 mm Hg and qualitative urine protein levels obtained from catheterized urine specimens ranged from 2+ to 4+. None of the patients had a history of chronic hypertension or renal disease. All patients received magnesium sulfate and one received hydralazine before delivery. Five had cesarean sections and three were delivered vaginally. Six of these patients had regional anesthesia for delivery and one received general anesthesia. All but one of the patients had normal fetal heart rate tracings. This fetal heart rate tracing showed a tachycardia with decreased variability. The umbilical arterial pH at delivery was 7.32. The neonate was born to a mother with severe preeclampsia at 27 weeks' gestation and had 1- and 5-minute Apgar scores of 3 and 8. This neonate subsequently died of severe respiratory distress and prematurity. The remaining seven neonates had 1- and 5-minute Apgar scores that were ≥8. Neonatal complications included two neonatal deaths—one caused by respiratory distress as noted and one caused by congenital cytomegalovirus pneumonia.

Group 5: Fetal distress. Ten patients delivered between 31 and 42 weeks' gestation had abnormal fetal heart rate tracings during labor (either severe variable decelerations, repetitive late decelerations, prolonged bradycardias, and/or tachycardias with decreased variability). Each of these was associated with an umbilical arterial pH at delivery ≤7.20 (range 6.85 to 7.20), with the exception of one fetus for whom an umbilical blood gas value was not obtained but whose scalp blood pH just before delivery was 7.20. Six of these patients were delivered by cesarean section and four had vaginal deliveries. One-minute Apgar scores ranged from 3 to 8 and all 5-minute Apgar scores were ≥8. Neonatal complications included one neonatal death attributed to asphyxia and probable sepsis.

Blood collection, plasma extraction, and ANP radioimmunoassay. All blood, with the exception of that used for the ANP degradation studies, was collected in

chilled test tubes containing ethylenediaminetetraacetic acid (1.0 mg/ml) and aprotinin (500 kallikrein inhibitor units per milliliter). The blood was centrifuged at 2000 *g* for 10 minutes at 4° C. The procedures we used for the plasma extractions and ANP radioimmunoassay have been previously described in detail.[16] In brief, the plasma was extracted under acidic conditions on C-18 Sep Pak columns (Waters Associates, Milford, Mass.). ANP was measured with a double-antibody radioimmunoassay with antihuman ANP (99-126) obtained from Peninsula Laboratories (Belmont, Calif.). Human ANP (99-126) was also used as the standard and label (Peninsula Laboratories). The interassay and intraassay variabilities were 8% and 4.5%, respectively.

Statistical analysis. All data are presented as the mean and standard error of the man. Paired or unpaired *t* tests (with Bonferroni correction for multiple comparisons) or one-way analysis of variance were used for statistical analysis. A p value <0.05 was considered significant except where the Bonferroni correction was used, in which case a p value <0.01 was significant.

This study had the approval of the institution's Human Subjects Committee and it was determined that consent forms were not necessary for the collection of umbilical cord blood.

Results

Table I shows the plasma ANP levels obtained in paired umbilical arterial and venous samples collected at the time of delivery. The levels in the umbilical artery were significantly higher than those in the umbilical vein ($p < 0.001$). Table I also shows the ANP levels in paired umbilical arterial samples. The ANP levels present in the umbilical artery at the time of delivery were almost 100% greater than those in the artery 10 minutes later ($p < 0.001$). The bottom of Table I shows the ANP levels in paired umbilical venous specimens. Again the levels in the umbilical vein at the time of delivery were greater than those 10 minutes later ($p < 0.001$).

Fig. 1 shows the ANP incubation studies done to determine if a peptidase that can degrade ANP is present in the umbilical circulation. The recovery of added ANP was 94% at 4° C and did not change when the plasma was incubated at 37° C for up to 30 minutes.

Fig. 2 shows the plasma ANP levels obtained in the umbilical artery at the time of delivery in the different patient groups studied. The average level in the normal population that underwent vaginal delivery at term was 51 ± 11 pg/ml. This level was not significantly different from the ANP levels obtained in the group delivered by cesarean section without labor or the group with prolonged pregnancies uncomplicated by fetal distress. The groups with preeclampsia and fetal distress both had significantly higher ANP levels (147 ± 41 and 334 ± 140 pg/ml, respectively) than the normal population at term ($p < 0.01$).

Comment

In this study we have attempted to identify some of the factors that may be involved in human fetal ANP metabolism and secretion. Our finding that umbilical arterial ANP levels are significantly greater than venous levels is in agreement with an earlier report by Yamaji et al.[13] and suggests that the placenta may be involved in the degradation or clearance of ANP. This is supported by a recent study that identified ANP receptors on the fetal side of the placenta.[12] Other investigators have failed to find any umbilical arterial-venous difference in ANP levels.[14] This could be related to the time interval from delivery to sampling of the umbilical vessels. In our study the average arterial-venous difference in ANP levels was 42 pg/ml when the samples were obtained at the time of delivery but was only 16 pg/ml when the samples were collected 10 minutes later.

The difference in ANP levels present in paired umbilical arterial (and paired umbilical venous) samples collected 10 minutes apart suggests that the umbilical vessels themselves are possible sites of ANP uptake or metabolism. This does not appear to be due to the presence of a circulating peptidase in the umbilical placental circulation because we did not observe any degradation of exogenous ANP in umbilical plasma after 30 minutes of incubation at 37° C. The umbilical vascular endothelium may contain receptors responsible for the uptake and clearance of ANP from the circulation. In adults, ANP receptors have been localized to the endothelium and smooth muscle of large and small vessels.[6]

Our results suggest that labor and route of delivery (vaginal vs. abdominal) have no significant effect on umbilical arterial ANP levels. Prolonged pregnancy (in the absence of fetal acidosis or maternal complications) also had no effect on ANP levels. However, both preeclampsia and fetal distress were associated with significantly higher ANP levels in the umbilical artery than were found in the normal control group. Although gestational age differences may account for the elevated ANP levels, we doubt this because ANP levels obtained at the time of cordocentesis have been shown to be unrelated to gestational age.[17] In addition, the present study shows that the gestational age difference between term and prolonged pregnancies is not associated with any changes in ANP levels.

There are several potential explanations for the elevated umbilical arterial ANP levels found in the group with preeclampsia. Doppler studies of the human umbilical placental circulation have shown an increase in umbilical vascular resistance in pregnancies complicated by preeclampsia.[18] The increased vascular resistance could stimulate ANP release from the heart through changes in myocardial work, a proposed determinant of ANP secretion.[4] The fetus may also be

relatively volume-depleted and the functioning placental mass may be decreased. Thus a decrease in the volume of distribution and/or a decrease in the clearance of ANP from the circulation may be responsible for the elevated ANP levels. This study cannot distinguish between these possibilities.

Umbilical arterial ANP levels were also elevated in the group with fetal distress. Because of our relatively small numbers we did not attempt to analyze our results with respect to the cause of the fetal distress. However, all subjects had in common an umbilical arterial pH (or scalp sample) of ≤7.20. Fetal acidosis has been reported to be associated with elevations in fetal plasma norepinephrine, epinephrine, and arginine vasopressin levels.[19, 20] Perfusion of the isolated rat heart with epinephrine has been shown to stimulate ANP secretion, an effect that appears to be mediated by both α- and β-adrenergic receptors.[2] Arginine vasopressin has been shown to stimulate ANP release in vivo presumably as a result of secondary hemodynamic changes.[3] Hypoxia can also stimulate ANP secretion by as yet poorly understood factors.[10] In the fetus hypoxia and acidosis may be accompanied by hypertension, which could stimulate ANP release through alterations in myocardial work. As noted for the group with preeclampsia, alterations in clearance or volume of distribution may also be partially responsible for the high ANP levels.

The physiologic significance of the high ANP levels present in the umbilical circulation of patients with preeclampsia or fetal distress requires further investigation. However, we postulate that ANP may be one factor by which the fetus attempts to regulate blood flow.

REFERENCES

1. Laragh JH, Atlas SA. Atrial natriuretic hormone: a regulator of blood pressure and volume homeostasis. Kidney Int 1988;34(suppl 25):S64-71.
2. Toth M, Ruskoaho H, Lang RE. Regulation of atrial natriuretic peptide secretion. J Hypertens 1986;4(suppl 6):S538-41.
3. Manning PT, Schwartz D, Katsube NC, Holmberg SW, Needleman P. Vasopressin-stimulated release of atriopeptin: endocrine antagonists in fluid homeostasis. Science 1985;229:395-7.
4. Rankin AJ. Mechanisms for the release of atrial natriuretic peptide. Can J Physiol Pharmacol 1987;65:1673-9.
5. Yandle TG, Richards AM, Nicholls MG, Cuneo R, Espiner EA, Livesey JH. Metabolic clearance rate and plasma half life of alpha-human atrial natriuretic peptide in man. Life Sci 1986;38:1827-33.
6. Jacobs JW, Vlasuk GP, Rosenblatt M. Atrial natriuretic factor receptors. Endocrinol Metab Clin North Am 1987; 16:63-77.
7. Itoh H, Nakao K, Shiono S, et al. Conversion of beta-human atrial natriuretic polypeptide into alpha-human atrial natriuretic polypeptide in human plasma in vitro. Biochem Biophys Res Commun 1987;143:560-9.
8. Brace RA, Cheung CY. Cardiovascular and fluid responses to atrial natriuretic factor in the sheep fetus. Am J Physiol 1987;253(Regulatory Integrative Comp Physiol 22):R561-7.
9. Ross MG, Ervin MG, Lam RW, Castro L, Leake RD, Fisher DA. Plasma atrial natriuretic peptide response to volume expansion in the ovine fetus. Am J Obstet Gynecol 1987;157:1292-7.
10. Cheung CY, Brace RA. Fetal hypoxia elevates plasma atrial natriuretic factor concentration. Am J Obstet Gynecol 1988;159:1263-8.
11. Ervin MG, Ross MG, Castro R, et al. Ovine fetal and adult atrial natriuretic factor metabolism. Am J Physiol 1988; 254(Regulatory Integrative Comp Physiol 23):R40-6.
12. Hatjis CG, Grogan DM. Atrial natriuretic peptide receptors in normal human placentas. Am J Obstet Gynecol 1988;159:587-91.
13. Yamaji T, Hirai N, Ishibashi M, Takaku F, Yanaihara T, Nakayama T. Atrial natriuretic peptide in umbilical cord blood: evidence for a circulating hormone in the human fetus. J Clin Endocrinol Metab 1986;63:1414-7.
14. Weil J, Bidlingmaier F, Dohlemann C, Kuhnle U, Strom T, Lang RE. Comparison of plasma atrial natriuretic peptide levels in healthy children from birth to adolescence and in children with cardiac diseases. Pediatr Res 1986; 20:1328-31.
15. Hughes EC, ed. Obstetric-gynecologic terminology. Philadelphia: FA Davis, 1972.
16. Castro LC, Arora C, Parvez S, Parvez H, Valenzuela G, Hobel CJ. Plasma atrial natriuretic peptide levels during the rat estrous cycle, pregnancy, and puerperium. Am J Obstet Gynecol 1989;160:15-9.
17. Weiner CP, Robillard JE. Atrial natriuretic factor, digoxin-like immunoreactive substance, norepinephrine, epinephrine, and plasma renin activity in human fetuses and their alteration by fetal disease. Am J Obstet Gynecol 1988; 159:1353-60.
18. Trudinger BJ, Giles WB, Cook CM. Flow velocity waveforms in the maternal uteroplacental and fetal umbilical placental circulation. Am J Obstet Gynecol 1985;152: 155-63.
19. Padbury JF, Roberman B, Oddie TH, Hobel CJ, Fisher DA. Fetal catecholamine release in response to labor and delivery. Obstet Gynecol 1982;60:607-11.
20. Stark RI, Daniel SS, Husain MK, Sanocka UM, Zubrow AB, James LS. Vasopressin concentration in amniotic fluid as an index of fetal hypoxia: mechanism of release in sheep. Pediatr Res 1984;18:552-8.

Impact of antenatal dexamethasone administration on respiratory distress syndrome in surfactant-treated infants

Elaine E. Farrell, MD, Richard K. Silver, MD, Lois V. Kimberlin, RN, Eileen S. Wolf, RN, and Joanne M. Dusik, RN

Evanston, Illinois

Neonatal lung disease is primarily responsible for the perinatal morbidity and mortality associated with preterm birth. Recently exogenous surfactant replacement therapy has been used to prevent or treat respiratory distress syndrome. As part of a multicenter, preventive trial between February 1986 and December 1988 using calf-lung surfactant extract, we treated 147 infants with single dose calf-lung surfactant extract. We analyzed this experience to evaluate the possibility that antenatal steroids may be additive (with calf-lung surfactant extract) in reducing both the incidence and severity of respiratory distress syndrome. Although a reduction in the incidence of respiratory distress syndrome was observed among the 33 neonates exposed to antenatal dexamethasone when compared with the 114 infants given calf-lung surfactant extract alone, the difference was not significant (37.7% vs. 24.2% $p = 0.15$). However, comparison of neonatal subsets previously shown to benefit most from steroid use revealed an additive effect between calf-lung surfactant extract and dexamethasone in reducing both the incidence and severity of respiratory distress syndrome. Of the 99 singleton pregnancies, only 2 of 16 infants treated with calf-lung surfactant extract and dexamethasone developed respiratory distress syndrome, compared with the 33 of 83 calf-lung surfactant extract cases ($p < 0.05$). A similar reduction was observed in infants between 28 and 32 weeks' gestation (calf-lung surfactant extract, 21 of 79 vs. calf-lung surfactant extract and dexamethasone, 0 of 24; $p < 0.05$). A reduction in disease severity was observed in male offspring (moderate or severe respiratory distress syndrome only; 22 of 63 vs. 2 of 22; $p < 0.05$). Potential confounding variables (e.g., gestational age at birth, birth weight, exposure to tocolytics before delivery, fetal pH at birth) were similar in all comparisons. We conclude that an additive effect between dexamethasone and calf-lung surfactant extract is observed in selected cases. It may therefore be appropriate to maximize antenatal steroid use in centers where exogenous surfactant is available. (AM J OBSTET GYNECOL 1989;161:628-33.)

Key words: Dexamethasone, respiratory distress, surfactant

Neonatal lung disease is primarily responsible for the perinatal morbidity and mortality associated with preterm birth. Until recently, specific therapy aimed at reducing the incidence of respiratory distress syndrome (RDS) has been limited to antenatal management strategies. These have included preterm labor prevention through risk assessment, early diagnosis, and aggressive use of tocolytic therapy and selected administration of antenatal steroids. The efficacy of steroid therapy in reducing the incidence of RDS has been confirmed in many studies,[1-3] including the national collaborative trial.[4] However, maternal steroid therapy is generally underutilized because of many factors, including the unanticipated nature of preterm delivery, the inability to delay delivery until steroid therapy can be completed, and the limited obstetric endorsement of this approach. In contrast, postnatal therapy with exogenous surfactant has shown great promise as both a prophylactic measure (to prevent RDS in neonates at risk) and a specific therapy (given after the diagnosis of RDS is made). Randomized preventative trials have demonstrated a significant reduction in the incidence of RDS with both bovine and human preparations of surfactant.[5-7] Improved outcome has also been reported in prospective trials designed to treat neonates with established RDS.[8, 9]

At first glance the encouraging results with surfactant replacement therapy, combined with the ability to prospectively identify and treat all neonates at risk, would favor surfactant treatment over antenatal steroid administration. However, in one report neonates exposed to both maternal steroid therapy and postnatal surfactant replacement had the lowest incidence of RDS when compared with patients receiving either treatment alone.[10] A synergistic response has also been identified in preterm animal models.[11, 12] As part of a multicenter,

From the Divisions of Neonatology and Maternal-Fetal Medicine at Evanston Hospital, and the Departments of Pediatrics and Obstetrics and Gynecology, Northwestern University Medical School.
Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.
Reprint requests: Richard K. Silver, MD, Evanston Hospital, 2650 Ridge Ave., Evanston, IL 60201.
6/6/14089

preventive trial with calf-lung surfactant extract,[13] we have treated infants born between 24 and 32 weeks' gestation with calf-lung surfactant extract. In this report we analyze our own experience to evaluate the possibility that antenatal steroids may be additive (with calf-lung surfactant extract) in reducing both the incidence and severity of RDS.

Material and methods

Study population. Between February 1986 and December 1988 calf-lung surfactant extract was administered to 147 neonates delivered from 121 patients, between 24 and 32 weeks of gestation. Obstetric complications included advanced preterm labor, single-agent tocolytic failure, bleeding placenta previa, and maternal conditions that warranted preterm delivery. Multiple births complicated 22 pregnancies (twins = 19, triplets = 1, quadruplets = 2) and accounted for 48 neonates (1 twin-acardiac pair). A total of 45 neonates were exposed to antenatal steroid therapy, but only 33 completed therapy before delivery. These infants were compared with the remaining 114 neonates treated with calf-lung surfactant extract alone.

Steroid administration. Steroid therapy was administered at the discretion of the managing obstetrician. The decision to treat was independent of consent for the calf-lung surfactant extract trial. Dexamethasone (5 mg) was given intramuscularly every 12 hours for a total of four doses. Repeat administration was accomplished every 7 days until either delivery or 32 weeks' gestation. A mature phospholipid profile (lecithin/sphingomyelin ratio $\geq 3:1$) precluded both steroid therapy and calf-lung surfactant extract administration.

Calf-lung surfactant extract. Calf-lung surfactant extract is derived from saline lavage of fresh calf lungs; its physical and chemical properties are similar to endogenous surfactant.[7] The infants in this study were enrolled as part of a phase III, uncontrolled, open trial sponsored by the Department of Pediatrics at Buffalo Children's Hospital. Administration of calf-lung surfactant extract followed an established protocol.[7] Immediately after delivery and before assisted ventilation, the neonatology staff intubated each infant and introduced calf-lung surfactant extract through a specially adapted endotracheal tube. The dose of calf-lung surfactant extract was chosen based on gestational age and estimated fetal weight by ultrasonography. For gestational ages <29 weeks or weights <1200 gm, 3 ml (105 mg) of calf-lung surfactant extract was administered. A dose of 210 mg was used in the remaining infants. After instillation, ventilation was established and the infant was stabilized and transferred to the special care nursery. Further treatments were provided in a routine fashion with the exception that endotracheal suctioning was withheld for the first 2 hours after calf-lung surfactant extract instillation.

Table I. Comparison of selected perinatal variables between calf-lung surfactant extract and calf-lung surfactant plus dexamethasone groups

	Calf-lung surfactant extract (N = 114)		*Calf-lung surfactant extract plus dexamethasone (N = 33)*	
Variables	*No.*	*%*	*No.*	*%*
Maternal transport	47	41.2	10	30.3
Membrane rupture before delivery	59	51.7	14	42.4
Multiple gestations	29	25.4	17	51.5*
Tocolytic therapy	113	99.1	33	100.0
Cesarean section	55	48.2	23	69.7†
Mean cord arterial pH	7.34 ± 0.10		7.33 ± 0.15	
Cord arterial pH <7.20	7	6.1	3	9.1

*$p < 0.01$.
†$p < 0.05$.

Definition of RDS. The diagnosis of RDS was made in those neonates who demonstrated (1) an oxygen requirement beyond 24 hours of life, (2) serial chest roentgenographic findings (reticulogranular pattern), and (3) a clinical course and ventilatory parameters compatible with the diagnosis. Respiratory distress syndrome was defined as mild if the FiO_2 remained <0.40 and the mean airway pressure was <8 mm H_2O. FiO_2 and mean airway pressure values of 0.40 to 0.69 and 8 to 11, respectively, defined moderate RDS, whereas neonates with FiO_2 >0.70 and mean airway pressure >12 were considered to have severe disease.

Data analysis. Maternal records were reviewed and both demographic and obstetric variables were coded. Neonatal data were obtained including categoric variables (e.g., the incidence and severity of RDS, intraventricular hemorrhage, pneumothorax and pulmonary interstitial emphysema, necrotizing enterocolitis and bronchopulmonary dysplasia), and continuous measures (e.g., specific ventilator parameters). Comparison between the two groups was accomplished with χ^2 analysis for discrete data and unpaired, two-tailed t tests for continuous variables. Significance was considered at $p < 0.05$.

Results

Comparison of maternal demographics and indications for delivery were similar in both the calf-lung surfactant extract only and calf-lung surfactant extract plus dexamethasone groups. Mean gestational age was the same in each group (29.3 weeks), and mean birth weights were also similar (calf-lung surfactant extract, 1340 gm vs. calf-lung surfactant extract plus dexa-

Table II. Comparison of neonatal outcome between calf-lung surfactant extract and calf-lung surfactant extract plus dexamethasone groups

Variables	*Calf-lung surfactant extract (N = 114)*		*Calf-lung surfactant extract plus dexamethasone (N = 33)*	
	No.	*%*	*No.*	*%*
Respiratory distress syndrome	43	37.7	8	24.2
Intraventricular hemorrhage	21	18.4	3	9.1
Intraventricular hemorrhage grade 3 or 4	10	8.8	1	3.0
Bronchopulmonary dysplasia	19	16.7	6	18.2
Patent ductus arteriosus	15	13.2	3	9.1
Pneumothoraces/PIE	14	12.3	5	15.2
Mortality	24	21.1	7	21.2
Mortality-respiratory cause	13	11.4	4	12.1

PIE, Pulmonary interstitial emphysema.

methasone, 1231 gm). Table I outlines other perinatal variables in this comparison. A greater proportion of multiple births was observed in the calf-lung surfactant extract plus dexamethasone group (17/33 vs. 29 of 114; $p < 0.01$), which accounted for part of the difference seen in the rate of cesarean delivery. Exposure to tocolytic therapy was equivalent in both groups, and no differences were identified in fetal acid-base status at birth (as measured by arterial cord pH or the proportion of acidemic values with pH <7.20).

Comparison of neonatal outcome is shown in Table II. Although a trend favoring neonates treated with calf-lung surfactant extract plus dexamethasone with regard to RDS and intraventricular hemorrhage was noted, the outcomes were similar. Because of the disparity in multiple births, we performed the same comparisons, restricting analysis to singleton pregnancies only. Table III summarizes these observations. A significant reduction in the incidence of RDS was observed; no infant had moderate or severe disease in the calf-lung surfactant extract plus dexamethasone group. Although no statistical differences in mortality were encountered, 11 of the 12 respiratory deaths occurred in the calf-lung surfactant extract group. In addition, 16 of 17 intraventricular hemorrhages were found in those neonates who received calf-lung surfactant extract only. The fetal acid-base status, male/female ratio, gestational age at delivery, and birth weights were all similar in this comparison.

Because the benefit of antenatal steroid therapy is less certain before 28 weeks' gestation, we also analyzed the data after excluding those infants delivered between 24 and 27 weeks of pregnancy (Table IV). A reduction in the incidence and severity of RDS was again observed in this calf-lung surfactant extract plus

Table III. Comparison of neonatal outcome between calf-lung surfactant extract and calf-lung surfactant extract plus dexamethasone groups: Singleton pregnancies (N = 99)

Variables	*Calf-lung surfactant extract (n = 83)*		*Calf-lung surfactant extract plus dexamethasone (n = 16)*	
	No.	*%*	*No.*	*%*
Respiratory distress syndrome	33	39.8	2	12.5*
Respiratory distress syndrome—moderate or severe	21	25.3	0	—*
Intraventricular hemorrhage	16	19.3	1	6.3
Intraventricular hemorrhage grade 3 or 4	8	9.6	0	—
Bronchopulmonary dysplasia	14	16.9	3	18.8
Pneumothoraces/PIE	13	15.7	4	25.0
Mortality	19	22.9	3	18.8
Mortality—respiratory cause	11	13.3	1	6.3
Gestational age at delivery (weeks, mean ± SD)	29.1 ± 3.1		29.9 ± 1.8	
Birth weight (gm, mean ± SD)	1289 ± 496		1313 ± 507	

PIE, Pulmonary interstitial emphysema.
*$p < 0.05$.

dexamethasone cohort. This reduction occurred despite a trend toward lower birth weight and lesser gestational age in the calf-lung surfactant extract plus dexamethasone group ($p < 0.1$ for both comparisons). The diagnosis of small for gestational age (<10th percentile birth weight) did not confound this comparison (calf-lung surfactant extract, $n = 4$; calf-lung surfactant extract plus dexamethasone, $n = 3$), and no differences in either the percentage of multiple births or in the male/female ratio were seen.

Since the benefit of antenatal steroid therapy is presumedly greatest in female and nonwhite infants,[4] analyses were also accomplished with gender and race as independent variables. As shown in Table V, female neonates experienced more favorable outcomes with regard to both respiratory disease and survival. These differences could not be attributed to more frequent steroid treatment in the female group. In fact, 22 (26%) of the males were exposed to dexamethasone compared with only 11 (18%) females. To determine if an inadequate response to antenatal steroids (by the male fetus) was responsible for the better female outcome, we analyzed the males separately (comparing steroid treated and untreated subsets, Table VI). A significant reduc-

Table IV. Comparison of neonatal outcome between calf-lung surfactant extract and calf-lung surfactant extract plus dexamethasone groups: Gestational age 28 to 32 weeks ($N = 103$)

Variables	*Calf-lung surfactant extract (n = 79)*		*Calf-lung surfactant extract plus dexamethasone (n = 24)*	
	No.	*%*	*No.*	*%*
Respiratory distress syndrome	21	27.8	1	4.2*
Respiratory distress syndrome—moderate or severe	13	16.5	0	—*
Intraventricular hemorrhage	10	12.6	1	4.2
Intraventricular hemorrhage grade 3 or 4	3	3.8	0	—
Bronchopulmonary dysplasia	5	6.3	1	4.2
Pneumothoraces/PIE	7	8.8	3	12.5
Mortality	10	12.7	2	8.3
Mortality—respiratory cause	3	3.8	1	4.2
Gestational age at delivery (weeks, mean ± SD)	31.0 ± 1.7		30.4 ± 1.4	
Birth weight (gm, mean ± SD)	1568 ± 410		1430 ± 390	

PIE, Pulmonary interstitial emphysema.
*$p < 0.05$.

Table V. Comparison of neonatal outcome: male versus female infants

Variables	*Male (N = 85)*		*Female (N = 62)*	
	No.	*%*	*No.*	*%*
Respiratory distress syndrome	37	43.5	14	22.6*
Respiratory distress syndrome—moderate or severe	25	29.4	6	9.7*
Intraventricular hemorrhage	13	15.3	15	24.2
Mortality	23	27.1	7	11.3†
Mortality—respiratory cause	15	17.6	2	3.2*
Gestational age at delivery (weeks, mean ± SD)	29.3 ± 2.9		29.4 ± 2.9	
Birth weight (gm, mean ± SD)	1315 ± 524		1316 ± 452	
Calf-lung surfactant extract plus dexamethasone infants	22	25.8	11	17.7

*$p < 0.01$.
†$p < 0.05$.

tion in moderate or severe RDS was observed in the calf-lung surfactant extract plus dexamethasone male cohort despite a disproportionate number of multiple births in that same group (55% vs. 14%; $p < 0.001$). An overall reduction in the incidence of RDS was also observed in the nonwhite neonates when compared with white infants (21% vs. 41%; $p < 0.05$) despite similar exposure to antenatal dexamethasone.

Comment

In this study we observed a consistent reduction in both the incidence and severity of RDS in selected neonates receiving calf-lung surfactant extract after exposure to antenatal dexamethasone. The fact that steroid administration was at the discretion of the managing physician may have introduced selection bias. However, potential confounding variables, as compared between the steroid-treated and untreated groups, were similar. The absence of a steroid-only cohort was not intentional. Since the introduction of the calf-lung surfactant extract trial at our institution, nearly uniform consent has been obtained so that all eligible infants have received the surfactant preparation. Therefore any neonate exposed to steroids would eventually receive calf-lung surfactant extract if delivered before 33 weeks' gestation. The study by Kwong et al.[10] had a steroid-only group for comparison because in that study steroid exposure constituted an exclusion criteria to calf-lung surfactant extract administration. They observed a synergism between betamethasone and calf-lung surfactant extract, but only a small number of infants were studied and the gestational age range (24 to 27 weeks' gestation) was limited. In contrast, the additive effect seen in our study was found among singleton pregnancies and occurred during the gestational age interval traditionally associated with successful induction of phospholipid synthesis.[4]

Our own analysis of very premature neonates (24 to 27 weeks) was hindered by the size of this particular cohort and its composition. Of the 45 infants <28 weeks' gestation at birth, only nine were exposed to antenatal steroids; seven of these were products of multiple gestations. A larger experience with more singleton pregnancies, or a randomized prospective trial of dexamethasone in very premature, calf-lung surfactant extract–treated neonates, might confirm the apparent synergism reported in the study by Kwong et al.[10]

The impact of gender on outcome was generally consistent with prior observations concerning steroid use. In animal models the "male disadvantage" has been attributed to delayed maturation of type II pneumocytes secondary to circulating testosterone.[11] In the collaborative trial,[4] steroid-exposed females experienced a significant reduction in RDS when compared with placebo controls (4.8% vs. 18.8%), whereas male neonates showed no apparent response (14.9% vs. 14.1%).

Table VI. Comparison of neonatal outcome among male infants: Calf-lung surfactant extract versus calf-lung surfactant extract plus dexamethasone ($N = 85$)

Variables	*Calf-lung surfactant extract (N = 63)*		*Calf-lung surfactant extract plus dexamethasone (N = 22)*	
	No.	*%*	*No.*	*%*
Respiratory distress syndrome	31	49.2	6	27.3*
Respiratory distress syndrome—moderate or severe	22	34.9	2	9.1†
Mortality	19	30.2	5	22.7
Mortality—respiratory cause	12	19.0	2	9.1
Gestational age at delivery (weeks, mean ± SD)	29.2 ± 3.1		29.5 ± 2.0	
Birth weight (gm, mean ± SD)	1351 ± 530		1213 ± 505	

*$p = 0.072$.
†$p < 0.05$.

Female infants also faired better in our study, but this may have been independent of steroid use, since no difference in the incidence of RDS was noted between the females treated with calf-lung surfactant extract plus dexamethasone or calf-lung surfactant extract (18% vs. 23%, respectively). In contrast the male infants demonstrated an additive response, with less respiratory distress syndrome in the calf-lung surfactant extract plus dexamethasone subset (49.2% vs. 27.3%; $p < 0.05$).

It is notable that the mean gestational age in our comparison (29 weeks) is significantly younger than that reported in collaborative trial (31 weeks), and the corresponding prevalence of RDS in our male infants (44%) differs from that of the collaborative trial (14%). Thus an impact of steroid treatment on the incidence of RDS may have been more readily identified in our study population.

It is tempting to speculate that the additive effect observed between steroids and calf-lung surfactant extract is directly related to increased phospholipid synthesis after enzymatic induction by dexamethasone. The addition of topical surfactant in the presence of enhanced, endogenous phospholipid production would increase the total surfactant content available to the neonatal lung. Our observation of clinical improvement associated with calf-lung surfactant extract plus dexamethasone in the same subsets of neonates traditionally thought to benefit from enhanced endogenous production supports this theory. In conflict with this interpretation are the reports of Ikegami et al.[11, 15] They observed an increase in preterm lung compliance in steroid- and surfactant-treated rabbit lungs but were unable to demonstrate a quantitative difference in alveolar surfactant pool sizes between steroid-exposed and unexposed groups. They suggested that antenatal steroids may impact on respiratory function by inducing structural modifications (e.g., maturation of the alveolar-capillary barrier providing for decreased protein leakage into the alveolar space) that prevent or limit lung injury. A study by Fiascone et al.[12] also supports the concept of a "nonsurfactant" effect of corticosteroids resulting in enhanced pulmonary compliance.

In summary, an additive effect between dexamethasone and calf-lung surfactant extract has been observed. We identified a decrease in both incidence and severity of RDS in those neonates exposed to steroid therapy and single-dose, prophylactic exogenous surfactant. Specific groups of neonates experiencing benefit included singleton infants, male infants, and those infants born between 28 and 32 weeks of gestation. It may therefore be appropriate to maximize antenatal steroid use in centers where exogenous surfactant is available. A prospective, randomized evaluation comparing calf-lung surfactant extract with calf-lung surfactant extract plus dexamethasone should be undertaken to confirm these observations.

REFERENCES

1. Liggins GC, Howie RN. A controlled trial of antepartum glucocorticoid treatment for prevention of the respiratory distress syndrome in premature infants. Pediatrics 1972; 50:515.
2. Taeusch HW, Frigoletto F, Kitzmiller J, et al. Risk of respiratory distress syndrome after prenatal dexamethasone treatment. Pediatrics 1979;63:64.
3. Papageorgiou AN, Desgranges MF, Masson M, et al. The use of antenatal betamethasone in the prevention of respiratory distress syndrome: a controlled, double blind study. Pediatrics 1979;63:73.
4. Collaborative group on antenatal steroid therapy. Effect of antenatal dexamethasone administration on the prevention of RDS. Am J Obstet Gynecol 1981;141:276.
5. Enhorning GE, Shennan A, Possmayer F, et al. Prevention of neonatal respiratory distress syndrome by tracheal instillation of surfactant: a randomized clinical trial. Pediatrics 1985;76:145.
6. Merritt TA, Hallman M, Bloom BT, et al. Prophylactic treatment of very premature infants with human surfactant. N Engl J Med 1986;315:785.
7. Kwong MS, Egan EA, Notter RH, Shapiro DL. Double blind clinical trial of calf-lung surfactant extract for the prevention of hyaline membrane disease in extremely premature infants. Pediatrics 1985;76:585.
8. Hallman M, Merritt TA, Jarvenpaa AL, et al. Exogenous human surfactant for treatment of severe respiratory distress syndrome: a randomized prospective clinical trial. J Pediatr 1985;106:963.
9. Raju TN, Viayasagar D, Bhat R, et al. Double-blind, controlled trial of single-dose treatment with bovine surfac-

tant in severe hyaline membrane disease. Lancet 1987; 1:651.
10. Kwong MS, Egan EA, Notter RH. Synergistic response of antenatal betamethasone and tracheal instillation of calf-lung surfactant extract at birth. Pediatr Res 1985;21: 458A.
11. Ikegami M, Jobe AH, Pettenazzo A, et al. Effects of maternal treatment with corticosteroids, T_3 TRH, and their combinations on lung function of ventilated, preterm rabbits with and without surfactant treatment. Am Rev Respir Dis 1987;136:892.
12. Fiascone JM, Jacobs HC, Moya FR, Mercuro MR, Lima DM. Betamethasone increased pulmonary compliance in part by surfactant independent mechanisms in preterm rabbits. Pediatr Res 1987;22:730.
13. Egan EA, Kwong M. Clinical results of multicenter open trial of Infasurf. Washington, DC: Ross Laboratory Special Conference, 1988.
14. Nielsen HC, Zinman HM, Torday JS. Dihydrotestosterone inhibits fetal rabbit pulmonary surfactant production. J Clin Invest 1982;69:611.
15. Ikegami M, Berry DD, Elkady T, et al. Corticosteroids and surfactant change lung function and protein leaks in the lungs of ventilated premature rabbits. J Clin Invest 1987;79:1371.
16. Elkady T, Jobe A. The effects of steroid and/or surfactant on lung rupture pressure in preterm rabbits. Pediatr Res 1986;20:428A.

Cocaine abuse during pregnancy: Peripartum prevalence and perinatal outcome

Mark G. Neerhof, DO, Scott N. MacGregor, DO, Sandy S. Retzky, DO, and Terence P. Sullivan, DO

Chicago, Illinois

The frequency of cocaine abuse during pregnancy has increased markedly in recent years. Cocaine abuse during pregnancy has been associated with significant perinatal morbidity and mortality. In this study a large population of pregnant women were tested for drug use at the time of their admission to the hospital for labor and delivery. During the study period a total of 138 (8%) of 1776 patients who were delivered of infants had positive urine toxicologic screening tests for cocaine. Moreover, the prevalence of peripartum cocaine abuse increased considerably during the study period. The perinatal outcomes of the 138 patients whose urine samples yielded positive test results for cocaine were compared with those of the 88 control patients with negative results. Cocaine use was associated with lower gestational age at delivery and higher incidences of preterm delivery, premature rupture of the membranes, and small-for-gestational-age infants. When compared with control infants, infants of mothers who used cocaine had more instances of neonatal morbidity and longer hospitalizations. In this study peripartum cocaine abuse was common, increased during the study period, and was associated with perinatal morbidity. (Am J Obstet Gynecol 1989;161:633-8.)

Key words: Cocaine abuse, prevalence, perinatal morbidity

The frequency of cocaine abuse has increased markedly in the United States during the last decade.[1] Among the reasons contributing to the epidemic abuse of this substance are the following: widespread availability, the seductive euphoria associated with its use, and the possibility of using this agent without intravenous injection. The increased abuse of cocaine has also led to the recognition of the potentially catastrophic complications.[2, 3] Reports of cocaine abuse in pregnancy have focused attention on the effects of cocaine on the fetus and newborn.[4-13] However, few data examining the prevalence of cocaine abuse among pregnant women have been published.[14] In this report we evaluated the prevalence of cocaine abuse among a large population of pregnant patients immediately before delivery and the perinatal outcome in these patients.

From the Departments of Obstetrics and Gynecology, Chicago College of Osteopathic Medicine and Northwestern University Medical School.
Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.
Reprint requests: Scott N. MacGregor, DO, 2650 Ridge Ave., Evanston, IL 60201.
6/6/13710

Material and methods

Beginning September 1, 1986, the Department of Obstetrics and Gynecology at Chicago Osteopathic Medical Center instituted a policy whereby all patients

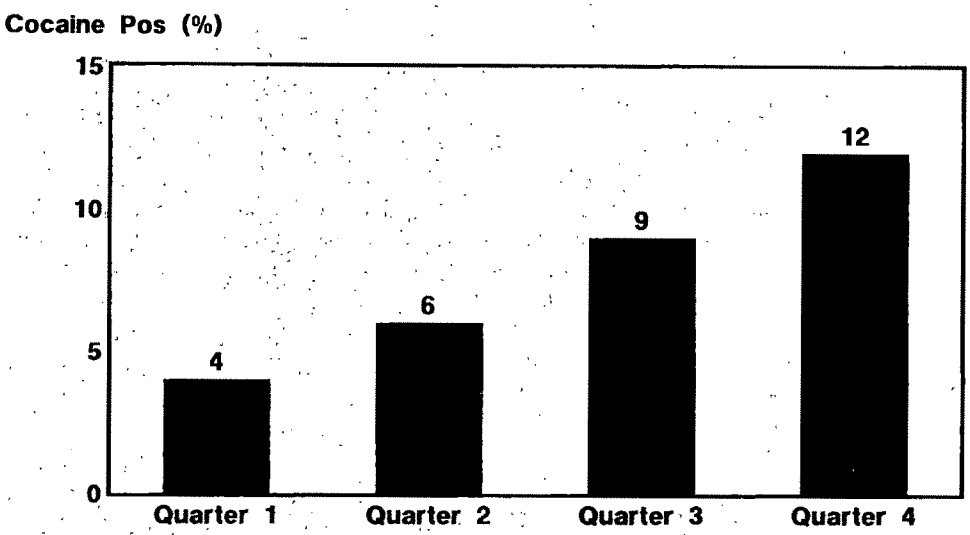


Fig. 1. Prevalence of cocaine-positive urine toxicologic screening at admission for labor and delivery by quarter. **Quarter 1*, Sept. 1986 to Feb. 1987 (14 positive tests of 397 tested); *quarter 2*, March to Aug. 1987 (24 of 408); *quarter 3*, Sept. 1987 to Feb. 1988 (40 of 457); *quarter 4*, March to Aug. 1988 (60 of 514).

Table I. Maternal characteristics

Characteristic	*Cocaine-positive group (mean ± SD) (n = 138)*	*Control group (mean ± SD) (n = 88)*	*Significance*
Maternal age (yr)	25.8 ± 4.7	23.9 ± 5.8	$p < 0.005$
Gravidity	3.8 ± 2.0	2.7 ± 1.6	$p < 0.0001$
Parity	2.2 ± 1.7	1.2 ± 1.2	$p < 0.0001$
Maternal hospitalization (days)	3.0 ± 1.9	2.7 ± 1.2	NS

admitted to the Labor and Delivery Unit underwent a urine toxicologic screening. This policy was instituted for the following reasons: (1) Cocaine abuse has been recognized with greater frequency among our obstetric population; (2) increased perinatal morbidity and mortality associated with cocaine abuse in pregnancy has been described[4-13]; (3) it was deemed important to identify both mothers in need of treatment for chemical dependency and infants in need of long-term neurodevelopmental follow-up. Urine toxicologic screening was performed with the Syva Emit urine toxicology assay kit (Syva Company, Palo Alto, Calif.). Substances screened included cocaine, opiates, methadone, amphetamines, barbiturates, benzodiazepines, and propoxyphene. Thorough drug histories and informed consents were obtained before urine toxicologic screening.

From September 1, 1986, to August 31, 1988, 1776 consecutive women delivered of their infants at our institution had urine toxicologic screening at the time of admission to the Labor and Delivery Unit. Those women with positive test results for cocaine composed the study population. A control group (n = 88) was selected from the general obstetric population for purposes of comparison. The controls were selected as follows: Every twentieth delivery during the study period was considered as a control candidate; those denying a history of cocaine abuse and having negative test results for cocaine were included in the control group; those with positive test results for cocaine were excluded, and the next woman delivered in succession who denied cocaine abuse and had negative test results for cocaine was considered as a control.

Multiple substance abuse was frequently observed among the study group. Those with positive screening results for cocaine alone (group 1) were compared with those who had positive screening results for cocaine plus additional illicit substances (group 2) in an attempt to delineate further the effects of multiple substance abuse on perinatal outcome.

Continuous data of the study and control groups were compared with the appropriate t test, and discrete data were compared by means of either χ^2 or Fisher's exact tests. Numerous obstetric and neonatal complications were examined; therefore pairwise comparisons with χ^2 or Fisher's exact tests were considered statistically significant for these parameters at $p < 0.01$ (based on Bonferroni's correction[15]).

Table II. Obstetric data

Parameter	*Cocaine-positive group (mean ± SD or No. and % of cases) (n = 138)*	*Control group (mean ± SD or No. and % of cases) (n = 88)*	*Significance*
Gestational age at delivery (wk)	37.6 ± 3.5	39.0 ± 2.4	$p < 0.001$
Infant birth weight (gm)*	2607 ± 655	3217 ± 612	$p < 0.001$
Route of delivery			
Vaginal	110 (80%)	76 (85%)	NS
Primary cesarean section	19 (14%)	11 (12%)	NS
Repeat cesarean section	8 (6%)	3 (3%)	NS
Preterm delivery (<37 wk)	35 (25%)	8 (9%)	$p < 0.005$
Premature rupture of the membranes	29 (21%)	3 (3%)	$p < 0.0005$
Small-for-gestational-age infants*†	43 (31%)	6 (7%)	$p < 0.0001$
Abruptio placentae	6 (4%)	0 (0%)	NS
Apgar scores ≤3‡			
1 min	13 (9%)	2 (2%)	NS
5 min	5 (4%)	0 (0%)	NS

*Cocaine-positive group includes two twin gestations ($n = 140$).

†Birth weight < 10th percentile by birth weight curves of Brenner et al.[16]

‡Cocaine-positive group includes two twin gestations and excludes three gestations complicated by intrauterine fetal death ($n = 137$).

Results

One hundred thirty-eight (8%) of 1776 women had positive test results for cocaine. The incidence of cocaine-positive urine toxicologic screens increased steadily during the study period (Fig. 1). Fourteen (4%) of 397 patients who were delivered of their infants during the first 6 months of the study had positive test results for cocaine. In contrast, during the last 6 months, 60 (12%) of 514 had positive screens for cocaine. Drug history alone was successful in identifying only 59 (43%) of the 138 women who had positive test results for cocaine. All patients who initially denied cocaine use but had positive urine toxicologic screens subsequently admitted to using cocaine after being confronted with the test results. Neonatal urine toxicologic screening was performed on 114 of the 140 infants whose mothers screened positive for cocaine, and 83 (73%) of these had positive screens for cocaine.

Maternal characteristics of the study and control groups are compared in Table I. Maternal age, gravidity, and parity were significantly higher in the study population than in the control group. Racial distribution and medical complications were similar in both groups.

Obstetric data of the two groups are summarized in Table II. The incidences of preterm delivery, premature rupture of the membranes, and small-for-gestational-age infants were significantly greater in the study group than in the control group. The frequencies of abruptio placentae and low Apgar scores (defined as ≤3) were greater in the study group than in the control group; however, these differences were not statistically significant.

Neonatal data are summarized in Table III. Among the study neonates, 41 (29%) displayed clinical evidence of withdrawal, including tremulousness, irritability, poor feeding, and diarrhea. Apnea and/or abnormal pneumograms were present in 20 (14%) study infants. Congenital anomalies were more common in the study infants and included ventricular septal defect ($n = 2$), atrial septal defect ($n = 1$), complete heart block ($n = 1$), inguinal hernia ($n = 2$), esophageal atresia ($n = 1$), hypospadias ($n = 1$), cleft lip and palate with trisomy 13 ($n = 1$), and polydactyly ($n = 1$). The perinatal mortality rate was higher in the study group than in the control group (50 vs. 0 per 1000, respectively). Intrauterine fetal death occurred in three study pregnancies and neonatal death occurred in four. The incidence of prolonged hospitalization was also significantly greater in the study group than in the control group.

Multiple substance abuse was frequently observed among the study group. Twenty-four of 138 (17%) study patients had positive urine toxicologic screens for illicit substances in addition to cocaine. Sixteen patients had positive results for one additional substance (15 for opiates and one for methadone), whereas eight were positive for two additional substances.

Comparisons of patients with positive screens for cocaine alone (group 1, $n = 114$) versus those with pos-

Table III. Neonatal data

Parameter	*Cocaine-positive group** *(mean ± SD or No. and % of cases)* *(n = 140)*	*Control group (mean ± SD or No. and % of cases)* *(n = 88)*	*Significance*
Withdrawal†	41 (30%)	1 (1%)	$p < 0.00001$
Respiratory distress syndrome†	12 (9%)	3 (3%)	NS
Apnea or abnormal pneumogram†	20 (15%)	0 (0%)	$p < 0.0005$
Congenital anomaly	10 (7%)	2 (2%)	NS
Perinatal death‡	7 (5%)	0 (0%)	NS
Neonatal hospitalization†			
Mean (days)	19.2 ± 86.0	5.1 ± 10.3	$p < 0.0001$
> 3 Days	110 (80%)	21 (24%)	$p < 0.00001$
> 10 Days	48 (35%)	9 (10%)	$p < 0.0005$

*Cocaine-positive group includes two twin gestations ($n = 140$).

†Cocaine-positive group excludes three gestations complicated by intrauterine fetal death ($n = 137$).

‡Intrauterine fetal and neonatal deaths.

Table IV. Comparison of study subgroups by patients of abuse

Parameter	*Group 1: Cocaine alone (mean ± SD or No. and % of cases)* *(n = 114)*	*Group 2: Multiple substances (mean ± SD or No. and % of cases)* *(n = 24)*	*Significance*
Gestational age at delivery (wk)	37.5 ± 3.7	37.8 ± 2.1	NS
Infant birth weight (gm)*	2644 ± 685	2421 ± 432	NS
Preterm delivery (< 37 wk)	28 (25%)	7 (29%)	NS
Small-for-gestational-age infants*†	32 (28%)	11 (46%)	NS
Withdrawal‡	28 (25%)	13 (54%)	$p < 0.005$
Congenital anomaly*	5 (4%)	5 (21%)	$p < 0.005$
Neonatal hospitalization‡			
> 3 Days	90 (80%)	20 (83%)	NS
> 10 Days	35 (31%)	13 (54%)	$p < 0.01$

*Group 1 includes two twin gestations ($n = 116$).

†Birth weight < 10th percentile by birth weight curves of Brenner et al.[16]

‡Group 1 includes two twin gestations and excludes three gestations complicated by intrauterine fetal death ($n = 113$).

itive screens for cocaine plus additional illicit substances (group 2, $n = 24$) are summarized in Table IV. Maternal age, gravidity, and parity were similar in both groups. In addition, obstetric data, including gestational age at delivery, infant birth weight, and frequencies of preterm labor and small-for-gestational-age infants, were similar in groups 1 and 2. Compared with infants of mothers abusing cocaine alone, infants of mothers abusing cocaine plus additional substances had significantly higher incidences of neonatal withdrawal, congenital anomalies, and prolonged hospitalization (>10 days).

Comment

The prevalence of cocaine abuse among the general population in the United States has increased dramatically during the past decade.[1] However, few data exist documenting the prevalence of cocaine abuse among pregnant women.[14] Our data suggest that peripartum cocaine abuse was common and increased dramatically during the study period among pregnant women delivered at our institution. The rising incidence of positive urine toxicologic screens appears to reflect an increasing prevalence of cocaine abuse among our obstetric population. There was no change in the testing methods during the study period, nor was there any appreciable change in the demographic characteristics of our obstetric population. Furthermore, our data very likely underestimate the actual prevalence of cocaine abuse in pregnancy. Cocaine metabolites are usually present in the urine for only 72 hours after ingestion; this study design, at best, only identified women abus-

ing cocaine within 72 hours before admission to the hospital. Those patients with positive test results for cocaine at the time of admission may represent only a fraction of those abusing cocaine at some time during pregnancy.

Previous reports have suggested that cocaine abuse in pregnancy may be associated with adverse perinatal outcome.[4-13] The perinatal morbidity that has been associated with cocaine abuse includes lower infant birth weight, lower mean gestational age at delivery, increased incidence of preterm delivery, and delivery of small-for-gestational-age infants. In addition, increased frequencies of abruptio placentae, congenital anomalies, and neonatal withdrawal have been observed. Our data support many of these findings. When compared with the control group, those women with positive test results for cocaine had lower gestational ages at delivery and lower infant birth weights, as well as higher incidences of preterm delivery, premature rupture of the membranes, and small-for-gestational-age infants. We also found that infants born to cocaine-abusing mothers had neonatal complications, including withdrawal and apnea or abnormal pneumograms, more frequently than control infants. In addition, the perinatal mortality rate in the cocaine-positive group was clinically alarming.

Several factors may have influenced our study results. First, neither the quantity of cocaine nor the pattern of abuse throughout pregnancy was available. An accurate estimation of the amount of cocaine used is problematic. We chose not to rely on patient recall for this information, since our data suggested that drug histories were not reliable among our patients. Our study identified only those women abusing cocaine immediately before delivery. Chasnoff et al.[13] have suggested that cocaine abuse during the third trimester may be associated with increased perinatal morbidity when compared with cocaine abuse limited to the first and second trimesters. Second, women abusing cocaine are likely to have abused multiple substances during their pregnancies, further confounding attempts to establish a cause-and-effect relationship, rather than an association, between a particular illicit substance and adverse perinatal outcome. In an effort to further delineate the impact of multiple substance abuse on perinatal outcome, cocaine-positive study patients were divided into two groups, those with positive screens for cocaine alone (group 1) and those with positive screens for cocaine plus additional illicit substances (group 2). Obstetric complications of these two groups were similar (Table IV). Infants of multiple-substance abusers were more likely to undergo withdrawal and require prolonged hospitalization than infants whose mothers had positive screens for cocaine alone. Those women abusing cocaine and additional substances during pregnancy may be at greatest risk for adverse perinatal outcome; however, significant perinatal morbidity was observed in both groups. Third, cocaine and multiple-substance abusers tend to have poor nutritional habits and adverse socioeconomic situations and often obtain limited prenatal care. Such factors may further influence differences in perinatal outcome between the cocaine-abusing group and the control group.

The prevalence of cocaine abuse and the frequency of adverse perinatal outcome associated with its use in our study emphasize the need to identify women abusing illicit substances. Identification during pregnancy would fascilitate increased antenatal surveillance, psychiatric and social service referral for substance-abusing mothers, and long-term neurodevelopmental follow-up for infants exposed to cocaine in utero. To our knowledge only one report has been published to date evaluating the prevalence of cocaine abuse in an obstetric population. Little et al.[14] reviewed data of 102 obstetric patients and estimated that the prevalence of cocaine abuse in their obstetric population was 9.8% on the basis of drug history. Drug history alone may be inadequate in identifying substance abusers. Indeed, in our study only 59 of 138 women who had cocaine-positive urine toxicologic test results admitted substance abuse on initial evaluation. Urine toxicologic screening may be beneficial in identifying illicit substance abusers, especially in a large urban population such as ours, so that proper maternal, prenatal, and neonatal care can be offered.

In conclusion, cocaine abuse during pregnancy appears to be alarmingly prevalent among our obstetric population and has increased dramatically. Our data also suggest an association between cocaine abuse during pregnancy and adverse perinatal outcome. Thus it appears that cocaine has contributed significantly to perinatal morbidity and mortality in our obstetric population. Complications in cocaine-exposed neonates associated with prolonged hospitalization may contribute significantly to health care costs and the utilization of health care resources.

REFERENCES

1. Abelson HI, Miller JD. A decade of trends in cocaine use in the household population. Natl Inst Drug Abuse Res Monogr Ser 1985;61:35-49.
2. Cregler LL, Mark H. Medical complications of cocaine abuse. N Engl J Med 1986;315:1495-9.
3. Isner JM, Estes NAM III, Thompson PD, et al. Acute cardiac events temporally related to cocaine abuse. N Engl J Med 1986;315:1438-43.
4. Acker D, Sachs BP, Tracey KJ, et al. Abruptio placentae associated with cocaine use. Am J Obstet Gynecol 1983;146:220-2.
5. Chasnoff IJ, Burns WJ, Schnoll SH, Burns KA. Cocaine use in pregnancy. N Engl J Med 1985;313:666-9.
6. Chasnoff IJ, Busey ME, Savich R, Stack CM. Perinatal

cerebal infarction and maternal cocaine use. J Pediatr 1986;108:456-9.
7. MacGregor SN, Keith LG, Chasnoff IJ, et al. Cocaine use during pregnancy: adverse perinatal outcome. Am J Obstet Gynecol 1987;157:686-90.
8. Chasnoff IJ, Burns WJ, Burns KA. Cocaine use in pregnancy: perinatal morbidity and mortality. Neurotoxicol Teratol 1987;9(4):291-3.
9. Ryan L, Ehrlich S, Finnegan L. Cocaine abuse in pregnancy: effects on the fetus and newborn. Neurotoxicol Teratol 1987;9(4):295-9.
10. Oro AS, Dixon SD. Perinatal cocaine and methamphetamine exposure: maternal and neonatal correlates. J Pediatr 1987;111:571-8.
11. Bingol N, Fuchs M, Diaz V, Stone RK, Gromisch DS. Teratogenicity of cocaine in humans. J Pediatr 1987;110: 93-6.
12. Cherukuri R, Minkoff J, Feldman J, Parekh A, Glass L. A cohort study of alkaloidal cocaine ("crack") in pregnancy. Obstet Gynecol 1988;72:147-51.
13. Chasnoff IJ, Griffith DR, MacGregor SN, Dirkes K, Burns KA. Temporal patterns of cocaine use in pregnancy: perinatal outcome. JAMA 1989;261:1741-4.
14. Little BB, Snell LM, Palmore MK, Gilstrap LC. Cocaine use in pregnant women in a large public hospital. Am J Perinatol 1988;5(3):206-7.
15. Ingelfinger JA, Mosteller F, Thibodeau LA, Ware JH. Biostatistics in clinical medicine. New York: MacMillan, 1987:159-61.
16. Brenner WE, Edelman DA, Hendricks CH. A standard of fetal growth for the United States of America. Am J Obstet Gynecol 1976;126:555-60.

Do the current standards for glucose tolerance testing in pregnancy represent a valid conversion of O'Sullivan's original criteria?

David A. Sacks, MD,[a] Salim Abu-Fadil, MD,[a] Jeffrey S. Greenspoon, MD,[b] and Nick Fotheringham, PhD[c]

Bellflower and Los Angeles, California

The venous plasma values for the 100 gm glucose tolerance test endorsed by the Second International Workshop-Conference on Gestational Diabetes represent an arbitrary conversion from O'Sullivan's original values. The latter were calculated from whole blood by means of the Somogyi-Nelson method. The factor used to convert the whole blood values to plasma glucose values was empirically derived in a population in whom pregnancy status was not stated. A conversion factor derived from a nonpregnant population may systematically overestimate plasma glucose concentration when applied to a pregnant population. Paired specimens obtained from 995 consecutive pregnant patients were analyzed by the Somogyi-Nelson method on whole blood and the glucose oxidase method on plasma. A conversion formula was derived and was used to estimate plasma glucose values and 95% confidence limits equivalent to whole blood values. Application of this formula yields plasma glucose oxidase glucose tolerance test values of fasting, 96 mg/dl; 1 hour, 172 mg/dl; 2 hours, 152 mg/dl; and 3 hours, 131 mg/dl. These values are all lower than those currently endorsed. (Am J Obstet Gynecol 1989;161:638-41.)

Key words: Gestational diabetes, glucose tolerance

In 1964 O'Sullivan and Mahan[1] published a study establishing criteria for the oral glucose tolerance test (GTT) in pregnancy. Two or more values (fasting, 90 mg/dl; 1 hour, 165 mg/dl; 2 hour, 145 mg/dl; 3 hour, 125 mg/dl) had to be equaled or exceeded to diagnose gestational diabetes. The glucose concentrations were determined from whole blood by the Somogyi-Nelson assay, a chemical method. Enzyme methods for measuring glucose (e.g., glucose oxidase) are more specific and have largely replaced chemical methods.[2] Furthermore, because estimates of glucose concentration in whole blood may be affected by the hematocrit,[3] they have been replaced by plasma assays.

The plasma glucose values endorsed first by the Na-

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Kaiser Foundation Hospital,[a] the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of Southern California/Los Angeles County Medical Center and Women's Hospital,[b] and Dixon Statistical Associates.[c]

Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.

Reprint requests: David A. Sacks, MD, Kaiser Foundation Hospital, 9400 East Rosecrans Ave., Bellflower, CA 90706.

6/6/13719

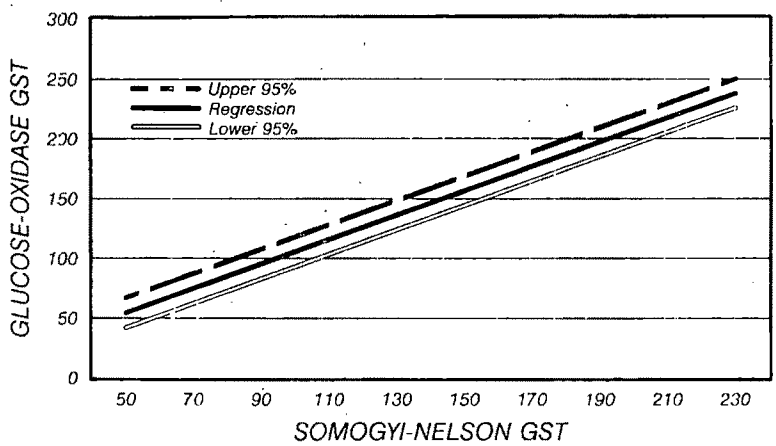


Fig. 1. Regression of plasma–glucose oxidase screening test results onto whole blood–Somogyi-Nelson values. *Line* in center represents mean; *upper* and *lower lines* delineate 95% confidence intervals. *GST*, Glucose screening test.

tional Diabetes Data Group[4] and subsequently by the Second International Workshop-Conference on Gestational Diabetes Mellitus (fasting, 105 mg/dl; 1 hour, 190 mg/dl; 2 hour, 165 mg/dl; 3 hour, 145 mg/dl) represent an arbitrary conversion from O'Sullivan's original values.[5] Although the factor used to convert the original whole blood–Somogyi-Nelson values to plasma enzyme assay values is not explicitly stated, it closely approximates the 13% to 16% reported in other studies. Those studies were notable for not explicitly stating the subjects' pregnancy status.[6, 7] As Schwartz and Brenner[8] noted, a factor derived from a nonpregnant population used to convert whole blood glucose to plasma glucose may systematically overestimate the plasma glucose in pregnant subjects. The purpose of this study was to empirically derive a formula to convert glucose concentrations in whole blood measured by the Somogyi-Nelson method to glucose concentrations in plasma obtained by the glucose oxidase method for use during pregnancy.

Material and methods

Nine hundred ninety-five consecutive predominantly Hispanic patients having routine 50 gm 1-hour glucose screening tests during pregnancy were studied. The glucose screening test was performed according to the recommendations of the Second International Workshop-Conference on Gestational Diabetes.[5] A total of 14 ml of blood was obtained from each patient on one occasion and separated equally into two tubes, each containing 14 mg of potassium oxalate as an anticoagulant and 17.5 mg of sodium fluoride to inhibit glycolysis. The tube used for the plasma assay was spun at 1570 *g* for 10 minutes to separate the plasma. The supernatant was assayed by an automated glucose oxidase method (Trendar Auto-Pak; HiChem, Inc., Anaheim, Calif.). The Somogyi-Nelson assay of whole blood was done by a standard manual method.[9] All testing was performed by the same laboratory. No changes in the technology of glucose assays occurred during the course of this study. The interassay coefficient of variation for normal controls was 2.3% for the glucose oxidase and 1.5% for the Somogyi-Nelson method.

Multiple linear regression analysis was used to evaluate the relationship between glucose assays with the Somogyi-Nelson and glucose oxidase methods and the effect of hematocrit on this relationship. These analyses were performed with BMDP Programs 2R and 6D. Demographic information was summarized with BMDP Program 2D.[10] The level of significance was set at $p < 0.05$.

Results

Among the 995 patients, 386 (38.8%) were nulliparous. The mean (±SD) maternal age was 26.1 ± 5.7 years. Two patients were tested in the first trimester, 234 (23.5%) in the second trimester, and 759 (76.3%) in the third trimester. The mean (±SD) glucose concentration was 109.1 ± 25.7 mg/dl for the whole blood–Somogyi-Nelson assay and 115.1 ± 26.7 mg/dl for the glucose oxidase assay. The respective ranges of values were 50 to 229 mg/dl and 47 to 235 mg/dl.

The formula for the regression line with the whole blood–Somogyi-Nelson assay as the independent variable is P = 4.391 + 1.015 WB, where P is plasma–glucose oxidase and WB is whole blood–Somogyi-Nelson. The 95% confidence interval of the slope was 1.001 to 1.029; the 95% confidence interval of the intercept was 4.03 to 4.75. The correlation coefficient between techniques was 0.976 ($p < 0.001$). The line of best fit and 95% confidence intervals are illustrated in

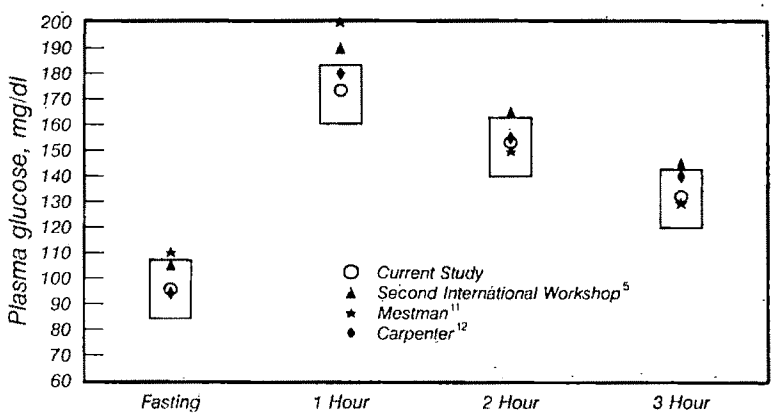


Fig. 2. Plot of criteria proposed by different references for glucose tolerance tests in pregnancy with 100 gm glucose load and glucose oxidase assay of plasma. Values in *boxes* represent 95% confidence intervals derived from data in this study for glucose oxidase assay of plasma, which would be equivalent to O'Sullivan's original GTT values.

Fig. 1. Application of the formula to O'Sullivan's whole blood–Somogyi-Nelson GTT values converts these to plasma glucose oxidase values of 96 mg/dl, 172 mg/dl, 152 mg/dl, and 131 mg/dl, respectively, for fasting, 1-hour, 2-hour, and 3-hour values. A comparison of these criteria with those published by others[5, 11, 12] with a 100 gm glucose load and a plasma–glucose oxidase assay is illustrated in Fig. 2. All values for the GTT proposed by Carpenter and Coustan[12] are within the 95% confidence intervals of the plasma–glucose oxidase equivalents derived from the current data.

Of the 995 patients studied, 572 had hematocrit samples drawn within 1 week of the glucose assays. Hematocrit values ranged from 26% to 43%. The slopes of the regression lines of P onto WB in the ranges 27.51% to 32.50%, 32.51% to 37.50%, and 37.51% to 42.50%, respectively, were not significantly different.

Comment

Several authors have used plasma–glucose oxidase values that they claimed were equivalent to the original Somogyi-Nelson–whole blood GTT criteria introduced by O'Sullivan and Mahan.[1] The conversion of these values requires consideration of both the laboratory method used and the blood component analyzed.

The initial step of the Somogyi-Nelson method is the preparation of a protein-free filtrate, which serves to remove nonglucose reducing substances found in red blood cells such as glutathione, glucuronic acid, ergothioneine, and ascorbic acid.[2] However, other hexoses (e.g., galactose, mannose) remain in the filtrate and may account for the higher "glucose" values reported for the Somogyi-Nelson method in comparison with enzyme methods.[2, 13] Technical factors within the Somogyi-Nelson assay also may alter test results. Mager and Farese[13] found significantly different results in the same specimens of whole blood by merely altering the sequence of the addition of reagents used in the preparation of the protein-free filtrate.

Because they confer maximum specificity of glucose estimations, enzyme methods (e.g., glucose oxidase and hexokinase) have largely replaced older chemical methods.[2] The hexokinase method is the current reference method, and the glucose oxidase method is the most commonly used glucose assay.[8] Glucose oxidase results correlate well with those obtained by the hexokinase assay.[2] Few studies have attempted to establish a quantitative relationship between Somogyi-Nelson and glucose oxidase assays. Mager and Farese[13] found that results obtained by Somogyi-Nelson assay were significantly higher (range, 2% to 6%) than those obtained by glucose oxidase when plasma was assayed. However, when these two methods were compared when whole blood was used, no consistent relationship was found.

Glucose assays of plasma offer significant advantages over those of whole blood. Interference from nonglucose reducing substances is minimized. Plasma more readily lends itself to automated assays than whole blood. Finally, and of critical relevance to pregnancy, whole blood assays for glucose may be influenced by hematocrit,[3] whereas plasma assays are not.

The concentration of glucose in plasma exceeds that in red blood cells. When the hematocrit is lower (the fewer the number of red cells per unit volume), the concentration of glucose in whole blood is higher and the difference between whole blood and plasma glucose values is lower.[3, 8] Because of the disproportionate expansion in plasma volume in relation to red blood cell mass during pregnancy,[14] pregnant patients may have lower hematocrit values than a nonpregnant population. Thus a factor used to convert whole blood to plasma glucose derived from a nonpregnant population may systematically overestimate plasma glucose

during pregnancy. O'Sullivan and Kantor[7] reported a mean difference of 13.4% between whole blood and plasma glucose when both were assayed by an automated ferricyanide method. Niejadlik et al.[6] found that plasma glucose values obtained by the hexokinase method exceeded by 13% the whole blood glucose values obtained by the Somogyi-Nelson method. In neither study was the subjects' pregnancy status noted.

As O'Sullivan and Kantor[7] reported, we were unable to detect a significant contribution by hematocrit to the differences in glucose values between assays. A potential explanation for this finding includes the narrow ranges of both observed hematocrit and glucose values. However, we did find a marked difference between our empirically derived conversion factor (approximately 5% to 6%) and those reported by others. It is of interest that the extrapolated values of Carpenter and Coustan,[12] which consider the effects of both method and blood component assayed, fall within the 95% confidence intervals of our empirically derived values (Fig. 2).

O'Sullivan's criteria for GTT in pregnancy are unique because they were both empirically derived and validated. Values that are 2 SDs above the mean of an unselected sample of 752 women were chosen because the proportion of patients meeting or exceeding these criteria approximated the prevalence of diabetes in the community (1.99%). In addition, when these criteria were applied to a second group of patients, it was found that those meeting criteria for gestational diabetes during pregnancy had a significantly higher incidence of diabetes 8 years after delivery than those who did not meet criteria for gestational diabetes.[1] Finally, O'Sullivan et al.[15] demonstrated significantly more perinatal losses among infants of women with gestational diabetes ≥25 years of age than among age-matched nondiabetic control women.

Unfortunately, both the Somogyi-Nelson laboratory method for glucose concentration and the blood component assayed (whole blood) in the 1964 study have more intrinsic variability than the enzyme assays of plasma in current use. Applying an arbitrary conversion factor to the original data may further detract from the accuracy of criteria proposed as equivalent. The ideal resolution of the issue would be to repeat O'Sullivan's study with an enzyme assay on plasma. Alternatively, critical examination of different proposed sets of GTT criteria relative to maternal, fetal, and neonatal outcome variables seems reasonable. It is our hope that the findings of this study will serve as a stimulus for further investigation.

REFERENCES

1. O'Sullivan JB, Mahan CM. Criteria for the oral glucose tolerance test in pregnancy. Diabetes 1964;13:278-85.
2. Todd-Sanford-Davidson. Carbohydrates. In: Henry JB, ed. Clinical diagnosis and management in laboratory methods. Philadelphia: WB Saunders, 1979 vol 1:158-60.
3. Dillon RS. Importance of hematocrit in interpretation of blood sugar. Diabetes 1965;14:672-4.
4. National Diabetes Data Group. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 1979;28:1039-57.
5. Summary and recommendations of the Second International Workshop-Conference on Gestational Diabetes Mellitus. Diabetes 1985;34(suppl 2):123-6.
6. Niejadlik DC, Dube AH, Adamko SM. Glucose measurements and clinical correlations. JAMA 1973;224:1734-6.
7. O'Sullivan JB, Kantor N. Variability of blood sugar levels with an automated method. Public Health Rep 1963; 78:1023-9.
8. Schwartz ML, Brenner WE. The need for adequate and consistent diagnostic classifications for diabetes mellitus diagnosed during pregnancy. AM J OBSTET GYNECOL 1982;143:119-24.
9. Fister HJ. "True dextrose" method. In: Manual of standardized procedures for spectrophotometric chemistry. New York: Standard Scientific Supply Corp., 1950:D-12a.1-4.
10. Dixon WJ. BMDP statistical software manual. Berkeley: University of California Press, 1988 vol 1.
11. Mestman JH. Outcome of diabetes screening in pregnancy and perinatal morbidity in infants of mothers with mild impairment in glucose tolerance. Diabetes Care 1980;3:447-52.
12. Carpenter MW, Coustan DR. Criteria for screening tests for gestational diabetes. AM J OBSTET GYNECOL 1982; 144:768-73.
13. Mager M, Farese G. What is "true" blood glucose? Am J Clin Pathol 1965;44:104-8.
14. Hytten F. Blood volume changes in normal pregnancy. Clin Haematol 1985;14:601-12.
15. O'Sullivan JB, Charles D, Mahan CM, Dandrow RV. Gestational diabetes and perinatal mortality rate. AM J OBSTET GYNECOL 1973;116:901-4.

How reliable is the fifty-gram, one-hour glucose screening test?

David A. Sacks, MD,[a] Salim Abu-Fadil, MD,[a] Jeffrey S. Greenspoon, MD,[b] and Nick Fotheringham, PhD[c]

Bellflower and Los Angeles, California

The Second International Workshop-Conference on Gestational Diabetes recommended screening all pregnant women with a 1-hour 50 gm oral glucose screening test, given without regard to time of day or time elapsed from the last meal. This study was designed to evaluate the reproducibility of that test, given under those clinical conditions. Thirty women with gestational diabetes and 80 control volunteers between 24 and 28 weeks' gestation were tested at the same time of day on two successive days. Of the 30 women with diabetes, three (10%) had glucose screening test results below the 135 mg/dl threshold on both days and 10 (33%) had results that straddled the threshold on successive days. Test results of 11 control women also straddled the threshold. Among these 21 women with results that straddled the threshold, there was no difference in mean glucose screening test results in diabetic women compared with nondiabetic ones. We conclude that, as currently recommended, the 1-hour glucose screening test is moderately reproducible. Reliance should not be placed on a single normal test result, particularly among patients with risk factors. (AM J OBSTET GYNECOL 1989;161:642-5.)

Key words: Gestational diabetes, screening for diabetes

The Second International Workshop-Conference on Gestational Diabetes recommended administering the 50 gm, 1-hour glucose screening test without regard to time of day or time elapsed from the last meal.[1] If the test administered under these clinical conditions is reproducible, then one may depend on a single glucose screening test result to reliably identify patients with gestational diabetes. However, if repetitive results of the same subject differ markedly, and especially if the test results straddle a threshold used to indicate that a glucose tolerance test (GTT) should be performed, then reliance on a single test may not be justified. The present study was designed to test the hypothesis that there is no significant difference in glucose screening test results obtained from the same subject on successive days.

Material and methods

Our protocol for screening for gestational diabetes has been previously reported.[2] Volunteers were recruited from two groups of predominantly Hispanic women. One group consisted of 84 obstetric patients who were not known to have glucose intolerance. These patients had a glucose screening test on two consecutive days between the twenty-fourth and twenty-eighth gestational weeks. Those whose test results equaled or exceeded 135 mg/dl on either or both days had a 3-hour GTT. Four of these 84 patients had gestational diabetes diagnosed by the GTT and are included with the gestational diabetic group for purposes of data analysis. The remaining 80 patients were termed controls.

The second group consisted of 26 women with gestational diabetes that was first diagnosed during the current pregnancy. These patients were identified by a glucose screening test result ≥135 mg/dl followed by a GTT that was diagnostic of gestational diabetes. After volunteering for the study, these 26 women had an additional glucose screening test on each of two consecutive days between the twenty-fourth and twenty-eighth gestational weeks. The GTT was not repeated in this group. None of the diabetic patients had initiated dietary and/or insulin therapy at the time the study was conducted.

On the first day of testing, the subjects were given a 1-hour, 50 gm glucose screening test, with a commercially prepared cola (Dolce-Hopping Bottling Co., Sunnyvale, Calif.). They were instructed to eat the same foods and engage in the same or similar physical activities at the same time of day on the following day and to return in exactly 24 hours for a second glucose screening test. The preparation for and conduct of both glucose screening and tolerance tests, as well as the criteria for defining diabetes, followed the recom-

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Kaiser Foundation Hospital,[a] Bellflower, the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Los Angeles County/University of Southern California Medical Center and Women's Hospital,[b] and Dixon Statistical Associates,[c] Los Angeles.

Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.

Reprint requests: David A. Sacks, MD, Kaiser Foundation Hospital, 9400 East Rosecrans Ave., Bellflower, CA 90706.

6/6/14469

mendations of the Second International Workshop-Conference on Gestational Diabetes.[1]

Gestational age was determined by clinical parameters including date of last menstrual period, early sizing, and sonographic examinations. Approval for this study was obtained from the Institutional Review Board of the Southern California Kaiser Permanente Research Program. A signed informed consent was obtained from each participant.

Plasma glucose assays were performed by an automated glucose oxidase method (Synchron CX3 System, Beckman Instruments, Brea, Calif.). Interassay coefficients of variation for normal controls (mean 90.6 mg/dl) and abnormal controls (mean 292.2 mg/dl), respectively, were 2.3% and 1.5%. Intraassay coefficients of variation for normal controls (mean 88.0 mg/dl) and abnormal controls (mean 294.4 mg/dl), respectively, were 1.8% and 1.0%.

Two-sample t tests and analysis of variance were used to compare mean glucose screening test values. Continuity-corrected χ^2 tests were used to compare frequencies of occurrence among patients. Product moment correlation coefficients and logistic regression analysis were used to evaluate relationships between variables. Descriptive statistics, t tests, χ^2 tests, correlation analysis, analysis of variance, and logistic regression were performed with BMDP Programs 2D, 3D, 4F, 8D, 2V, and LR.[3] Statistical results were considered significant at a level of $p < 0.05$.

Results

The mean ± SD for maternal age was 27.6 ± 6.4 years. Of the 110 patients, 43 (39%) were nulliparous. The mean ± SD for number of weeks' gestation on the first day of testing was 26.6 ± 1.4. The mean ± SD for time interval from first to second glucose screening test was 24 ± 2.6 hours.

The individual day-to-day glucose screening test results are displayed in Fig. 1. The mean day-to-day glucose screening test values for diabetic and control groups are shown in Table I. On both days, the mean value for the diabetic group was significantly greater than that of the control group. A two-way analysis of variance demonstrated no significant day-to-day differences in mean values for either diabetic or control group. The mean of the absolute day-to-day differences in values for women with diabetes approached but did not achieve a statistically significant difference from that of the control group (Table I).

Ten women with diabetes and 11 control women had glucose screening test values ≥135 mg/dl on only 1 day. Five of the values in the diabetic group equaled or exceeded the threshold on the first day of testing and five on the second day (Fig. 1). In contrast with the entire patient sample, there was no significant difference in mean glucose screening test results between these "straddler" diabetics and controls (Table I).

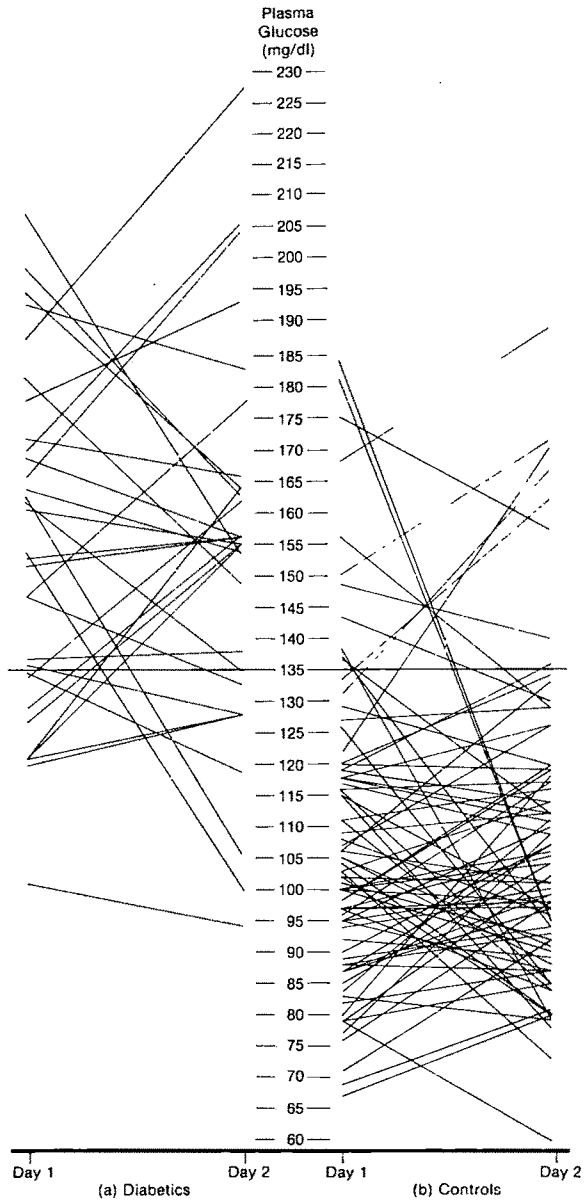


Fig. 1. Plot of glucose screening test results on day 1 and day 2 for gestational diabetic and control groups. A *line* connects values on successive days from the same individual. A threshold of 135 mg/dl was used to indicate that a GTT should be performed.

Fasting hyperglycemia was defined by a fasting plasma glucose level ≥105 mg/dl on the GTT. Among the 30 with gestational diabetes, fasting hyperglycemia occurred with approximately equal frequency in patients whose two glucose screening test results were below threshold (1 of 3, 33%), straddled the threshold (3 of 10, 30%), or exceeded the threshold (6 of 17, 35%).

The parameters investigated as independent risk fac-

Table I. Mean day-to-day differences in glucose screening test results

	All patients (N = 110)			*Straddlers (n = 21)*		
	Diabetic (n = 30)	*Control (n = 80)*	*p*	*Diabetic*	*Control*	*p*
GST, day 1 (mean) (mg/dl)	155.4	108.2	<0.001	136.7	143.5	NS
GST, day 2 (mean) (mg/dl)	153.9	106.9	<0.001	138.4	124.3	NS
ΔGST (mean) (mg/dl)	24.2	17.2	0.054	31.3	47.2	NS
GST (range) (mg/dl)	96-227	60-189		99-169	78-184	

GST, Glucose screening test; *ΔGST*, day-to-day difference in GST values; *NS*, not significant. Straddlers are those patients whose test results equaled or exceeded the 135 mg/dl threshold on only 1 day.

tors were: family history of diabetes, obesity (defined as a prepregnancy weight ≥150 pounds), and age ≥25 years.[2] All of the 30 women with gestational diabetes had at least one risk factor. Seven of these 30 (23%) had only one risk factor, 11 (37%) had two, and 12 (40%) had all three. In contrast, significantly fewer (61 of 80 or 76%; $p < 0.05$) control women had one or more risk factors.

Comment

It is important to note that the selection of a 135 mg/dl threshold was somewhat arbitrary.[2,4] In general, the lower the threshold selected, the greater the sensitivity and the lower the specificity.[5]

This study was designed to be optimally consistent with the recommendations of the Second International Workshop-Conference on Gestational Diabetes.[1] Patients were therefore tested without regard to time of day or time elapsed from the last meal. Although there is objective evidence of patient compliance with temporal requirements (mean interval of 24 hours between glucose screening tests), compliance with instructions to maintain the same activity and diet on both days was not closely monitored. Coustan et al.[4] demonstrated that the ingestion of a mixed meal 1 hour before a glucose screening test significantly lowered test results in women with gestational diabetes in comparison with those tested after fasting. However, the absence of significant differences in 1-hour postglucose values relative to time after the last meal has been reported among nondiabetic pregnant[4] and nonpregnant[6] women. The degree to which diet, activity, and time interval from and content of the last meal may influence the reproducibility of the glucose screening test may be investigated by testing a patient sample in which these variables are more rigorously controlled (e.g., hospitalized patients).

A number of other studies have indicated that, despite rigid control of diet and activity, consecutive glucose challenge test results are poorly reproducible. Sisk et al.[7] noted a correlation coefficient of 0.66 in successive values 15 days apart in 96 male prisoners tested 1 hour after a 50 gm glucose load. Ackland et al.[8] reported significant day-to-day differences among seven male laboratory personnel undergoing a 50 gm glucose tolerance test on three successive days. Among 31 male prisoners who met criteria for diabetes, McDonald et al.[9] found that none did so on all six glucose tolerance tests administered over a year's time. However, Owens et al.,[10] reporting on four normal men who received 50 gm glucose challenges every 3 to 4 hours on 2 days of testing during two successive weeks, found no significant differences at most fixed time intervals after each challenge.

The positive correlation between magnitude of mean plasma glucose values and day-to-day variability also has been previously reported. Various authors[9, 11, 12] have noted both peak absolute value and maximal test-to-test differences 1 hour after the glucose challenge. In addition, Harding et al.[13] found a positive correlation between the area under the glucose tolerance curve and between-test variability.

The reasons for variability in results within the same subject from one test to another are not clear. In evaluating 52 women who had third-trimester GTTs during each of three pregnancies, O'Sullivan and Mahan[14] found that the majority of variation with repetitive testing was due to biologic variation within, more-so than between, individuals. Harding et al.[13] postulated that intrasubject differences in glucose concentrations are due to day-to-day fluctuations in glucose absorption and distribution, as well as hepatic glycolysis and gluconeogenesis.

The clinical consequences of this day-to-day variation in 1-hour postglucose screening in pregnancy are illustrated by the findings of the current study. Reliance on a single glucose screening test value <135 mg/dl would have resulted in missing eight of 30 (27%) cases of diabetes on any single given day (Fig. 1). Three of these cases of gestational diabetes would have been missed on both days. Had all cases of diabetes been ascertained from a group of women with known gestational diabetes, the latter number might have been larger. There did not appear to be a relationship be-

tween fasting hyperglycemia and whether the glucose screening test results were below, straddled, or met or exceeded the designated threshold. The relationship between fasting hyperglycemia in pregnancy and increased perinatal mortality has been previously noted.[15] Finally, it was not possible to distinguish glucose screening test results of "straddler" diabetics from those of "straddler" controls on the basis of day-to-day mean test values or mean differences in test results (Table I).

In conclusion, performing the 1-hour glucose screening test in pregnancy without regard to time of day or time elapsed from the last meal offers the advantages of simplicity and acceptability, and thus patient compliance. However, the data in this study suggest that a substantial proportion of cases of gestational diabetes may be missed by reliance on a single glucose screening test. From these data, it seems unlikely that a glucose screening test value ≤95 mg/dl would be associated with gestational diabetes. We therefore suggest that with values above this level reliance not be placed on a single "normal" test result, particularly among patients with identifiable risk factors.

REFERENCES

1. Summary and recommendations of the second international workshop-conference on gestational diabetes mellitus. Diabetes 1985;34(suppl 2):123-6.
2. Sacks DA, Abu-Fadil S, Karten GK, Forsythe AB, Hackett JR. Screening for gestational diabetes with the one-hour 50-g glucose test. Obstet Gynecol 1987;70:89-93.
3. Dixon WJ. BMDP statistical software manual. Berkeley, California: University of California Press, 1988 vol 1.
4. Coustan DR, Widness JA, Carpenter MW, Rotondo L, Pratt DC, Oh W. Should the fifty-gram, one-hour plasma glucose screening test for gestational diabetes be administered in the fasting or fed state? Am J Obstet Gynecol 1986;154:1031-5.
5. Carpenter MW, Coustan DR. Criteria for screening tests for gestational diabetes. Am J Obstet Gynecol 1982; 144:768-73.
6. Phillips N, Duffy T. Effect of age, hours since last food, time of day and ketonuria on 1-hour glucose tolerance. Health Serv Rep 1972;87:649-57.
7. Sisk CW, Burnham CE, Stewart J, McDonald GW. Comparison of the 50 and 100 gram oral glucose tolerance test. Diabetes 1970;19:852-62.
8. Ackland JD, Clayton H, Mitchell B. Reproducibility of a glucose tolerance test. J Appl Physiol 1962;17:119-22.
9. McDonald GW, Fisher GF, Burnham C. Reproducibility of the oral glucose tolerance test. Diabetes 1965;14:473-80.
10. Owens DR, Wragg KG, Biggs PI, Luzio S, Davies CJ, Jones MK. The reproducibility of serial meal and oral glucose tolerance tests in normal subjects. Diabete Metab 1981; 7:25-33.
11. Freeman H, Looney JM, Hoskins RG. "Spontaneous" variability of oral glucose tolerance. J Clin Endocrinol 1942;2:431-4.
12. Rushforth NB, Bennett PH, Steinberg AG, Miller M. Comparison of the value of the two and one-hour glucose levels of the oral GTT in the diagnosis of diabetes in Pima Indians. Diabetes 1975;24:538-46.
13. Harding PE, Oakley NW, Wynn V. Reproducibility of oral glucose tolerance data in normal and mildly diabetic subjects. Clin Endocrinol 1973;2:387-95.
14. O'Sullivan JB, Mahan CM. Glucose tolerance test. Variability in pregnant and nonpregnant women. Am J Clin Nutr 1966;19:345-51.
15. Gabbe SG. Effects of identifying a high risk population. Diabetes Care 1980;3:486-8.

Glycemic control in gestational diabetes mellitus—How tight is tight enough: Small for gestational age versus large for gestational age?

Oded Langer, MD, Judith Levy, MD, Lois Brustman, MD, Akolisa Anyaegbunam, MD, Ruth Merkatz, RN, and Michael Divon, MD

Bronx, New York

The relationship between optimal levels of glycemic control and perinatal outcome was assessed in a prospective study of 334 gestational diabetic women and 334 subjects matched for control of obesity, race, and parity. All women with gestational diabetes mellitus were instructed in the use of a memory-based reflectance meter. They were treated with the same metabolic goal according to a predetermined protocol. Three groups were identified on the basis of mean blood glucose level throughout pregnancy (low, ≤86 mg/dl; mid, 87 to 104 mg/dl; and high, ≥105 mg/dl). The low group had a significantly higher incidence of small-for-gestational-age infants (20%). In contrast, the incidence of large-for-gestational-age infants was 21-fold higher in the mean blood glucose category than in the low mean blood glucose category (24% vs. 1.4%, $p < 0.0001$). An overall incidence of 11% small-for-gestational-age and 12% large-for-gestational-age infants was calculated for the control group. A significantly higher incidence of small-for-gestational-age infants (20% vs. 11%, $p < 0.001$) was found between the control and the low category. In the high mean blood glucose category an approximate twofold increase was found in the incidence of large-for-gestational-age infants when compared with the control group ($p < 0.03$). No significant difference was found between the control and mean blood glucose categories (87 to 104 mg/dl). Our data suggest that a relationship exists between level of glycemic control and neonatal weight. This information is helpful in targeting the level of glycemic control while optimizing pregnancy outcome in gestational diabetes comparable to the general population. (Am J Obstet Gynecol 1989;161:646-53.)

Key words: Gestational diabetes, level of control, intrauterine growth retardation, macrosomia

In recent years tight glycemic control became a standard of care in the management of the pregnant diabetic in order to reduce perinatal mortality and morbidity. This resulted in the reduction of perinatal mortality to rates comparable to those observed in the normal population.[1,2] In contrast, the incidence of perinatal morbidity as reflected by metabolic disorders and macrosomia remains relatively high.[3,4] Perinatal mortality was prevented by recognition of the need for stringent metabolic control in the pregnant diabetic. However, paucity of information is available regarding the optimal level of glycemia that should be targeted to reduce perinatal morbidity.

Tight glycemic control may result in acute and chronic nutritional deprivation, which are linked to retarded fetal growth and substrate limitations. Fetal insulin levels are responsive to changes in substrate availability in either direction. The fetus grows to the limits allowed by the supply of critical nutrients. It can be anticipated that there will be a significant difference in neontal size in relation to the level of glycemic control in the mother.

In the early 1970s, Pedersen[5] recognized that maternal hyperglycemia is a cause for fetal hyperinsulinemia, leading to excess fetal growth. In contrast, we and other researchers have shown that relative maternal hypoglycemia is associated with intrauterine growth retardation (IUGR).[6,7] In this study we sought to answer the research question: What are the optimal levels of glycemic control that will maximize perinatal outcome? To resolve this issue, we investigated the association between different levels of glycemic control and neonatal weight size (large-for-gestational-age (LGA) vs. small-for-gestational-age (SGA) infants). We also evaluated the relationship between neonatal weight and selected maternal characteristics (e.g., obesity, treatment modality).

Material and methods

A prospective study of 334 gestational diabetic and 334 matched control nondiabetic women was under-

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, The Albert Einstein College of Medicine.
Supported in part by an educational grant from Miles Laboratories.
Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.
Reprint requests: Oded Langer, MD, Department of Obstetrics and Gynecology, The University of Texas Health Science Center, 7703 Floyd Curl Dr., San Antonio, TX 78284-7836.
6/6/14090

Table I. Patient characteristics

	Women with gestational diabetes (n = 334)	*Control subjects (n = 334)*
Age (yr)	31 ± 5*	27 ± 6
Weight change (kg)	14 ± 7	15 ± 7
Race (%)		
White	39	35
Black	38	37
Hispanic	29	28
Obesity (%)	52	49
Body mass index	28 ± 6	27 ± 6
Gestational age at delivery (wk)	39 ± 2	39 ± 2
Family history of diabetes (%)	40	38
Previous history of gestational diabetes (%)	10	—
Oral glucose tolerance test results		
Fasting	103 ± 16	84 ± 8
1 hr	209 ± 28	141 ± 26
2 hr	187 ± 33	120 ± 21
3 hr	140 ± 38	98 ± 22

*$p < 0.05$.

Table II. Characteristics of patients with gestational diabetes according to neonatal size grouping

	Neonatal birth weight groups		
Maternal data	*SGA (n = 38)*	*AGA (n = 264)*	*LGA (n = 34)*
Age (yr)	31 ± 6	31 ± 5	32 ± 6
Parity (%)			
0	62	41	30
1-2	22	47	60
>3	16	12	10
Race (%)			
White	36	37	31
Black	49	38	19
Hispanic	15	25	50
Obesity (%)	58	49	71
Previous gestational diabetes (%)	6	9	18
Mean arterial pressure (mm Hg)	87 ± 13	85 ± 9	84 ± 10
Glucose profile			
Mean blood glucose (mg/dl)	88 ± 8	92 ± 8	107 ± 15
Interquartile range	27 ± 11	30 ± 11	34 ± 14
No. of reversals	3 ± 1	4 ± 2	6 ± 4
Average excursion	31 ± 10	27 ± 11	24 ± 13

taken from 1985 to 1988. Women were matched for obesity, race, parity, and gestational age at delivery. Gestational diabetes mellitus was diagnosed in accordance with the National Diabetes Data Group Criteria and normal individuals by a normal oral glucose tolerance test (all values normal) during the third trimester.[8]

To promote optimal metabolic control, the gestational diabetic participants were instructed in the use of a memory-based reflectance meter to assure accurate and reliable collection of blood glucose data.[9] They were requested to test their blood seven times per day. The quality of control was assessed at the weekly or biweekly clinic visit. Self-monitoring blood glucose data were analyzed for four parameters: mean, interquartile range (variability) distance between the 25th and 75th percentiles, reversals (fluctuation) in median glucose curve, and level of excursion from the median.[10]

The treatment modalities included either diet alone or a combination of insulin and diet. Treatment assignments were determined on the basis of fasting plasma and mean blood glucose levels. Subjects were counseled by a nutritionist in diet management, with a regimen of three meals and four snacks daily. Diet control involved caloric restriction based on maternal body weight.[11, 12] Maternal size was determined by body mass index (body mass index = weight/height^2 in kilograms per meter). Obese patients (body mass index ≥27) were restricted to 25 calories per kilogram of body weight, and nonobese patients to 30 calories per kilogram. Insulin therapy was initiated when fasting plasma glucose, mean blood glucose, or both were >95 mg/dl. Insulin

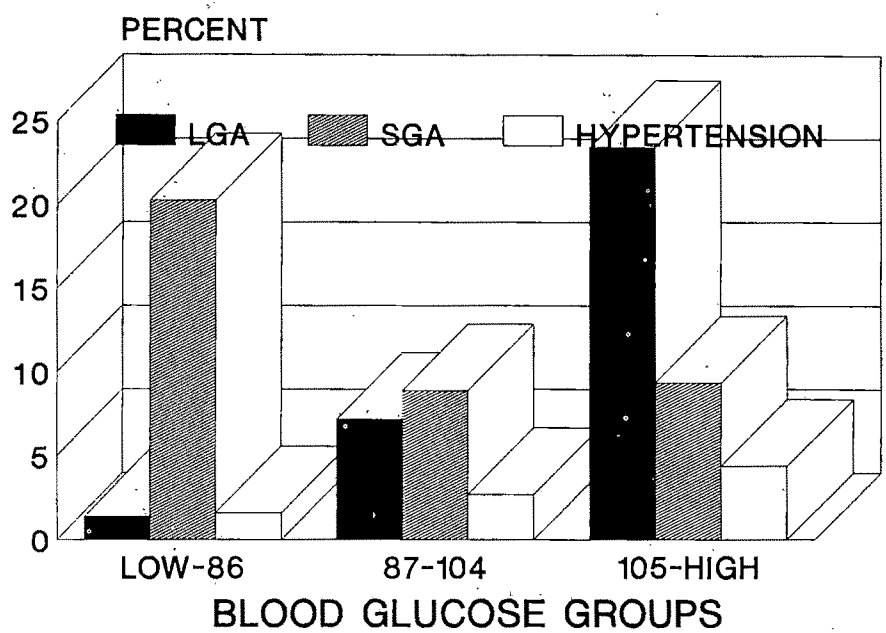


Fig. 1. Association among neonatal size, level of glycemic control, and hypertension.

was administered by multiple injections of regular and NPH insulin synchronized with the diet.[11]

The goal of treatment was to maintain a fasting blood glucose level of 60 to 80 mg/dl, preprandial and bedtime levels of 70 to 90 mg/dl, postprandial levels of <120 mg/dl, and an overall mean <95 mg/dl.

Infants were considered large for gestational age when their birth weight was greater than or equal to the 90th percentile and small for gestational age when their birth weight was less than or equal to the 10th percentile.[13] Clinical data (e.g., last menstrual period), early ultrasonographic result (<20 weeks) when available, pelvic examination, and newborn examination were used to assess gestational age. Neonatal metabolic complications included hypoglycemia (<35 mg/dl), hypocalcemia (<7.5 mg/dl), hyperbilirubinemia (>12 mg/dl), and polycythemia (>65%).

Since we previously identified three clusters on the basis of overall mean blood glucose,[14] in this study they were used to analyze the relationship between level of glycemia and neonatal size. This resulted in three groups: low, ≤86 mg/dl (n = 69); mid, 87 to 104 mg/dl (n = 180); and high, ≥105 mg/dl (n = 85). Data analyses included univariate and bivariate statistical techniques with parametric and nonparametric tests. Categoric data were analyzed with the χ^2 and Fisher's exact tests. Continuous data were analyzed by Student's t test.

Analysis of variance was used to test for differences between the three groups. To determine the extent to which the variables contribute to neonatal size, a stepwise multiple regression was performed. Both tests of significance and the magnitude of the relationships expressed as the variance of the dependent variable that can be accounted for by the independent variable were used to report regression results. Variables contributing 3% of the total variance were considered noteworthy of discussion. In all cases the F values are significant at the 0.01 level or better.

Results

Study sample characteristics. A total of 334 gestational diabetics were identified and participated in this prospective study. They represented a 2.6% incidence of gestational diabetes. The control group was drawn from the group of all women referred for oral glucose tolerance tests from 1985 to 1988; controls were matched for race, obesity, and gestational age at delivery. As shown in Table I, there was a significant difference only in maternal age. Further analysis compared gestational diabetic patient characteristics on the basis of neonatal weight (SGA, appropriate for gestational age (AGA), LGA). The average maternal age was approximately 31 years and was similar for all groups. The mean gestational ages at diagnosis and delivery were comparable. The majority of patients with AGA and LGA infants had been delivered at least once before; the rate of multiparity ranged from 59% to 70%. In contrast, the multiparity rate of the mothers with SGA infants was 38%. Approximately two thirds of patients with LGA infants (71%) were obese and one third (30%) had a previous history of gestational diabetes. This compared to 58% and 11% for SGA, and 49% and 16% for AGA groups, respectively. Characterization of level of glycemic control by means of the ambulatory glucose profile revealed a significant difference between the groups. The mothers with SGA infants were found to have the most stable level of

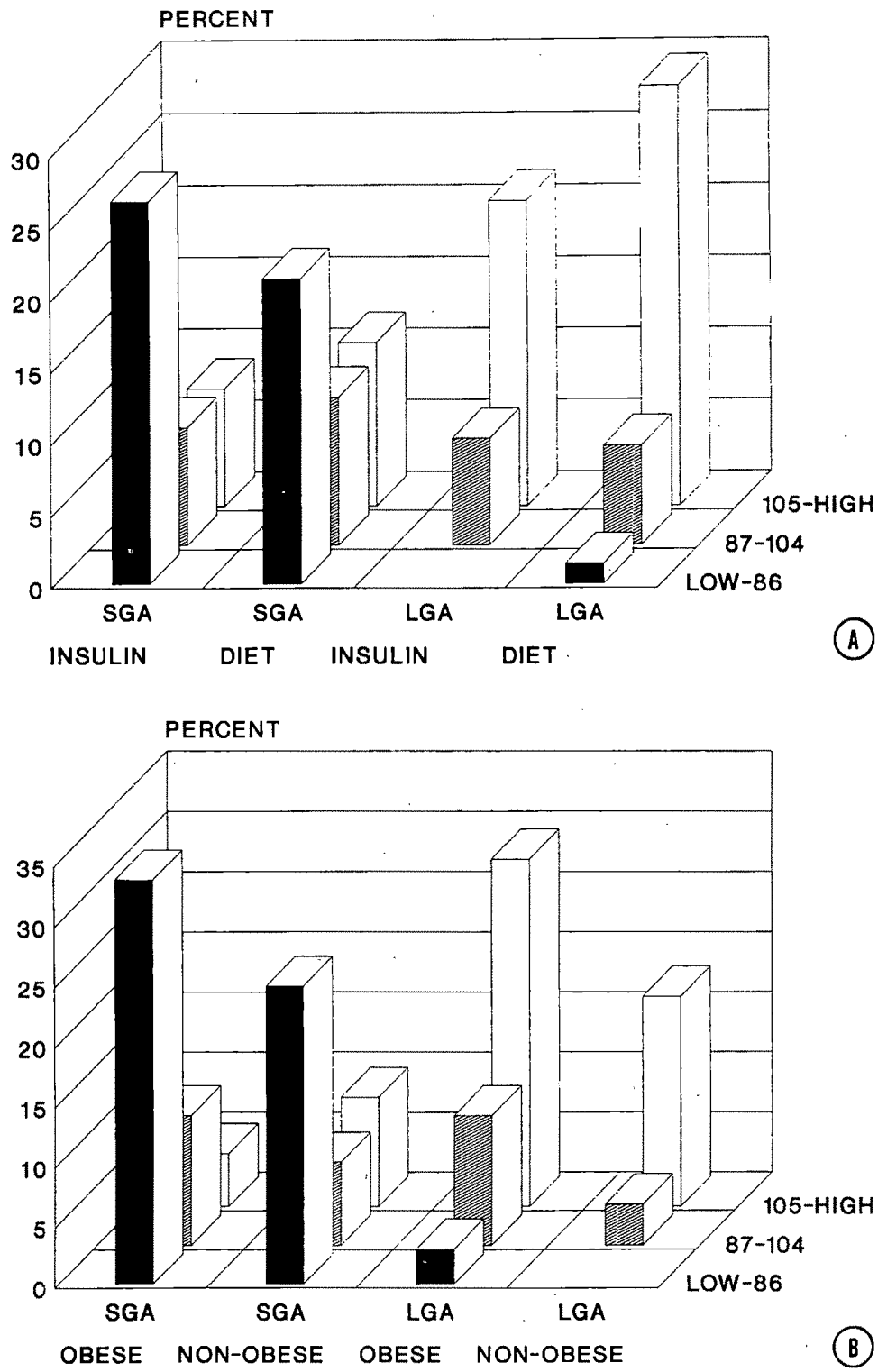


Fig. 2. A, Incidence of LGA, SGA, and treatment modalities. **B,** Incidence of LGA, SGA, and maternal size.

glycemic control (reversals), the lowest mean blood glucose, with a narrow variability indicated by a 27 mg range (Table II).

Association between level of glycemia and neonatal weight. The relationship between level of glycemic control and incidence of SGA and LGA infants is shown in Fig. 1. The relative risk for having an SGA infant in the low (≤86 mg/dl) category (mean blood glucose 81 ± 6 mg/dl) was 2.56 with a 95% confidence interval of 1.24 to 5.29 in comparison with the other groups. No

Table III. Outcome of pregnancy by neonatal size groups

	SGA (n = 38)	*AGA (n = 264)*	*LGA (n = 34)*
Maternal weight change (kg)	12 ± 7*	14 ± 9	17 ± 8
Hypertensive disorder (%)	13	2	3
Gestational age at delivery (wk)	38 ± 2	39 ± 2	39 ± 1
Previous LGA infant (%)	3	17	35
Previous stillbirth (%)	8	12	9
Previous SGA infant (%)	3	6	—
Primary cesarean section (%)	30	13	18
Neonatal birth weight (gm)	2426 ± 291	3231 ± 400	4047 ± 247
Polycythemia (>60%) (%)	5	1	8
Hyperbilirubinemia (>12 mg/dl) (%)	12	4	4
Hypoglycemia (<35 mg/dl) (%)	7	5	8
Hypocalcemia (<7.5 mg/dl) (%)	7	—	4
Respiratory distress syndrome (%)	7	2	—
Neonatal intensive care unit admission (%)	17	6	12

*SGA versus LGA, $p < 0.006$.

significant difference exists in the two mean blood glucose categories (mid, 87 to 104 mg/dl, mean blood glucose 95 ± 5, and high, ≥105 mg/dl, mean blood glucose 114 ± 7 mg/dl) in the incidence of SGA (8.9% vs. 9.4%, respectively).

In contrast, a relative risk of 20.92 (95% confidence interval, 2.73 to 160.42) for developing LGA infants was found for the high mean blood glucose category when compared with the low mean blood glucose category. In addition, a relative risk of 3.95 (95% confidence interval, 1.86 to 8.40) for developing LGA infants was found between the high and the mid mean blood glucose categories. A relative risk of 2.61 (95% confidence interval, 1.20 to 5.69) was found between the mid and low categories.

Association among level of control, maternal size, treatment modality, and neonatal size. Overall 32% (107) of the 334 participants were managed by diet alone and 68% (227) were treated with insulin. The prevalence of SGA and LGA infants by treatment modality for the three categories of level of glycemic control is shown in Fig. 2, *A*. For patients in the mid mean blood glucose range, the proportion of small infants was unrelated to treatment regimen (10% for diet, and 8% for the insulin groups, p = NS). There was a comparable rate of large infants (6.9% and 7.4%, respectively). No significant difference was found between treatment modalities and the incidence of LGA and SGA infants in the low and high categories of mean blood glucose. Within the low mean blood glucose category, the relative risk for developing SGA vs. LGA infants was 17.31 (95% confidence interval of 2.20 to 60.76). The risk for developing LGA infants within the high category was 2.96 (95% confidence interval of 1.22 to 7.17). Each of these categories unilaterally was found to be significantly different from all other categories ($p < 0.001$).

The data were analyzed to determine the relationship between maternal obesity and LGA and SGA infants. Significantly ($p < 0.04$) more LGA and SGA infants were born to obese (14% and 13%) than nonobese mothers (6% and 10%, respectively).

Fig. 2, *B*, demonstrates that the rate of large infants was significantly higher in the high mean blood glucose category regardless of maternal obesity. Furthermore, in the low mean blood glucose category, a trend was observed for a greater disparity for risk of SGA infants regardless of maternal size. No significant difference in weight change during pregnancy in the three mean blood glucose categories was found.

Pregnancy outcome. Comparison between the control and gestational diabetes subjects for the entire study population revealed a higher incidence of hypertensive disorder in the control group (13% vs. 3%, $p < 0.05$, respectively). No significant difference was found in the incidence of hypertension among the three blood glucose categories in the gestational diabetic patients. The mean weight gain change and primary cesarean section rate (13% vs. 14%) showed no difference between the control and the gestational diabetic subjects. In the control group, an overall incidence of 11.7% SGA and 12.2% LGA infants was calculated. Further analysis between control and low (≤86 mg/dl) category showed the highest incidence of SGA infants (11% vs. 20%) with a relative risk of 1.93 (95% confidence interval of 1.03 to 3.75), and the lowest incidence of LGA infants (12.2% vs. 1.4%) relative risk 6.03 (95% confidence interval of 1.28 to 69.5). In the high mean blood glucose category (≥105 mg/dl), an approximately twofold increase was found in the incidence of LGA infants when compared with the control group (relative risk 2.22, 95% confidence interval, 1.23 to 3.99). Finally, no significant difference was

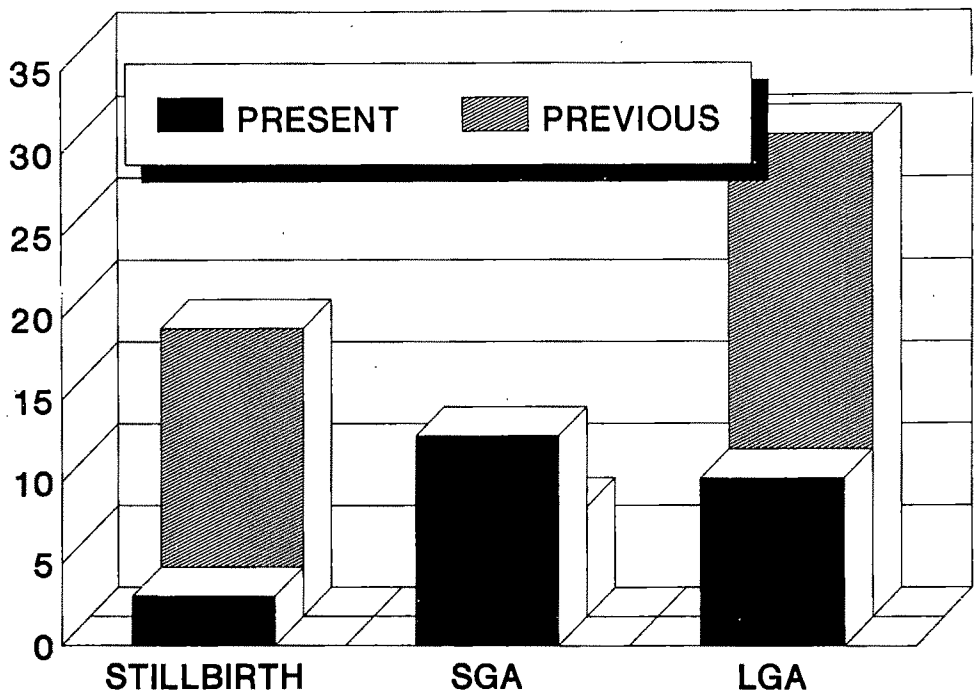


Fig. 3. Pregnancy outcomes: previous versus present pregnancy.

found between the control and mid mean blood glucose category.

The gestational diabetes study population was further stratified on the basis of neonatal weight into three groups: SGA, AGA, and LGA. The neonatal outcomes and selected maternal characteristics by neonatal weight are listed in Table III. Infants were similar with respect to gestational age at delivery (mean, 39 ± 1 weeks) for all patients. Polcythemia was documented in approximately 5% of all neonates, and was most common in the LGA infants ($p < 0.04$).

Hyperbilirubinemia affected 12% of the SGA infants, compared with 4% for each of the other two groups. The incidence of neonatal intensive care unit admission was twofold higher for LGA infants and threefold higher for the SGA infants when compared with AGA infants ($p < 0.03$).

Two stepwise multiple regressions (gestational diabetes and control) were performed to determine the relative contribution of each variable to neonatal size. In the first (gestational diabetes), 21 independent variables were analyzed. Three variables were noteworthy with respect to contribution to neonatal size. Mean blood glucose, weight gain, and gestational age at delivery accounted for 44% of the total variance. Race, parity, hypertension, obesity, and previous large infant, although entered into the regression, did not meet the criteria for inclusion in the regression results. In the second analysis (control), weight gain in pregnancy, body mass index, gestational age at delivery, fasting plasma glucose on glucose tolerance test, and previous LGA infant accounted for 36% of the variance.

Finally, further analysis used patient obstetric history as the comparison for present pregnancy outcome. Previous macrosomia was recorded in 29% of all previous pregnancies. Of these, 78% of the mothers did not have LGA infants in the current pregnancy ($p < 0.02$). There was one stillbirth in this series (3%) of a diet-treated patient at a gestational age of 37 weeks. This is in contrast to an 18% stillbirth rate in previous pregnancies (Fig. 3).

Comment

A key finding in this study is that the incidence of SGA infants of gestational diabetic mothers is significantly related to maternal glucose level. The results further support three distinct mean blood glucose groups: low, ≤86 mg/dl (mean, 81 ± 6 mg/dl) with the highest incidence of IUGR; mid category, 87 to 104 mg/dl (mean, 95 ± 5 mg/dl) comparable to the general population; high category, ≥105 mg/dl (mean, 114 ± 7 mg/dl), which was characterized by the highest rate of LGA infants.

It is widely accepted that complications in the pregnant diabetic (mainly perinatal mortality and congenital malformations) are the consequence of hyperglycemia.[1, 15] However, considerable controversy exists regarding the means for lowering the incidence of perinatal morbidity in mothers with gestational diabetes. The concept that maternal glycemic control can influ-

ence perinatal morbidity was supported by several investigators[14, 15] and refuted by others.[3, 16] The majority of these studies failed to include a control group to reflect a normal population. Additionally, researchers also have debated what criteria constitutes the optimal level of glycemic control. Several key factors contribute to this controversy. Lack of uniformity in the diagnostic criteria creates diverse study populations.

There is inconsistency in the definition of large infants. Some investigators define LGA infants as those with birth weights greater than the 90th percentile, using divergent growth curves, whereas others define macrosomic infants as those weighing >4000 to 4500 gm. The addition of LGA infants contributes to the incidence of infants weighing >4000 gm. Therefore the relatively high proportion of affected infants may remain unrecognized and enhances the scope of the problem.

Furthermore, there is lack of agreement regarding treatment criteria for the patient with gestational diabetes (e.g., insulin initiation) and the appropriate method to verify the accuracy of the glucose data (e.g., frequency, method of testing, and patient compliance). Thus it is problematic to determine the optimal level of glycemia that will maximize pregnancy outcome.[3, 4]

We addressed these issues by maximizing uniformity in our study population by use of the National Diabetes Data Group criteria, adherence to strict calculation and confirmation of gestational age and treatment of all patients with the same protocol using memory-based reflectance meters to assure accurate and reliable data. The standard growth curve comparable to our general population was used. In addition, a matched control from our normal population was chosen to remove selection bias.

The results strongly support the efficacy of stringent metabolic control for the reduction of the incidence of LGA infants. The data further demonstrate that a relationship exists between level of glycemic control and neonatal outcome. The fact that mean blood glucose explained 26% (of total 44% variance) of the contribution to neonatal size further underscores the significance of tight glycemic control. In this group of patients, optimal control was achieved in 75%, and only 25% (high mean blood glucose category) experienced fluctuations in level of control during the study period. This deviation from optimal control for the small number of patients probably accounts for the predictability between mean blood glucose and neonatal size.

The low mean blood glucose category resulted in a high incidence of IUGR (20%). Could this finding be explained by the presence of hypertensive disorder, a factor well known to be associated with SGA infants? The overall incidence of hypertensive disorder in our diabetic subjects was 3%, with the lowest incidence in the low mean blood glucose group (1.6%).

In contrast, the incidence of SGA infants remains virtually the same in the mid and high groups (9% overall). Thus it appears that a mean blood glucose level <87 mg/dl increased the incidence 2.55-fold for the development of IUGR, resulting in a high incidence of neonatal intensive care unit admissions, and a higher incidence of respiratory distress syndrome.

Another concern with previous studies is the failure to analyze separately the obese and nonobese mothers with respect to neonatal outcome. Evaluation of the effect of treatment modalities and maternal obesity on the incidence of SGA infants revealed that the risk appears to be directly related to mean blood glucose levels with two points of demarcation: 87 and 105 mg/dl. The first level signals the risk for IUGR and the second the risk for macrosomia.

Previous studies[3] suggested a high incidence of repeat occurrence of large infants in mothers with a previous history of macrosomia. We found that 78% of the mothers with gestational diabetes who had at least one previous macrosomic infant completed the current pregnancy with an AGA infant. Furthermore, tight verified metabolic control was achieved in 75% of our diabetic patients.

In summary, there is little doubt that absence of insulin in fetal circulation is associated with severe fetal growth retardation. It is also well recognized that macrosomia is a consequence of fetal hyperinsulinemia. Although multiple risk factors are cited as plausible causes for alteration in fetal growth, it appears that level of glycemic control plays a major role. Thus undertreatment and overtreatment of diabetic individuals can result in iatrogenic growth-retarded or macrosomic fetuses. Furthermore, targeting the level of glycemic control to a range of 87 to 104 mg/dl (mean, 95 ± 5 mg/dl) will result in neonatal size comparable to the general population.

REFERENCES

1. Coustan DR. Pregnancy in diabetic women. N Engl J Med 1988;319:1663-5.
2. Mazze RS, Langer O. Primary, secondary, and tertiary prevention: program for diabetes in pregnancy. Diabetes Care 1988;11(suppl 3):263-8.
3. Willman SP, Leveno KJ, Guzick DS, Williams ML, Whalley PJ. Glucose threshold for macrosomia in pregnancy complicated by diabetes. AM J OBSTET GYNECOL 1986;154: 470-5.
4. Gyves MT, Schulman PK, Merkatz IR. Results of individualized intervention in gestational diabetes. Diabetes Care 1980;3:495-6.
5. Pederson J. The pregnant diabetic and her newborn. 2nd ed. Baltimore: Williams & Wilkins, 1977:213.
6. Langer O, Damus K, Maiman M, Divon M, Levy J, Bauman W. A link between relative hypoglycemia-hypoinsulinemia during oral glucose tolerance tests and

intrauterine growth retardation. AM J OBSTET GYNECOL 1986;155:711-16.
7. Sokol RJ, Kazzi GM, Kalhan SC, Pillay SK. Identifying the pregnancy at risk for intrauterine growth retardation: possible usefulness of the intravenous glucose tolerance test. AM J OBSTET GYNECOL 1982;143:220-23.
8. National Diabetes Data Group. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 1979;28:1039-57.
9. Langer O, Mazze R. Diabetes in pregnancy: evaluation of self-monitoring performance and glycemic control with memory-based reflectance. AM J OBSTET GYNECOL 1986; 155:635-7.
10. Mazze RS, Lucido D, Langer O, Hartmann K, Rodbard D. Ambulatory glucose profile: representation of verified self-monitored blood glucose data. Diabetes Care 1987; 10(suppl 1):111-17.
11. Langer O, Anyaegbunam A, Brustman L, Guidetti D, Mazze RS. Gestational diabetes: insulin requirements in pregnancy. AM J OBSTET GYNECOL 1987;157:669-75.
12. Langer O. Scientific rationale for management of diabetes in pregnancy: recent approaches with innovative computer-based technology. Diabetes Care 1988;11(suppl 1):67-72.
13. Brenner WE, Edelman DA, Hendricks CH. A standard of fetal growth for the United States of America. AM J OBSTET GYNECOL 1976;126:555-64.
14. Langer O, Mazze RS. The relationship between large-for-gestational-age infants and glycemic control in women with gestational diabetes. AM J OBSTET GYNECOL 1988; 59:1478-83.
15. Landon MB, Gabbe SG, Piana R, Mennuti MT, Main EK. Neonatal morbidity in pregnancy complicated by diabetes mellitus: predictive value of maternal glycemic profiles. AM J OBSTET GYNECOL 1987;156:1089-95.
16. Lavin JP, Lovelace DR, Miodovnik M, Knowles HC, Barden TP. Clinical experience with one hundred seven diabetic pregnancies. AM J OBSTET GYNECOL 1983;147:742-52.

Make researching easier with the Ten-Year Cumulative Index (1978-1987) to the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY

The Ten-Year Cumulative Index is a complete guide to more than 30,000 pages of original articles, case reports, letters, and editorials published in the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY from 1978 through 1987 (volumes 130 to 157). Numbering more than 800 pages, the Cumulative Index includes both a subject and an author index. This hard-cover volume is $64.50 ($69.00 international). Prices include shipping. *Payment must accompany all orders and must be in U.S. funds, drawn on a U.S. bank.*

Contact The C.V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146 USA; phone (314) 872-8370, ext. 394. In the U.S., call toll-free: 1-800-325-4177, ext. 394. Please allow six weeks for delivery.

Maternal hemoglobin F levels may have an adverse effect on neonatal birth weight in pregnancies with sickle cell disease

Akolisa Anyaegbunam, MD, Henny H. Billett, MD, Oded Langer, MD, Lois Brustman, MD, Claudia Berger, BA, Linda Wyse, MD, Ronald L. Nagel, MD, and Irwin R. Merkatz, MD

Bronx, New York

A total of 26 patients with sickle cell disease were followed up through 32 pregnancies. There was no correlation between days in hospital or number of painful crises and either birth weight or birth weight percentile. The number of dense irreversibly sickled and least deformable cells was negatively correlated with birth weight percentile ($r = -0.63$, $p < 0.01$). Patients' initial hemoglobin levels were positively correlated with birth weight percentile ($r = 0.52$, $p < 0.004$). Hemoglobin F, on the other hand, was significantly inversely correlated with birth weight percentile. Nine pregnancies with small-for-gestational-age infants had an average hemoglobin level of 9.1% ± 4.5%. In contrast, patients who were delivered of appropriate-for-gestational-age infants (23 pregnancies) had an average hemoglobin F level of 3.6% ± 2.9% ($p < 0.01$). We conclude that total hemoglobin levels and dense cells are correlated with birth weight percentile; moreover, the higher the maternal hemoglobin F levels the higher the risk of small-for-gestational-age infants. We speculate that although high hemoglobin levels may be beneficial to the fetus, high maternal hemoglobin F levels could increase the desaturation of non-F cells and induce placental obstruction. (Am J Obstet Gynecol 1989;161:654-6.)

Key words: Sickle cell disease, pregnancy, hematology

Sickle cell disease is a clinically heterogeneous disorder with some patients virtually asymptomatic, whereas others exhibit severe disease.[1] The disease may have an adverse effect on fetal growth and a high prevalence of low birth weight babies has been reported.[2] Although the degree of anemia has been shown to influence birth weight percentile, there is a paucity of information with regard to which, if any, hematologic parameters in sickle cell disease influence neonatal outcome. This study attempts to examine this question by investigating the relationship between several hematologic factors and neonatal birth weight percentile.

Material and methods

All pregnant women with sickle cell disease were initially seen in the first trimester of pregnancy. Prenatal visits were scheduled every 2 weeks until 34 weeks' gestation, and then weekly thereafter. Whenever possible, the hematologic parameters used for analysis were those obtained in the previous nongravid state. If the patient was first seen when pregnant, the values obtained on the earliest visit or well after delivery (>2 months) were used.

Table I. Selected patient characteristics

	Mean ± SD
Maternal age (yr)	26.0 ± 4.8
Birth weight (gm)	2754 ± 463.0
Gestational age at delivery (wk)	38.4 ± 1.8
Hemoglobin (gm/dl)	8.4 ± 1.1
Hemoglobin F (%)	5.8 ± 4.3
Dense cells (%)	13.8 ± 10.0
Hospitalization (days)	30 ± 49
Reticulocyte count (%)	12.6 ± 5.1

Complete blood counts were performed on blood drawn into tubes containing ethylenediaminetetraacetic acid and processed within 8 hours on an automated cell counter (Technicon HI, Tarrytown, N.Y.). Isopyknic red blood cell continuous density gradients were performed by the technique previously described by Fabry and Nagel.[3] Density gradients were photographed and the resultant transparencies were evaluated by densitometry. Four fractions (SS1, SS2, SS3, and SS4) were defined with SS1 representing the lightest cells and SS4 representing the densest fraction of sickle cells and containing the major portion of irreversibly sickled cells.

All patients had solubility studies and hemoglobin electrophoreses performed by both cellulose and agar gel electrophoresis. Hemoglobin F was measured by alkaline denaturation. Reticulocyte counts were per-

From the Departments of Obstetrics and Gynecology and Medicine, Albert Einstein College of Medicine.
This study was supported in part by National Institutes of Health grants HL21016 and HL38655.
Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.
Reprint requests: Akolisa Anyaegbunam, MD, Department of Obstetrics and Gynecology, Room 703, Albert Einstein College of Medicine, 1825 Eastchester Road, Bronx, NY 10461.
6/6/13912

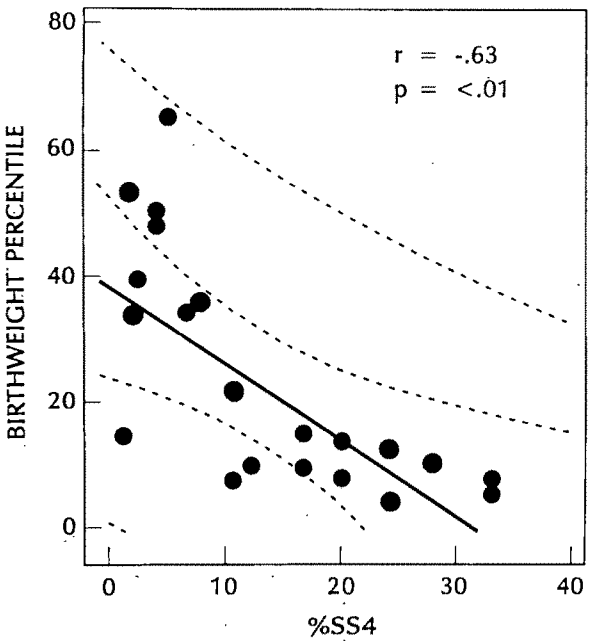


Fig. 1. Regression of maternal percentage of dense cells (SSA) on birth weight percentile.

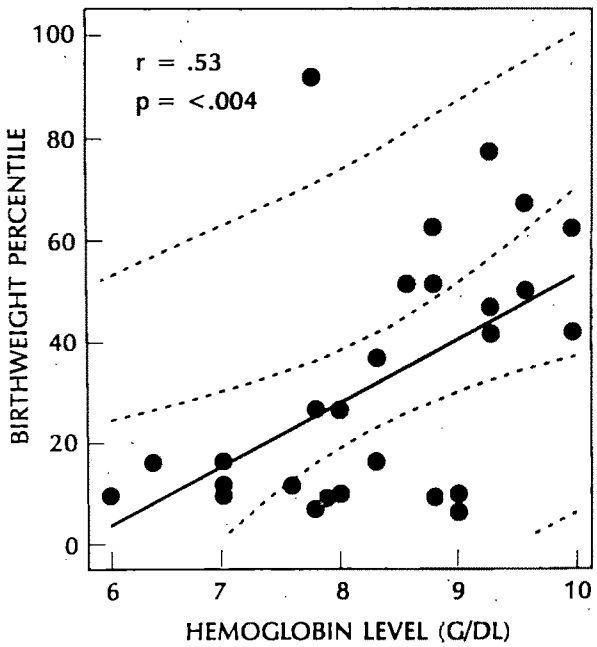


Fig. 2. Regression of initial hemoglobin levels on birth weight percentile.

formed with methylene blue. Bilirubin levels were obtained from routine laboratory serum multichannel analyzers.

Small for gestational age was defined as neonatal birth weight less than the tenth percentile for gestational age.[4]

Distribution statistics, regression analysis, and paired Student *t* tests were performed with a Lotus data base with the Statgraphics statistics program (STSC-PlusWare, Rockville, Md.) on an IBM-AT computer.

Results

A total of 26 patients with sickle cell disease were followed-up through 32 pregnancies. Selected patient characteristics are listed in Table I. The densest and least deformable cells (SS4) were negatively and significantly correlated with birth weight percentile ($r = -0.63$, $p < 0.01$) (Fig. 1). Patients' initial hemoglobin levels were positively and significantly correlated with birth weight percentile ($r = 0.52$, $p < 0.004$) (Fig. 2). Hemoglobin F, on the other hand, was significantly inversely correlated with birth weight percentile ($r = -0.59$, $p < 0.03$) (Fig. 3). Nine of the 32 pregnancies (28%) resulted in small-for-gestational-age births and their mean maternal hemoglobin F level was 9.1 ± 4.5%. In contrast, those patients who were delivered of appropriate-for-gestational-age infants had significantly lower mean hemoglobin F levels 3.6 ± 2.9% ($p < 0.01$).

There was no correlation with days in hospital or number of painful crises and either birth weight or birth weight percentile. In addition, there was no relationship between birth weight percentile and the number of transfusions received during pregnancy, reticulocyte count, mean cell volume, serum bilirubin level or lactic dehydrogenase level.

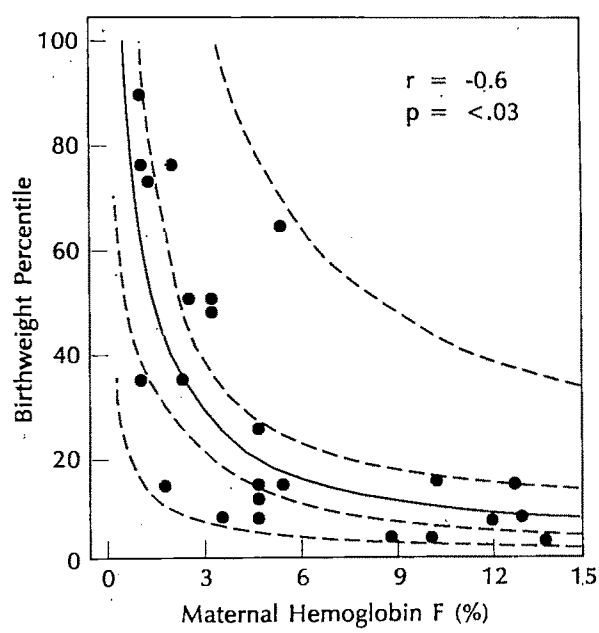


Fig. 3. Regression of maternal hemoglobin F level on birth weight percentile.

Comment

Other investigators have reported that anemic patients tend to have smaller infants with increased risk

of fetal death[5, 6]; hence it is not surprising that pregnant patients with sickle cell disease are delivered of lighter babies. What is surprising is the extent of this positive correlation between birth weight and maternal hemoglobin level, despite the lower oxygen affinity of the sickle hemoglobin secondary to both increase in 2,3-diphosphoglycerate level and intrinsic low affinity of the polymer of hemoglobin S, which should predict better oxygenation than might otherwise be expected for the level of hemoglobin.

Many of the adverse consequences in patients with sickle cell anemia are a result of vasoocclusion induced by sickle cell adhesion and precapillary and capillary obstruction and the subsequent selective trapping of denser sickle cells.[7, 8] In our patient population, the denser and less deformable cells (SS4) were inversely related with neonatal birth weight percentile, which suggests that dense cells may play a role in these phenomena.

The finding that the maternal hemoglobin F level was inversely related to neonatal birth weight was unexpected. High hemoglobin F levels have been associated with decreased hemolysis, higher hemoglobin levels, and fewer dense cells.[9] For most patients with sickel cell anemia, hemoglobin F is unevenly distributed in cells, with some cells containing a large amount of hemoglobin F (F cells) and others with little or no hemoglobin F.[10] Subgroups of patients with sickle cell anemia, the Saudi Arabian and Indian populations and carriers of Senegal haplotype, as well as those with hereditary persistence of hemoglobin F, exist that have higher hemoglobin F values, more F cells, and generally milder disease.[9, 12, 13] It would have been more reasonable to assume that hemoglobin F, with its associated milder disease, would also have a favorable effect on neonatal outcome.

The negative effect of hemoglobin F on birth weight percentile could be explained if the higher hemoglobin F levels in the mother interfere with the ability of the fetus to compete for oxygen at critical stages of development. However, Charache et al.[14] have demonstrated that high affinity hemoglobins with oxygen half-saturation pressure (P 50) levels lower than that of hemoglobin A do not compromise fetal outcome. An alternative explanation is that the delivery of maternal oxygen in the placenta would fall predominantly on the red blood cells that do not contain hemoglobin F because of their lower oxygen affinity. Under these circumstances, and particularly as a result of hemodynamic stasis prevalent in the sinusoids of the placenta, the blood cells that do not contain hemoglobin F would become selectively even more deoxygenated and particularly rigid. The larger the percentage of cells that contain hemoglobin F, the smaller the percentage of low affinity cells and consequently the lower the saturation of these non-F cells. Although fewer, the non-F cells without hemoglobin F in mothers with higher hemoglobin levels will be much more rigid than their counterparts and could induce sludging in general and obstruction of the venular outlets of the sinusoids. This interpretation is in agreement with the data of Charache[14] and our observations of a patient with hereditary persistence of hemoglobin F and 33% hemoglobin F who was delivered of an infant in the 75th percentile. In both instances the hemoglobin is distributed equally in all cells and would not promote selective desaturation of non-F cells. In addition, it may help to explain why transfusion does not appear to be beneficial in pregnancies with sickle cell disease.[2, 15]

We thank Dr. Mary E. Fabry for her help with the gradients, and Kathy Conroy and Fanya Schonbuch for their technical assistance.

REFERENCES

1. Nagel RL, Fabry ME, Billett HH, Kaul DK. Sickle cell painful crises: a multifactorial event. Prog Clin Biol Res 1987;240:361-80.
2. Cunningham FG, Pritchard JA, Mason R. Pregnancy and sickle cell hemoglobinopathies: results with and without prophylactic transfusions. Obstet Gynecol 1983;62:419-24.
3. Fabry ME, Nagel RL. Heterogeneity of red cells in the sickler: a characteristic with practical clinical and physiological implications. Blood Cells 1982;8:9-15.
4. Brenner WE, Ecledman DA, Hendricks CH. A standard of fetal growth for the United States of America. Am J Obstet Gynecol 1976;126:555.
5. McFee JG. Anemia in pregnancy—a reappraisal. Obstet Gynecol Surv 1973;11:769.
6. Desforges J. Anemia complicating pregnancy. J Reprod Med 1973;3:111.
7. Kaul DK, Fabry ME, Nagel RL. Vasoocclusion by sickle cells: evidence for selective trapping of dense red cells. Blood 1986;68:1162-6.
8. Mankad VN, Williams JP, Harpen M, Longnecker CT, Brogdon B, Moore B. Magnetic resonance imaging, percent of dense cells and serum prostanoids as tools for objective assessment of pain crisis: a preliminary report. Prog Clin Biol Res 1987;240:337-50.
9. Billett H, Sutton M, Bouhassira E, Croizat C, Driscoll C, Fabry M, and Nagel RL. Senegal haplotype is associated with less anemia than the other haplotypes in sickle cell anemia. Clin Res 1989;37:377A.
10. Kleihauer E, Braun H, Betke K. Demonstration von fetalem haemoglobin in den erythrocytes eines blutausstrichs. Klin Wochenschr 1957;35:637.
11. Kar BC, Kulosik AE, Sird S, Satapathy RK, Kulosik M, Serjeant BE, Serjeant GR. Sickel cell disease in Orissa State, India. Lancet 1986;2:1198.
12. Conley CL, Weatherall DJ, Richardson SN, Shepard MK, Charache S. Hereditary persistence of fetal hemoglobin: a study of 79 affected persons in 15 negro families in Baltimore. Blood 1963;21:261.
13. Nagel RL, Farry EF, Pagner J, Zohoun I, Wajcman H, Baudin V, Labie D. Hematologically and genetically distinct forms of sickel cell anemia in Africa. N Engl J Med 1985;312:880-4.
14. Charache S, Catalano P, Burns S. Pregnancy in carriers of high affinity hemoglobins. Blood 1985;65:713.
15. Koshy M, Burd L, Wallace D, Moawad A, Baron J. Prophylactic red-cell transfusion in pregnant patients with sickle cell disease. N Engl J Med 1988;319:1447-51.

Pneumonia during pregnancy: Has modern technology improved maternal and fetal outcome?

Nancy E. Madinger, MD,[a] Jeffrey S. Greenspoon, MD,[b] and A. Gray Ellrodt, MD[c]
Los Angeles, California

The maternal death rate and rate of preterm delivery associated with pneumonia during pregnancy have not decreased since the introduction of antibiotics four decades ago. We retrospectively reviewed 25 cases of pneumonia during pregnancy; these cases occurred among 32,179 deliveries. Medical complications included: bacteremia, 16%; empyema, 8%; atrial fibrillation, 4%; respiratory failure necessitating mechanical ventilation, 20%. Obstetric complications occurring during the pneumonia episode included: preterm labor, 44%; preterm delivery, 36%. A patient with cystic fibrosis died; one stillbirth and two neonatal deaths occurred. Underlying maternal disease was significantly associated with maternal medical complications ($p = 0.023$) and preterm delivery ($p = 0.012$). Significant medical and obstetric complications continue to occur frequently despite modern antimicrobial, tocolytic, and supportive therapy. Underlying maternal disease, including acquired immunodeficiency syndrome and cystic fibrosis, was associated with preterm delivery and neonatal and maternal death. (Am J Obstet Gynecol 1989;161:657-62.)

Key words: Pneumonia, pregnancy, complications, preterm delivery

Pneumonia is an infrequent complication of pregnancy, reported to occur in 1 per 118 to 2288 deliveries.[1-4] It has long been recognized to jeopardize the well-being of both the mother and fetus,[1, 2, 5] although maternal complications have decreased markedly throughout this century.[2-4] The introduction of antibiotics dramatically reduced maternal mortality but did not appreciably affect the rate of preterm delivery.[2]

The spectrum of community-acquired pneumonia in adults has recently been expanded to include several "new" pathogens, including *Legionella pneumophila, Pneumocystis carinii,* and *Chlamydia psittaci* TWAR strain.[6, 7] Additionally, an increasing proportion of pneumonias are secondary to gram-negative organisms, while a declining incidence of *Streptococcus pneumoniae* and *Staphylococcus aureus* has been noted.[7]

Women with chronic respiratory diseases and women who are immunocompromised (from infection, malignancy, or chemotherapeutic agents) are now surviving into reproductive years and are attempting pregnancy. Respiratory infections commonly encountered in such patients may lead to an increase in pneumonias during pregnancy, which may differ from the community-acquired pneumonias described in adults. The impact of this group of patients on the incidence and type of pulmonary pathogens in an obstetric population has not been systematically described. Previous studies of pneumonia during pregnancy either were conducted before the recognition of unusual pathogens that cause pneumonias or consisted of isolated case reports.[8-11] We reviewed all cases of pneumonia in pregnant patients admitted to a university-affiliated, community hospital to determine if the natural history of this medical complication of pregnancy has changed in recent years.

Methods and material

The records of 25 patients with a diagnosis of pneumonia and pregnancy admitted to Cedars-Sinai Medical Center (CSMC) from January 1983 to March 1988 were reviewed. This study was approved by the Human Research Review Committee at CSMC. CSMC is an 839-bed community hospital serving both private and health maintenance organization patients. Patients were identified by a computerized search of medical center records accessed by discharge diagnosis according to the International Classification of Diseases, 9th revision, Clinical Modification (ICD-9-CM).

The criteria for inclusion in the study included: (1) clinical syndrome compatible with pneumonia (pulmonary symptoms and/or fever), (2) radiographic evidence of a pulmonary infiltrate, (3) absence of another noninfectious process that would account for the findings, and (4) time course consistent with community-acquired pneumonia.

Twenty-four of the 25 cases of pneumonia during pregnancy were identified by computer search. Cases incorrectly coded or misclassified as pneumonia, i.e., the physician selected a diagnosis not substantiated by

From the Department of Internal Medicine,[a] the Division of Critical Care Medicine, Department of Internal Medicine,[b] and the Division of General Internal Medicine,[c] Cedars-Sinai Medical Center, and the Department of Obstetrics and Gynecology, University of Southern California School of Medicine.[b]
Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.
Reprint requests: Jeffrey S. Greenspoon, MD, Room 5K40, Women's Hospital, 1240 North Mission Road, Los Angeles, CA 90033.
6/6/14294

Table I. Demographic information

	Range	*Mean ± SD*
Age (yr)	15-38	28.5 ± 6.1
Gravidity	1-9	3.6 ± 2.3
Parity	0-5	1.4 ± 1.4
Gestational age at time of admission (wk)	21-39	30.8 ± 5.2
Gestational age at time of delivery (wk)	25-41	35.2 ± 5.0
Maternal hospital stay (days)	3-35	10.0 ± 8.4

the medical record, were not included. One additional case, involving a patient with acquired immunodeficiency syndrome (AIDS), was identified only by alternate sources and is included among the 25 cases.

The charts were abstracted and data entered into the MR-TOD (Retriever Data Systems, Seattle, Washington) data base for analysis. Contingency table analysis was performed with Fisher's exact test or Student's *t* test. All cases were reviewed by two of the authors.

An etiologic agent of pneumonia was assigned if any one of the following criteria were fulfilled: (1) positive blood culture for a known pulmonary pathogen in the absence of another source (when the culture results from blood and sputum were inconsistent, the case was classified according to the blood culture result); (2) sputum culture; (3) viral culture; (4) histologic or cytologic findings from material obtained by bronchoscopy. Patients were categorized as having an unknown cause if a Gram stain was available without confirmatory culture or if sputum culture was incompatible with the clinical course. For example, in a patient whose sputum was colonized with *Haemophilus influenzae,* the illness was cured with erythromycin.

The microbiologic evaluation was deemed inadequate if sputum Gram stain, sputum culture, and blood culture were not obtained. The designation "underlying maternal disease" included patients with asthma, cystic fibrosis, AIDS, and severe pregnancy-induced hypertension.

Results

Demographics. Twenty-five patients with pneumonia during pregnancy were identified. During the 63-month study period there were 32,179 deliveries, yielding an incidence of 0.78 per 1000 deliveries (1 case per 1287 pregnancies). Demographic information is shown in Table I. The racial background included 13 white, 10 black, and 2 Hispanic women. No cases were identified during the first trimester of pregnancy. Underlying maternal disease was present in 6 of 25 (24%) patients: two had asthma; one had cystic fibrosis; one had AIDS; two had severe pregnancy-induced hypertension.

Table II. Pathogenic organisms causing pneumonia during pregnancy

	No.	*%*
Streptococcus pneumoniae	4	16
Haemophilus influenzae	2	8
Pseudomonas aeruginosa	1	4
Influenza A	1	4
Pneumocystis carinii	1	4
No pathogen identified	11	44*
Inadequate microbiologic evaluation	5	20
Total	25	100

*Including two patients with gram-positive cocci in the sputum and a clinical course typical for *Streptococcus pneumoniae* and one patient with viridans *Streptococcus* bacteremia.

Eight patients smoked tobacco and five abused alcohol and/or illicit drugs; only one patient used a parenteral route of drug administration. No identifiable relationship was found between outcome (maternal medical complication or preterm delivery) and the following parameters: age; race; obstetric history; tobacco, drug, or alcohol abuse; or presenting symptoms. Although the mean hospital stay was 10 days, more than half of the patients were discharged within 1 week.

Diagnosis. All patients in the study group had presenting symptoms and/or signs suggesting pneumonia; however, the diagnosis of pneumonia was initially made in only 20 of 25. Two patients had preterm labor with prominent peritoneal signs compatible with an acute condition in the abdomen and underwent emergency appendectomy. In both cases the appendix was normal, and pneumonia was diagnosed as the cause of illness after operation. Pyelonephritis and preterm labor were the initial diagnoses in one patient, and two additional patients had preterm delivery soon after admission and pneumonia was diagnosed the next day. A delay in the diagnosis of pneumonia resulted in inadequate collection of culture specimens, and therefore no pulmonary pathogens were identified in this group.

Microbiology. The cause of pneumonia was determined in 9 patients (Table II). The most common pathogen was *Streptococcus pneumoniae* (four cases); two were identified by sputum culture and two by blood culture. *Haemophilus influenzae* was identified in two patients. In one who had respiratory failure, blood, pleural, and pericardial cultures were positive. This case was reported elsewhere.[12] The single case of *Pseudomonas aeruginosa* pneumonia occurred in a patient with cystic fibrosis who had a long history of similar infections. *Pneumocystis carinii* pneumonia was the initial clinical illness in the patient with AIDS.

Eleven of 25 patients (44%) had no pathogen identified. Serologic studies for causes of atypical pneumonia were completed in only one of these patients.

Two patients were presumed to have *Streptococcus pneumoniae* on the basis of sputum Gram stain, clinical presentation, and response to appropriate antibiotics. Three cases were most consistent with *Mycoplasma pneumoniae,* although confirmatory serologic studies were not done. Although one patient had bacteremia caused by a viridans *Streptococcus,* this organism was not considered to be the pulmonary pathogen. Five patients (20%) had an inadequate microbiologic evaluation. Antibiotics empirically used with a successful clinical cure in patients without an etiologic diagnosis included erythromycin, ampicillin, cefazolin, and cefuroxime. No adverse antibiotic effect was noted in any case.

Maternal complications. All of the major complications occurred in 12 patients (Table III). Severe maternal disease was a significant risk factor for medical complications. Five of 6 (83%) patients with underlying maternal disease developed maternal medical complications of pneumonia, compared with 5 of 19 without underlying maternal disease ($p = 0.023$). Preterm delivery during pneumonia occurred in 2 of 4 women with bacteremia, in 5 of 6 women with mechanical ventilation of the lungs, and in 5 of 6 women with underlying maternal disease.

The single maternal death occurred in a 20-year-old woman, gravida 2, para 0, with one elective abortion; this woman had cystic fibrosis and pulmonary function tests predicted a poor maternal pregnancy outcome.[13] She developed an overwhelming *Pseudomonas* pneumonia with hypoxia and an intractable respiratory acidosis despite maximal ventilatory support. Arterial blood gas values with an inspired oxygen concentration of 60% were pH 7.25, P_{CO_2} 75 torr, P_{O_2} 58 torr, bicarbonate 32 mEq/L, oxygen saturation 84%. At 29 weeks' gestation, a cesarean section was performed during the terminal stage of her illness. Her infant developed hyaline membrane disease and died of *Pseudomonas* sepsis in the neonatal period.

Obstetric complications. Obstetric outcomes in 21 of the 25 patients for whom complete delivery data were available are shown in Fig. 1. Eleven patients (44%) had preterm labor. Preterm labor was common both in patients with underlying maternal illness (2 of 6) and in those without underlying maternal illness (9 of 19). The proportion of patients with preterm labor was not different between groups ($p = 0.66$).

Five of the 11 (45%) had successful tocolysis. Two of these 5 and 2 patients who did not require tocolysis were lost to follow-up and are not included in Fig. 1. Two patients had preterm delivery during a later hospitalization despite successful tocolysis and clinical cure of pneumonia during the initial admission. One of these patients had pyelonephritis, which was cured at the time pneumonia developed; the other had a twin pregnancy and required a cervical cerclage for an incompetent cervix while she was ill with pneumonia. She was delivered of her infant 50 days later with clinical evidence of abruptio placentae.

Table III. Major medical and obstetric complications

Complication	*No. of patients*	*%*
Bacteremia	4	16
Empyema	2	8
Mechanical ventilation	5	20
Pneumothorax	1	4
Pericardial tamponade	1	4
Atrial fibrillation	1	4
Appendectomy	2	8
Maternal death	1	4
Neonatal death	3	12
Preterm delivery during active pneumonia	9	36

Severe maternal disease was a significant risk factor for preterm delivery during the episode of pneumonia. Preterm delivery during the course of pneumonia occurred in 5 of the 6 patients with underlying maternal disease compared with 4 of 19 without underlying maternal disease ($p = 0.012$). Delivery data were unavailable for four patients, including one with underlying disease, asthma.

Although 12 of the febrile patients had fetal tachycardia (fetal heart rate >160 beats/min) when they were initially seen, this appeared to be unrelated to outcome. Fetal distress during the episode of pneumonia was observed in only one case, in a patient with AIDS, who had an intrapartum stillbirth of a 25-week fetus.

Perinatal complications. There was one stillbirth and two neonatal deaths in this series. The perinatal death rate in this study was 3 of 26 (including one set of twins), or 115.4 per 1000 births. The patient with cystic fibrosis whose neonate died was described above. One patient with asthma, who required mechanical ventilation, developed preterm labor and was delivered at 26 weeks' gestation. The neonate died of complications of prematurity.

Comment

Historical perspective. Historically, pneumonia during pregnancy was thought to occur more frequently and to be more severe than in the nonpregnant population. In 1939, Finland and Dublin[1] described pneumococcal pneumonia in the preantibiotic era and noted that 13% of cases of pneumonia occurring in women of reproductive age were among pregnant patients. The incidence of pneumonia among hospitalized obstetric patients was 0.63%. Bacteremia and mortality were highest in the obstetric cases, and maternal mor-

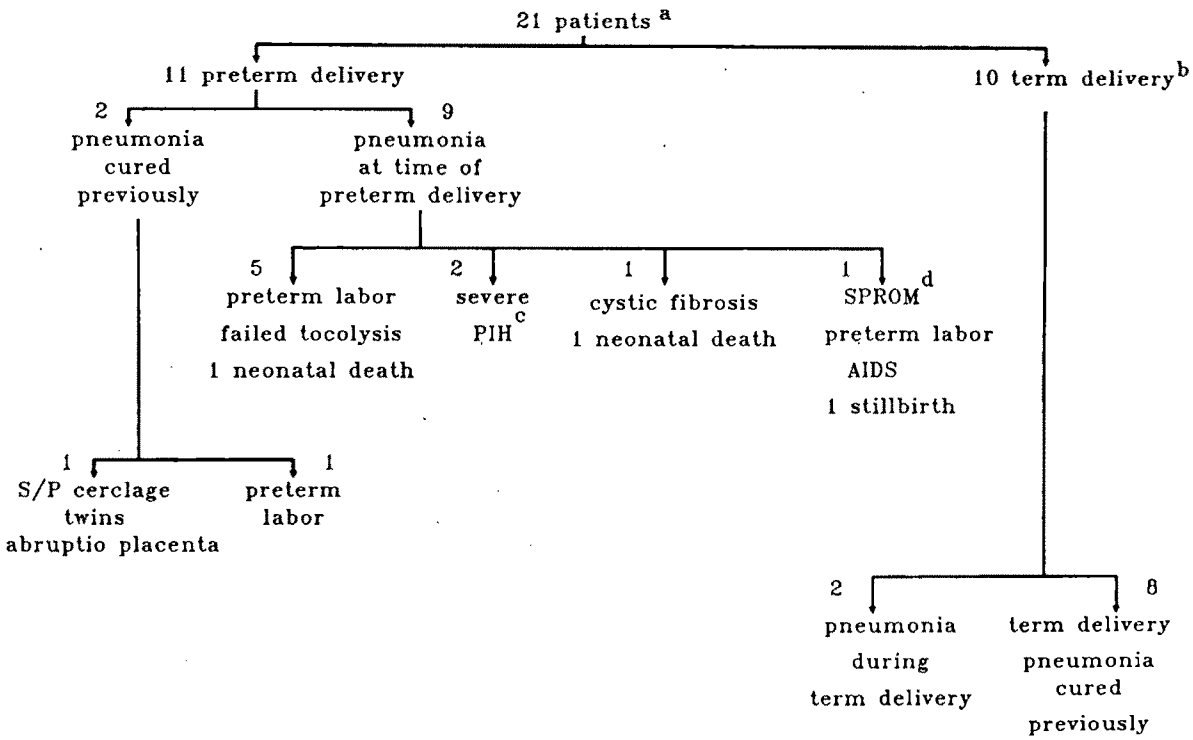


Fig. 1. Flow diagram of the obstetric outcomes of patients with pneumonia during pregnancy. *a*, The figure does not include four patients who were discharged undelivered after the pneumonia was cured and then were lost to follow-up. *b*, Includes one patient who was delivered at 36 weeks during an episode of pneumonia. *c*, Severe pregnancy-induced hypertension was the indication for preterm delivery in both patients. *d*, Spontaneous preterm premature rupture of the membranes occurred during the episode of preterm labor.

tality was greatest in patients who were delivered of their infants or were near term.

Oxorn[2] stated that mortality declined significantly with the advent of antibiotics. However, preterm delivery was unaffected by the availability of antimicrobials and involved nearly half the cases. Mortality was again felt to be increased in those patients who delivered or were near term.

In 1965, Hopwood[3] reported 23 cases from a military-based population and noted an association of tobacco use and the incidence of pneumonia. The Collaborative Perinatal Study[14] provides the largest prospective series of pneumonia during pregnancy. The perinatal death rate was 7 in 119 births (58.8 per 1000 births).

Benedetti et al.[4] reported a decline in the incidence of pneumonia, a reduction in preterm delivery, and a major decrease in maternal mortality in 1982. The overall improvement was attributed to modern obstetric care and a population who presented early in the course of disease and received antibiotics promptly.

Maternal complications. The high complication rate in our series does not differ from rates in other reports published during the modern era (Table IV). Maternal death rates declined after the introduction of antibiotics but have remained essentially unchanged since 1955. The rate of preterm delivery is unaltered over the past three decades. One explanation for this lack of change may be that the population being described is different. Previous studies did not mention patients requiring mechanical ventilation, suggesting that our patients may have been more severely ill. Certainly, at least five patients would have died during the episode of pneumonia if ventilatory support and other critical care modalities had been unavailable.

Moreover, this series included several patients with chronic illness who would not have been part of an obstetric population in the past. We report one death involving a patient with cystic fibrosis who had prognostically ominous Shwachman-Kulczycki clinical scores and pulmonary function tests.[13] She had been counselled about the potentially adverse outcome of pregnancy and had undergone an elective abortion of her first pregnancy. Her decision to continue her second pregnancy resulted in a tragic outcome for both the mother and the neonate. In general, patients with mild to moderately severe cystic fibrosis apparently tolerate pregnancy, and the subgroup at risk for deterioration can be clinically identified and counseled before conception.[13]

Table IV. Comparison of the incidence of pneumonia during pregnancy and the rates of preterm delivery and maternal death

	No. of pneumonia cases (per No. of obstetric admissions)	*Incidence (per No. of deliveries)*	*Preterm delivery*		*Maternal deaths*	
			No.	*%*	*No.*	*%*
Finland and Dublin,[1] 1939	164/25,891	1/158	32/74	43	18/74	24
Oxorn,[2] 1955 (before antibiotics)	35/ND	ND	9/19	47	7/35	20
Oxorn, 1955 (after antibiotics)	29/ND	ND	11/23	48	1/29	3.5
Hopwood,[3] 1955	23/2720	1/118	ND		2/23	8.7
Collaborative Perinatal Study,[14] 1972	119/39,215	1/330	ND		ND	
Benedetti et al.,[4] 1982	39/89,219	1/2288	3/20	15*	0/39	0
Current series	25/32,179	1/1287	9/21	43*	1/25	4
Subtotals						
Preantibiotic era	NC	NC	41/93	44†	25/109	23
Antibiotic era	206/163,333	1/793	23/64	36†	4/116	3.4

NC, Not calculable; *ND,* no data reported.
*Not statistically different ($p = 0.085$).
†Not statistically different ($p = 0.308$).

The patient with AIDS survived her first episode of *Pneumocystis carinii* pneumonia as well as pregnancy, in contrast to most case reports of maternal AIDS in pregnancy.[9-11] Additional maternal deaths caused by pneumonia in pregnancy may occur as concomitant infection with the human immunodeficiency virus becomes more prevalent in our society. Preterm labor and failure of tocolysis are common in pregnant patients with AIDS and active pulmonary disease. Perimortem cesarean section in terminally ill patients may be appropriate under some circumstances.

Obstetric complications. Previous reports described the rate of preterm delivery but not that of preterm labor (although the two rates may have been similar before the routine use of effective tocolytic agents). Although Benedetti et al.[4] noted that preterm delivery was less frequent in their series than in previous reports they did not state if this was the result of the use of tocolytic therapy. In our study 11 of 25 patients (44%) had preterm labor; only 5 of 11 (45%) had successful tocolysis. The reason for the high rate of preterm labor associated with pneumonia or other infections outside of the reproductive tract is not well understood. The cervicovaginal microflora and/or the host's inflammatory response to infection have been implicated as a cause of preterm labor. One postulated mechanism is the local production of prostaglandins.[15] A similar inflammatory response to infection in the lung might produce prostaglandins that could circulate to the uterus and produce preterm labor. It is conceivable that pneumonia triggers a process that leads to preterm labor early in the course of pneumonia and is not affected by antibiotics or tocolytics.[16]

Perinatal complications. The perinatal death rate of 115.4 per 1000 births is higher than that reported by the Collaborative Perinatal Study despite major technologic advances in diagnosis, monitoring, supportive care, and treatment of premature neonates. The two neonatal deaths in this series resulted from complications of preterm delivery. The stillbirth occurred in a 25-week fetus; intervention was considered inappropriate in this case because of immaturity. All three perinatal deaths occurred in pregnancies complicated by underlying maternal disease, pneumonia, and preterm delivery.

Study design. Although all the cases of pneumonia during pregnancy that we identified were reviewed, we recognize the limitations of retrospectively retrieving cases via ICD-9-CM codes. For this reason we searched alternate sources and were able to identify only one additional case. We were aware of several cases that were also identified by the search. There were no missing medical charts of the pneumonia episode, which might have resulted in misleading conclusions.[17]

The retrospective design of this and other reports of pneumonia may provide a biased description of the etiologic agents causing pneumonia because there may be variation in the completeness of the microbiologic evaluation. There was no systematic attempt to determine the cause of atypical pneumonia in our series of patients. The proportion of patients without an identifiable agent in our study, 64%, is higher than the proportion reported in prospective studies of nonpregnant adults, as frequently as 57%.[18] As in the study of Benedetti et al.,[4] the most common pathogen in our series was *Streptococcus pneumoniae.* Even the frequency

of pneumococcal pneumonia may be underestimated because this is a fastidious organism that may not grow from the sputum culture and therefore would not be identified unless seen on Gram stain or identified from blood cultures. Conversely, there is a danger of falsely attributing the cause of pneumonia to *Streptococcus pneumoniae* because the sputum of 35% to 60% of normal individuals may be colonized with this organism.[19] In view of changing trends in the causes of adult pneumonias, it would be best to interpret our results in terms of the wide range of pathogens that cause pneumonia during pregnancy but not to assign a specific frequency to each agent.[7]

Only one pregnant patient received outpatient treatment of pneumonia before admission. Because preterm labor is a frequent complication of pneumonia, initial in-patient evaluation and treatment may be prudent. The evaluation might include Gram stain and culture of sputum,[20] blood cultures, skin testing for tuberculosis and coccidioidomycosis, serologic tests for respiratory pathogens, and viral cultures. More aggressive methods of obtaining a diagnosis, such as bronchoscopy, are warranted in some patients. Until a prospective study of pregnant patients is undertaken, empiric antibiotic coverage should be based on data collected from the nonpregnant population with similar underlying diseases.

Although the incidence of pneumonia during pregnancy remains low, significant morbidity occurs in many of these cases, especially if the mother suffers from underlying illness. In 40% of the cases reviewed in this series, patients experienced potentially life-threatening complications such as sepsis, respiratory failure, and cardiac tamponade. In addition, preterm labor and delivery remain a major problem, despite the use of modern tocolytic agents.

REFERENCES

1. Finland M, Dublin TD. Pneumococcic pneumonias complicating pregnancy and the puerperium. JAMA 1939; 112:1027-32.
2. Oxorn H. The changing aspects of pneumonia complicating pregnancy. AM J OBSTET GYNECOL 1955;70:1057-63.
3. Hopwood HG. Pneumonia in pregnancy. Obstet Gynecol 1965;25:875-9.
4. Benedetti TJ, Valle R, Ledger WJ. Antepartum pneumonia in pregnancy. AM J OBSTET GYNECOL 1982; 144:413-7.
5. Ransdell RC. Pneumonia and pregnancy. Am Med 1905; 9:237-9.
6. Marrie TJ, Grayston JT, Wang S-P, Kuo C-C. Pneumonia associated with the TWAR strain of *Chlamydia*. Ann Intern Med 1987;106:507-11.
7. Fick RB, Reynolds HY. Changing spectrum of pneumonia—news media creation or clinical reality? Am J Med 1983;74:1-8.
8. Soper DE, Melone PJ, Conover WB. Legionnaire disease complicating pregnancy. Obstet Gynecol 1986;67:10S-2S.
9. Wetli CV, Roldan EO, Fojaco RM. Listeriosis as a cause of maternal death: an obstetric complication of the acquired immunodeficiency syndrome (AIDS). AM J OBSTET GYNECOL 1983;147:7-9.
10. Jensen LP, O'Sullivan MJ, Gomez-del-Rio M, Setzer ES, Gaskin C, Penso C. Acquired immunodeficiency (AIDS) in pregnancy. AM J OBSTET GYNECOL 1984;148:1145-6.
11. Minkoff H, deRegt RH, Landesmann S, Schwarz R. *Pneumocystis carinii* pneumonia associated with acquired immunodeficiency syndrome in pregnancy: a report of three maternal deaths. Obstet Gynecol 1986;67:284-7.
12. Weingarten S, Weinberg H, Fang M, Meyer R. *Hemophilus influenzae* pericarditis in two adults. West J Med 1986; 145:690-4.
13. Palmer J, Dillon-Baker C, Tecklin JS, et al. Pregnancy in patients with cystic fibrosis. Ann Intern Med 1983;99:596-600.
14. Niswander KR, Gordon M, eds. The women and their pregnancies: The Collaborative Perinatal Study of the National Institute of Neurological Diseases and Stroke. Philadelphia: WB Saunders, 1972:230-3.
15. MacGregor JA. Prevention of preterm birth: new initiatives based on microbial-host interactions. Obstet Gynecol Surv 1988;43:1-14.
16. Ellrodt AG. Sepsis and septic shock. Emerg Med Clin North Am 1986;4:809-40.
17. Westgren M, Divon M, Greenspoon J, Paul R. Missing hospital records: a confounding variable in retrospective studies. AM J OBSTET GYNECOL 1986;155:269-71.
18. White RJ, Blainey AD, Harrison KJ, et al. Causes of pneumonia presenting to a district general hospital. Thorax 1981;36:566-70.
19. Barrett-Connor E. The nonvalue of sputum culture in the diagnosis of pneumococcal pneumonia. Am Rev Respir Dis 1970;103:845-8.
20. Murray PR, Washington JA II. Microscopic and bacteriologic analysis of expectorated sputum. Mayo Clin Proc 1975;50:339-44.

Comparison of predelivery versus postdelivery Kleihauer–Betke stains in cases of fetal death

John Owen, MD, Charles M. Stedman, MD, and Tommy L. Tucker, MT(ASCP)
Birmingham, Alabama

A prospective study was undertaken to determine whether the delivery process could introduce a significant amount of fetal cells into the maternal circulation in patients with a fetal death. Sixty-six cases of fetal death were studied over a 39-month period. All patients had both predelivery and postdelivery Kleihauer-Betke stains performed. Of these, three (4.6%) had a massive fetomaternal hemorrhage. None of the postdelivery stains showed evidence of a significant fetomaternal hemorrhage unless results of the antepartum stain had also been positive. We conclude that the delivery process itself does not simulate a massive fetomaternal hemorrhage in cases of fetal death. (AM J OBSTET GYNECOL 1989;161:663-6.)

Key words: Fetomaternal hemorrhage, fetal death, Kleihauer-Betke stain

The association between massive fetomaternal hemorrhage and poor perinatal outcome has been well described.[1,2] Fetomaternal hemorrhage has been reported to occur in patients with various obstetric complications, but may also occur without a recognizable antecedent event. Nevertheless the exact pathophysiology of this disorder remains obscure. Previous series[3] have reported that the incidence of massive fetomaternal hemorrhage in otherwise unexplained cases of fetal death is as high as 14%, while the incidence of fetal death from massive fetomaternal hemorrhage is <0.1%.[2] However, most series included patients who only had a postpartum fetal cell stain. Hence, fetal cells could have entered the maternal circulation during delivery. We therefore sought to determine whether the delivery process could simulate a massive fetomaternal hemorrhage by introducing fetal cells into the maternal circulation after the death had occurred.

Material and methods

Since November 1985 a protocol has been in effect for the evaluation of women with a fetal death at ≥24 weeks' gestational age. This evaluation includes a fetal cell stain of the maternal blood before and after delivery. Fetal cell stains were performed using the Simmler acid elution kit according to manufacturer directions. A minimum of 5000 cells on each slide were counted, and positive control slides were made with each maternal sample batch by means of stored cord blood samples to assure adequate stain efficiency.

The predelivery maternal blood samples were collected into vials containing ethylenediaminetetraacetic acid at the time of diagnosis (n = 4), at the time of admission for delivery (n = 56), or both (n = 6). The postpartum samples were collected in similar fashion within 4 hours of delivery. All samples were processed and interpreted by one of the physician-authors within 72 hours of collection. If processing could not be performed immediately after collection, the blood was stored at 3° C.

In each case clinical information from the antepartum, labor, delivery, and postpartum records was reviewed by one of the physician-authors for factors that may have contributed to the fetal death. If a direct cause-and-effect relationship could be established (i.e., massive abruptio placentae), the case was deemed certain. If complications that may have caused the fetal death were present, the case was termed possible (i.e., hypertensive disease), and if no cause or association could be found, the case was assigned an unknown classification. Since autopsies, placental microscopic examinations, and genetic studies were encouraged but inconsistently obtained, this information was not used in the clinical assignments of causality.

To estimate the magnitude of the fetomaternal hemorrhage expressed as a proportion of the total fetoplacental blood volume, the fetoplacental blood volume was calculated with a combination of the formula established by Morris[1]—Fetoplacental blood volume = 162 ml/kg—and the birth weight. Then the volume of fetal cells in the maternal circulation was calculated by use of the following formula[3]:

From the Department of Obstetrics and Gynecology and the Department of Clinical Pathology, University of Alabama at Birmingham.
Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.
Reprint requests: John Owen, MD, Department of Obstetrics and Gynecology, University of Alabama at Birmingham, Birmingham, AL 35294.
6/6/13911

Table I. Identified causes and associations of fetal death

Certain	
True knot	2
Massive abruptio placentae	6
Acute fatty liver	1
Uterine rupture	1
	10
Possible	
Presumed cord accident	2
Maternal hypertension	11
Growth retardation or oligohydramnios	9
Substance abuse	2
Collagen disease	1
Diabetes mellitus	2
Fetal congenital anomaly	1
Extreme postterm	1
	29
Uncertain	
No cause identified	27

$$\frac{\text{No. of fetal cells}}{\text{No. of maternal cells}} \times 2400 = \text{Volume of fetal cells in maternal circulation (ml)}$$

Assuming a nominal baseline fetal hematocrit of 50%, the proportion of the estimated fetoplacental blood volume from an acute hemorrhage into the maternal circulation is twice the volume of fetal cells detected from the fetal cell stain divided by the estimated fetoplacental blood volume. Although this is only a crude estimate because of the assumptions made in the calculations, it may serve as a gauge of the severity of the hemorrhage in a more meaningful fashion than the simple calculation of the volume of fetal cells in the maternal circulation.

When appropriate, data are expressed as the mean ± 1 SD. Confidence limits for binomial proportions are derived from the exact computational method.

Results

A total of 66 patients entered the study. The mean maternal age was 24 years (range, 13 to 40 years) with a racial distribution of 62% black and 38% white women. There were 35 primiparous and 31 multiparous women. The mean gestational age at diagnosis was 34 weeks; 13 patients were between 24 and 30 weeks, 31 were between 31 and 36 weeks, and the remaining 22 women were ≥37 weeks' gestation. Sixty-four of the 66 patients came either to the clinic for their obstetric care or to the labor suite for acute care and were found to have a fetal demise. One patient suffered a fetal death on the antepartum ward, and there was one intrapartum fetal death.

Ten patients were assigned to the certain group, 29 to the possible group, and 27 to the unknown group. The causes and associations discovered for those in the last two groups are listed in Table I. Of the 27 patients with an unknown classification, three (11.1%, 95% confidence limits 2 and 27) had evidence of a massive fetomaternal hemorrhage on their antepartum stains.

The first patient, a 28-year-old, para 0-0-2-0, white female woman, was seen near term with unreliable gestational criteria, spontaneous contractions, and absent fetal movement for 2 days. An ultrasonographic examination documented the absence of fetal cardiac activity. Except for smoking less than 10 cigarettes a day during her pregnancy, no antepartum risk factors could be established. At the initial cervical examination, the cervix was dilated 5 cm and labor was complicated by amnionitis. A fetal cell stain on admission showed an 8.7% fetomaternal hemorrhage, equivalent to 209 ml of fetal cells or approximately 160% of estimated fetoplacental blood volume. In addition, the rhesus typing returned Du positive. Because of the magnitude of the fetomaternal hemorrhage, we postulated that rhesus-positive cells from the fetus may have caused the Du factor to appear falsely positive. Therefore she was treated with eight vials of Rh immune globulin. The postpartum stain showed 6.6% fetal cells. Rhesus typing and an indirect Coombs test repeated 1 year later demonstrated that she was Du-negative and had not been become sensitized from the transfusion of rhesus-positive fetal cells. She was delivered of a 2070 gm female stillborn infant with no obvious anomalies. An autopsy was declined, and no placental pathologic examination was performed. No cause for the fetal death could be established.

The second patient was a 23-year-old, para 0-0-1-0, rhesus-positive, white woman, who was seen at 36 weeks' gestation with absent fetal movement for 7 days. An ultrasonographic examination confirmed the absence of fetal cardiac activity. The only identifiable risk factor was maternal smoking of <20 cigarettes per day. Labor was induced with oxytocin, and she was delivered of a macerated, but otherwise normal-appearing, 2785 gm male stillborn infant. The admission fetal cell stain showed a fetomaternal hemorrhage of 3.8% corresponding to 91 ml of fetal blood or approximately 40% of the estimated fetoplacental blood volume; the postpartum stain showed 2% fetal cells. An autopsy was declined, but placental pathologic examination revealed hemorrhagic endovasculitis, which has been shown to be associated with poor perinatal outcomes[6]; however, a consistent association with fetomaternal hemorrhage has not yet been established.

The third patient was a 21-year-old, para 1-0-0-1, rhesus-positive, black woman who was seen at 42 week's gestation complaining of absent fetal movement for 1 day. After ultrasonographic confirmation of the fetal death, she was discharged home to await spontaneous

labor. Two days later she was delivered of a 2850 gm female stillborn infant with no obvious anomalies. An autopsy and placental pathologic examination failed to reveal another cause for the fetal death. The fetal cell stain at diagnosis showed 7.1% fetal cells. This was equivalent to 170 ml of fetal cells, or approximately 74% of the estimated fetoplacental blood volume. The fetal cell stains performed 2 days later on admission and again post partum showed 4.8% and 6.1% fetal cells, respectively.

A total of 63 patients demonstrated no evidence of a significant fetomaternal hemorrhage (<10 fetal cells per 5000 maternal cells), and these were considered as negative. Therefore in an unselected series of 66 patients with a fetal death at ≥ 24 weeks' gestation, three (4.6%, 95% confidence limits 2 and 12) were attributable to a massive fetomaternal hemorrhage. The incidence of the delivery process simulating a massive fetomaternal hemorrhage was 0 in 63 cases (0%, 95% confidence limits 0 and 6).

Comment

Although the exact cause of massive fetomaternal hemorrhage remains poorly defined, its association with perinatal morbidity and mortality has been well documented. A number of newer methods have become available for the detection of fetal cells in the maternal circulation, but the acid elution stain still remains a popular technique.[7] Although its use has been questioned for the precise quantification of fetomaternal hemorrhage, it is rapidly performed with commercially available reagents, and it has been well described in the obstetric literature.

Although it has generally been accepted that the finding of a massive fetomaternal hemorrhage meant that the cause of fetal death was simple exsanguination, this has never been conclusively demonstrated. However, evidence that strongly suggests this as the cause includes the reports of live-born infants with severe anemia and abnormal fetal heart tracings in association with massive fetomaternal hemorrhage.[1, 8] However, the possibility that fetal cells may enter the maternal circulation in large quantities after fetal death has already occurred has never been adequately studied. We were unable to demonstrate this effect in any of our five patients who had a negative antepartum stain at diagnosis and another stain performed at the time of admission for delivery. Similarly, the effects of delivery on the process of fetomaternal hemorrhage after fetal death has remained a viable question. After fetal death has occurred normal clotting mechanisms are activated, autolysis commences, and the hydrostatic pressure in the fetal compartment is lost. We postulate that these factors preclude the entry of a large quantity of fetal cells into the maternal circulation.

Although the precise definition of the term massive fetomaternal hemorrhage remains elusive, its effects on the fetus are probably a result of both the magnitude and chronicity of the blood loss as well as the current fetoplacental blood volume. The finding of 25 ml of fetal red blood cells in the maternal circulation is more significant at 20 weeks' gestation (the entire estimated fetoplacental blood volume) than at 40 weeks' gestation (10% of the fetoplacental blood volume). We would estimate that an acute hemorrhage of 20% or more of the fetoplacental blood volume could be clinically significant, but the minimum hemorrhage required to cause fetal death is not known. A review of six case reports of fetal death from fetomaternal hemorrhage[3, 9] demonstrates a range from 23% to 51% of the estimated fetoplacental blood volume at the time of sample collection, whereas in cases of maternal trauma and poor perinatal outcome, the percentage of the estimated fetoplacental blood volume found in the maternal circulation was between 14% and 34%.[10] However, important variables that can affect the estimation of fetal blood loss with fetal cell stains include the rate of entry of fetal cells into the maternal circulation and the rate at which the fetal cells are cleared, particularly when an ABO or rhesus incompatibility exists. Since the exact time of fetal death can seldom be established, the possibility exists that an exsanguinating hemorrhage could occur and the fetal cells be destroyed before a fetal cell stain is performed. These topics have never been adequately addressed in previous studies on fetomaternal hemorrhage and fetal death. Currently there are insufficient data in the literature to establish the optimum management of a pregnancy complicated by a fetomaternal hemorrhage in which fetal death has not also occurred, although an intrauterine transfusion might be considered if signs of fetal decompensation are present.[11]

We conclude that the incidence of massive fetomaternal hemorrhage in cases of fetal death is low and that the delivery process itself does not simulate a fetomaternal hemorrhage by introducing fetal cells into the maternal circulation. We recommend that a fetal cell stain be obtained in all cases of fetal death to establish the diagnosis of a massive fetomaternal hemorrhage. In addition, careful microscopic examination of the placenta may also be of value and needs further study. However, few data exist to determine the prognosis of future pregnancies in those women who have suffered this condition.

REFERENCES

1. Fliegner JRH, Fortune DW, Barrie JU. Occult fetomaternal haemorrhage as a cause of fetal mortality and morbidity. Aust NZ J Obstet Gynaecol 1987;27:158.
2. Van de Putte I, Renan M, Vermylen C. Counting fetal

erythrocyte as a diagnostic aid in perinatal death and morbidity. Am J Obstet Gynecol 1972;114:850.
3. Laube DW, Schauberger CW. Fetomaternal bleeding as a cause for "unexplained" fetal death. Obstet Gynecol 1982;60:649.
4. Morris JA, Hustead RF, Robinson RG, et al. Measurement of fetoplacental blood volume in the human previable fetus. Am J Obstet Gynecol 1974;118:927.
5. Molleson PL. Quantitation of transplacental hemorrhage. Br Med J 1972;3:31.
6. Sander CH, Kinnane L, Stevens NG, Echt R. Haemorrhagic endovasculitis of the placenta: a review with clinical correlation. Placenta 1986;7:551.
7. Scott JR, Warenski JC. Tests to detect and quantitate fetomaternal bleeding. Clin Obstet Gynecol 1982;25:277.
8. Clark SL, Muller FC. Sinusoidal fetal heart rate pattern associated with massive fetomaternal transfusion. Am J Obstet Gynecol 1984;149:97.
9. Fay RA. Feto-maternal haemorrhage as a cause of fetal morbidity and mortality. Br J Obstet Gynaecol 1983;90:443.
10. Rose PG, Strohm PL, Zuspan FP. Fetomaternal hemorrhage following trauma. Am J Obstet Gynecol 1985;153:844.
11. Cardwell MS. Successful treatment of hydrops fetalis caused by fetomaternal hemorrhage: a case report. Am J Obstet Gynecol 1988;158:131.

Uterine rupture: Are intrauterine pressure catheters useful in the diagnosis?

M. Hellen Rodriguez, MD, Damon I. Masaki, MD, Jeffrey P. Phelan, MD, and Fidelia G. Diaz, MA

Los Angeles, California

The usefulness of the intrauterine pressure catheter in the diagnosis of uterine rupture was assessed by review of 76 cases of uterine rupture, 39 of which were monitored with an intrauterine pressure catheter. The classic description of a loss of intrauterine pressure or cessation of labor was not observed in any of the patients. However, an increase in baseline intrauterine pressure was observed in four patients with an intrauterine pressure catheter. The increase in pressure was associated with severe variable decelerations such that by itself the intrauterine pressure catheter added little to the diagnosis of uterine rupture. (Am J Obstet Gynecol 1989;161:666-9.)

Key words: Uterine rupture, intrauterine pressure catheter

The intrauterine pressure catheter has the advantage of providing the clinician with quantitative measurements of uterine resting tone as well as the duration and intensity of contractions.[1] Because loss of intrauterine pressure and cessation of labor are thought to be classic signs of uterine rupture,[2] it has been taught that an intrauterine pressure catheter would be helpful in making the diagnosis. As a consequence of this and the fear of possible uterine rupture in patients who undergo a trial of labor after a prior cesarean section, it has become common practice in many institutions to monitor these patients with an intrauterine pressure catheter.

From the Department of Obstetrics and Gynecology, University of Southern California School of Medicine, Los Angeles County—University of Southern California Medical Center.
Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.
Reprint requests: M. Hellen Rodriguez, MD, San Gabriel Valley Perinatal Medical Group, 1135 S. Sunset Ave., Suite 402, West Covina, CA 91793.
6/6/13715

Uterine rupture has been extensively reviewed by several authors.[3-6] However, review of the literature shows there is a scarcity of data that support or disclaim the use of the intrauterine pressure catheter in patients at risk of rupture. In this article the value of the intrauterine pressure catheter in making the diagnosis of uterine rupture is assessed.

Material and methods

This is a retrospective study that consisted of chart and monitor strip review of all cases of uterine rupture at the Los Angeles County–University of Southern California Women's Hospital between June 1979 and April 1988. The definition that was used for uterine rupture was that of Plauche et al., "cases of complete separation of the wall of the pregnant uterus with or without expulsion of the fetus, which endangered the life of the mother and/or fetus. Asymptomatic uterine scar dehiscences were excluded."[6]

The patients in the study were divided into three groups on the basis of the type of uterine monitoring

Table I. Maternal characteristics of each study group

	Group A (n = 8): No uterine monitor	*Group B (n = 29): External tocometer*	*Group C (n = 39): IUPC*
Maternal age (yr)	31.3 ± 7.1*	28.5 ± 7.2*	29.9 ± 6.2*
Parity			
0	1:8 (13%)	1:29 (3%)	0:39 (0%)
1	1:8 (13%)	8:29 (28%)	18:39 (46%)
2	1:8 (13%)	9:29 (31%)	13:39 (33%)
3	1:8 (13%)	4:29 (14%)	3:39 (8%)
≥4	4:8 (50%)	7:29 (24%)	5:39 (13%)
Gestational age (wk)	35.8 ± 4.8*	38.3 ± 2.6*	39.8 ± 2.0*
Previous cesarean section	4:8 (50%)	24:29 (83%)	32:39 (82%)
Use of oxytocin	0:8 (0%)	6:29 (21%)†	25:39 (64%)
Use of epidural anesthetic	0:8 (0%)	4:29 (14%)	10:39 (25%)

IUPC, Intrauterine pressure catheter.
*Values are mean ± SD.
†Statistically significant difference when compared with group C, $p < 0.05$.

Table II. Diagnostic signs and symptoms of uterine rupture

	Group A (n = 8): No uterine monitor	*Group B (n = 29): External tocometer*	*Group C (n = 39): IUPC*
No sign or symptom	1:8 (13%)	0:29 (0%)	2:39 (5%)
Fetal death	3:8 (38%)*†	1:29 (3%)	1:39 (3%)
Pain	5:8 (63%)*†	4:29 (14%)	5:39 (13%)
Hemorrhage	1:8 (13%)	5:29 (17%)	7:39 (18%)
Fetal distress	4:8 (50%)	23:29 (79%)	32:39 (82%)
Decreased baseline uterine pressure	NA	NA	0:39 (0%)
Loss of uterine contraction	NA	0:29 (0%)	0:39 (0%)
Recession of presenting part	NA	2:29 (7%)	2:39 (5%)

IUPC, Intrauterine pressure catheter.
NA, Not applicable.
*Statistically significant difference when compared with group B, $p < 0.05$.
†Statistically significant difference when compared with group C, $p < 0.05$.

they received. Group A did not have uterine monitoring; these patients went to surgery within 30 minutes of entering the hospital because of fetal or maternal compromise. Patients in group B were monitored with an external tocodynamometer and patients in group C were monitored with a standard open-ended intrauterine pressure catheter. The three groups were compared in terms of fetal and maternal outcome as well as diagnostic signs and symptoms of uterine rupture with χ^2 analysis (or Fisher's exact test for total 2×2 table frequencies <40), one-way analysis of variance, or the Kruskal-Wallis rank test depending on the scale of measurement. After an overall significant result was achieved, pairwise comparisons were conducted with appropriate multiple comparison procedures with $\alpha = 0.05$.

Results

During the study period there were 138,853 deliveries at Women's Hospital. A total of 84 patients (one case per 1653 deliveries) met the definition for uterine rupture. There were 8 patients who were not included in the study because of failure to obtain the complete charts with the monitor strip. Of the 76 patients included in the study, eight patients went directly to surgery (group A), 29 patients had external tocodynamometry only (group B), and 39 had an intrauterine pressure catheter (group C).

Table I presents the maternal characteristics of each group. There is no statistically significant difference between groups with respect to admission characteristics. As expected, no patients in group A received oxytocin or epidural anesthetics because they went immediately to surgery. The number of patients who received oxytocin is significantly higher in group C than that of group B. This is a consequence of our protocol, which strongly encourages the use of an intrauterine pressure catheter in patients who receive oxytocin. The use of epidural anesthetics or the history of a prior cesarean section was not statistically different between the groups.

Table II presents the signs and symptoms that led to the suspicion of uterine rupture. In three patients there was no suspicion of rupture and the diagnosis was made at the time of cesarean section for other indications. One patient in group A who underwent an elective

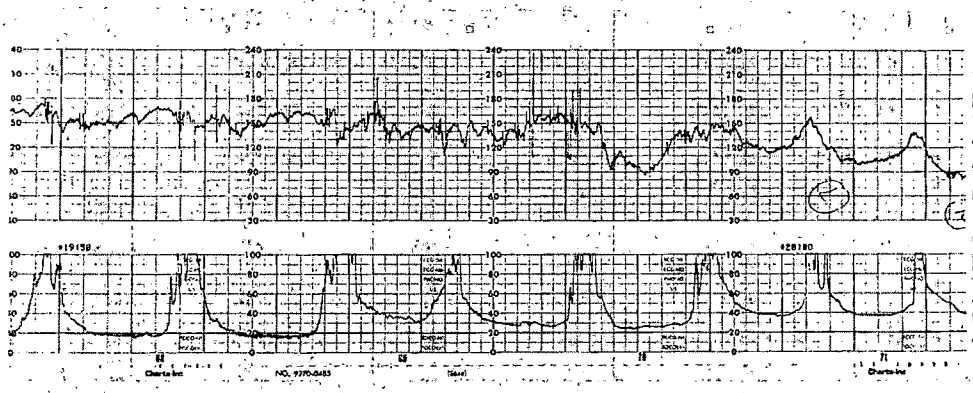


Fig. 1. Monitor tracing demonstrates fetal heart rate decelerations, increase in uterine tone, and continuation of uterine contractions in patient with uterine rupture monitored with intrauterine pressure catheter.

Table III. Maternal and fetal outcome

	Group A (n = 8): No uterine monitor	*Group B (n = 29): External tocometer*	*Group C (n = 39): IUPC*
Intrauterine or neonatal death	3:8 (38%)*,†	1:29 (3%)	2:39 (5%)
1-minute Apgar score‡			
0-3	5:8 (63%)	6:29 (21%)	9:39 (23%)
4-6	2:8 (25%)	5:29 (17%)	9:39 (23%)
≥7	1:8 (13%)	18:29 (62%)	21:39 (54%)
5-minute Apgar score‡			
0-3	3:8 (38%)	2:29 (7%)	4:39 (10%)
4-6	3:8 (38%)	4:29 (14%)	2:39 (5%)
≥7	2:8 (25%)	23:29 (79%)	33:39 (85%)
Birth weight (gm)	3000 ± 917§	3139 ± 621†,§	3526 ± 543§
Estimate blood loss (ml)	2887 ± 1281*,†,§	1416 ± 995§	1752 ± 921§
Hysterectomy	3:8 (38%)	7:29 (24%)	10:39 (25%)
Maternal death	1:8 (13%)	0:29 (0%)	1:39 (3%)

IUPC, Intrauterine pressure catheter.
*Statistically significant difference when compared with group B, $p < 0.05$.
†Statistically significant difference when compared with group C, $p < 0.05$.
‡Statistically significant difference when group A is compared with either group B or C, $p < 0.05$.
§Values are mean ± SD.

repeat cesarean section because of a previous classical uterine incision was found to have a prolapsed fetal arm through a rupture of the prior incision. Two patients in group C met criteria for failure to progress and at the time of cesarean section were incidentally found to have ruptured uteri. The most common sign or symptom to uterine rupture in all groups was fetal distress that preceded pain and hemorrhage. None of the patients including those with an intrauterine pressure catheter, demonstrated a loss of uterine tone or cessation of labor. It is of interest that four patients with an intrauterine pressure catheter demonstrated an increase in baseline uterine tone (Fig. 1) from an average of 15 to 35 mm Hg at the time of diagnosis.

Table III presents the maternal and fetal outcomes in each of the groups. The mortality and morbidity for both the infant and the mother are higher in group A and are not significantly different between groups B and C. Therefore the use of the external tocodynamometer versus the intrauterine pressure catheter did not appear to affect the neonatal or maternal outcomes.

Comment

Loss of uterine tone and cessation of labor have been thought to be classic signs in making the diagnosis of uterine rupture. Because the intrauterine pressure catheter provides the clinician with quantitative measurements of uterine activity, its use has been advocated in patients who are at increased risk of uterine rupture such as patients who undergo a trial of labor after a previous cesarean section.

In this study we reviewed diagnostic signs and symptoms of uterine rupture in 76 cases. None of the patients with or without an intrauterine pressure catheter had a decrease in uterine resting tone or cessation of labor. It is noteworthy that four of 39 patients (10%)

with an intrauterine pressure catheter showed an increase of uterine baseline tone. Whether this increase in tone caused or resulted from the uterine rupture remains unanswered. In all four cases the increase in tone was accompanied by severe variable decelerations that led to immediate cesarean delivery. Therefore the information obtained by the intrauterine pressure catheter was not helpful in making the diagnosis of uterine rupture in any of the patients in our study.

In conclusion:

1. The classic description of a decrease in uterine tone or the cessation of labor at the time of uterine rupture was not observed.

2. There is no difference in fetal or maternal morbidity or mortality when an intrauterine pressure catheter is used versus an external tocodynamometer in patients with uterine rupture.

3. Fetal distress occurred in 78% of cases and was the most common finding in uterine rupture.

4. The usefulness of the intrauterine pressure catheter in making the diagnosis of uterine rupture is not supported by this study.

REFERENCES

1. Paul RH. Fetal heart rate and uterine activity monitoring: what every practitioner must know about the equipment. In: Mishell DR, Brenner PF, eds. Management of common problems in obstetrics and gynecology. 2nd ed. New Jersey: Medical Economics Company Inc., 1988:228-33.
2. Huff RW. Postpartum hemorrhage. In: Pauerstein CJ, ed: Clinical obstetrics. New York: J Wiley and Sons, 1987: 927-8.
3. Schrinsky DC, Benson RC. Rupture of the pregnant uterus: a review. Obstet Gynecol Surv 1978;33:217-32.
4. Golan A, Sandbank O, Rubin A. Rupture of the pregnant uterus. Obstet Gynecol 1980;56:549-54.
5. Eden RD, Parker RT, Gall SA. Rupture of the pregnant uterus: a 53-year review. Obstet Gynecol 1986;68:671-4.
6. Plauche WC, Almen MV, Muller R. Catastrophic uterine rupture. Obstet Gynecol 1984;64:792-7.

Correction

In the article by Salvador and Koos, "Effects of regular and decaffeinated coffee on fetal breathing and heart rate" (Am J Obstet Gynecol 1989;160:1043-7), Fig. 1 on page 1044 was incorrect as published. The correct illustration is shown here.

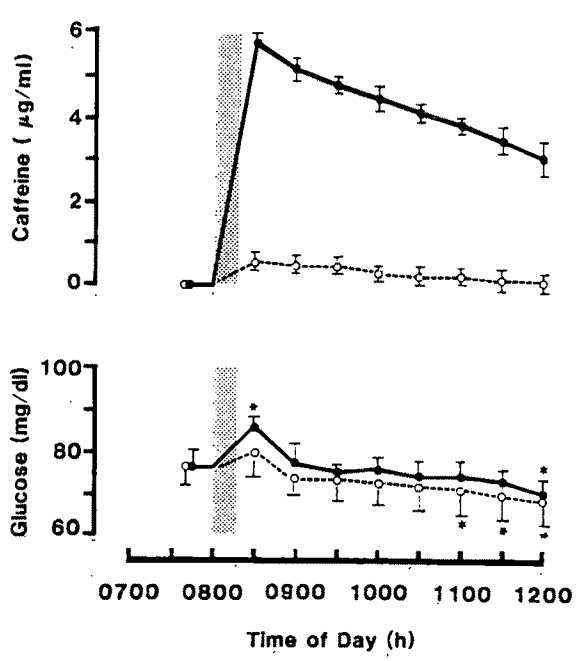


Fig. 1. Serum caffeine and glucose concentrations with regular (—) or decaffeinated (——) coffee. *Shaded vertical bar* represents mean time of coffee consumption. *$p < 0.05$.

The effect of continuous epidural analgesia on cesarean section for dystocia in nulliparous women

James A. Thorp, MD,[a] Valerie M. Parisi, MD, MPH,[b] Peter C. Boylan, MB,[a] and Dennis A. Johnston, PhD[c]

Houston, Texas

Epidural analgesia in labor is generally accepted as safe and effective and therefore has become increasingly popular. However, little is known regarding the effect of epidural analgesia on the incidence of cesarean section for dystocia in nulliparous women. During the first 6 months of 1987 we studied 711 consecutive nulliparous women at term, with cephalic fetal presentations and spontaneous onset of labor. Comparison of 447 patients who received epidural analgesia in labor with 264 patients who received either narcotics or no analgesia was performed. The incidence of cesarean section for dystocia was significantly greater ($p < 0.005$) in the epidural group (10.3%) than in the nonepidural group (3.8%). There remained a significantly increased incidence ($p < 0.005$) of cesarean section for dystocia in the epidural group after selection bias was corrected and the following confounding variables were controlled by multivariate analysis: maternal age, race, gestational age, cervical dilatation on admission, use of oxytocin, duration of oxytocin use, maximum infusion rate of oxytocin, duration of labor, presence of meconium, and birth weight. The incidence of cesarean section for fetal distress was similar ($p > 0.20$) in both groups. There were no clinically significant differences in frequency of low Apgar scores at 5 minutes or cord arterial and venous blood gas parameters between the two groups. This study suggests that epidural analgesia in labor may increase the incidence of cesarean section for dystocia in nulliparous women. (Am J Obstet Gynecol 1989;161:670-5.)

Key words: Epidural, labor, cesarean section, dystocia, nullipara

The rising cesarean section rate, from 5% to 25% over the past 25 years, has resulted in major cost increases and higher maternal morbidity and mortality without proved benefit to the mother or newborn. One of the major factors in the cesarean "epidemic" is the rise in the incidence of cesarean section for dystocia in nulliparous women. Vaginal delivery after cesarean birth is performed in only a small minority of cases nationwide, and therefore the rise in cesarean section for dystocia in nulliparous women is responsible for a rise in repeat cesarean section in multiparous women. Thus cesarean section for dystocia in nulliparous women represents the single most important factor in the cesarean "epidemic."

The use of epidural labor analgesia also has increased dramatically over recent years, and the effect of epidural analgesia on the incidence of cesarean section is controversial. In the large body of literature concerning epidural analgesia in labor, there are suprisingly few controlled studies that address the effects of epidural analgesia on cesarean section rates.[1-4] One controlled, retrospective study demonstrated an increased incidence of cesarean section associated with epidural analgesia in labor.[1] To our knowledge, there are no controlled studies that specifically examine the effect of epidural analgesia on the incidence of cesarean section for dystocia in nulliparous women. Studying an exclusively nulliparous population is important since nulliparous women, in contrast to mutigravid ones, are especially predisposed to dystocia.[5,6] The purpose of this study is to assess the effects of epidural analgesia in labor on the incidence of cesarean section for dystocia in nulliparous women.

From the Division of Maternal-Fetal Medicine, Department of Obstetrics, Gynecology, and Reproductive Sciences,[a] and Department of Pediatrics,[b] University of Texas Medical School and the M. D. Anderson Cancer Center.[c]

Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.

Reprint requests: James A. Thorp, MD, St. Luke's Perinatal Center, 4400 Wornall Road, Kansas City, MO 64111.

6/6/14312

Material and methods

In the first 6 months of 1987, 711 consecutive nulliparous women with cephalic fetal presentations and singleton gestations were managed according to the principles of active management of labor.[5] Labor was diagnosed by regular, painful uterine contractions and cervical effacement and usually was accompanied by either bloody show or rupture of the membranes. Patients presenting with fetal distress were not included in this study. After the diagnosis of labor was established, amniotomy was performed. If 2 cm of cervical dilatation did not occur within 2 hours of amniotomy, oxytocin augmentation was initiated. Oxytocin was administered by controlled intravenous infusion (model

960, IMED Corp., San Diego) of 20 units diluted in 1 L of lactated Ringer's solution. Infusion was begun at 4 mU/min, was increased by 4 mU/min every 15 minutes, and did not exceed a maximum dose of 36 mU/min. If uterine activity exceeded a frequency of seven contractions in 15 minutes, the oxytocin infusion rate was not increased.

Patients were offered either narcotic agents or epidural analgesia if they requested assistance with pain relief during labor. Epidural analgesia was provided by an obstetric anesthesia department, with 24-hour in-house staffing. Each patient received an intravenous bolus infusion of 1000 ml of lactated Ringer's solution before induction of epidural analgesia. When the cervix was dilated to at least 3 cm, the patient was placed in the lateral decubitus or sitting position and an epidural catheter was placed via the L2-3 or L3-4 interspace and advanced 2 to 3 cm cephalad. Epidural analgesia was not routinely administered at 3 cm of cervical dilatation but was administered thereafter, on request by the patient. Initial bolus injection of bupivacaine 0.25% was then followed by a continuous infusion of 6 to 12 ml/hr of a 0.125% solution. Epidural infusions were adjusted to maintain a dermatomal level of T10-12. Continuous epidural infusion was continued in the second stage of labor as long as the patient achieved progress. Continuous electronic fetal monitoring was performed in all patients. Arterial and venous umbilical cord blood samples were obtained immediately after delivery and placed on ice. The samples for cord blood gas measurement were obtained by preheparinized 3 ml syringes (Marquest Medical products, Englewood, Colo.) to obtain a consistent concentration and preparation of heparin.

The following factors were compared in the epidural and nonepidural groups by means of χ^2 analysis or Student's t test as appropriate: maternal age, race, gestational age, cervical dilatation on admission, duration of labor, number of patients requiring oxytocin augmentation, duration of oxytocin use, maximum dose of oxytocin required, type of delivery, birth weight, frequency of 5-minute Apgar scores <7, and frequency of low umbilical cord pH values. The incidences of cesarean section for dystocia and for fetal distress were compared by χ^2 analysis by the Yates correction when appropriate. One-way analysis of variance and Scheffé test for groups with significant differences were used to compare the birth weights in the following four analgesia subgroups: those having no analgesia in labor, those having narcotic analgesia only, those having epidural analgesia only, and finally those having both epidural and narcotic analgesia.

For the purpose of definition in this study, cesarean sections for dystocia included those performed for arrest of the active phase in the first stage of labor, arrest of descent in the second stage of labor, i.e., fetus in persistent occipitoposterior position, minor deflexion or asynclitism, and cephalopelvic disproportion. Multivariate analysis was then performed with the linear logistic regression analysis technique,[7] and the following factors were controlled: maternal age, race, gestational age, cervical dilatation on admission, use of oxytocin, duration of oxytocin use, maximum infusion rate of oxytocin, total duration of labor, presence of meconium, and birth weight. There were six patients who received epidural analgesia after an established abnormal rate of dilatation (<1 cm/hr) and subsequently underwent cesarean section for dystocia. To address the selection bias even further, these six patients were deleted from the epidural group, and the incidence of cesarean section for dystocia was again compared in the epidural and nonepidural groups, both by χ^2 analysis and by multivariate analysis as described above. Data base and statistical analysis was assisted by computer software (Symphony, Lotus Development Corp., Cambridge, Mass.; Abstat, Anderson-Bell, Parker, Colo.; SPSS, SPSS Inc., Chicago).

Results

Of the 711 consecutive nulliparous women at term with spontaneous onset of labor, 142 (20%) received no analgesia in labor, 122 (17%) received narcotic analgesia only, 378 (53%) received epidural analgesia only, and 69 (10%) received epidural and narcotic analgesia. The epidural group consisted of 447 patients receiving only epidural or epidural combined with narcotic analgesia, and the nonepidural group consisted of 264 patients having either no analgesia or narcotic analgesia. The epidural and nonepidural groups are compared in Table I. Patients in the nonepidural and epidural groups had similar ages, gestational weeks, and racial background (45% black, 36% white, and 14% Hispanic).

Oxytocin augmentation was used in 397 (55.8%) of the 711 patients in this study. There was a significantly greater frequency ($p < 0.05$) of oxytocin augmentation in the epidural group (73%) than in the nonepidural group (27%). The duration of oxytocin use, maximum oxytocin dose, duration of labor, and birth weights were significantly greater ($p < 0.005$) in the epidural group than in the nonepidural group. Cesarean section for fetal distress occurred with similar frequency ($p > 0.20$) in both groups. The incidence of cesarean section for dystocia in the epidural group (10.3%) was significantly greater ($p < 0.005$) than that in the nonepidural group (3.8%). When macrosomic infants (≥4000 gm) were removed from the nonepidural and epidural groups, there remained a significant increase in incidence of cesarean section for dystocia in the nonepidural group (8 of 253 [3.2%] vs. 36 of 402 [9.0%], $p < 0.002$).

Multivariate analysis, controlling for the 10 factors listed above, demonstrated a significant increase in in-

Table I. Comparison of nonepidural and epidural groups

	Nonepidural group (n = 264)	*Epidural group (n = 447)*	*p Value**
Maternal age (yr)	23.1 ± 5.4	23.8 ± 5.4	NS
Gestation (wk)	39.5 ± 1.2	39.7 ± 1.2	NS
Cervical dilatation on admission (cm)	4.8 ± 2.6	3.0 ± 1.4	<0.05
Oxytocin augmentation	71 (27%)	326 (73%)	<0.05
Duration of oxytocin (hr)	3.1 ± 1.7	5.0 ± 3.0	<0.05
Maximum oxytocin (mU/min)	14.2 ± 8.4	17.9 ± 9.6	<0.05
Birth weight (gm)	3255 ± 456	3400 ± 466	<0.05
Total duration of labor (hr)	4.7 ± 2.8	8.6 ± 3.1	<0.05
Cesarean section for fetal distress	5 (1.9%)	9 (2.0%)	NS
Cesarean section for dystocia	10 (3.8%)	46 (10.3%)	<0.05†
	10 (3.8%)	40 (9.1%)	<0.05†
5 min Apgar score < 7	5 (1.9%)	2 (0.4%)	NS
Cord arterial pH	7.24 ± 0.07 (n = 233)	7.24 ± 0.07 (n = 414)	NS
Cord venous pH	7.32 ± 0.06 (n = 242)	7.32 ± 0.06 (n = 431)	NS
Cord arterial pH ≤ 7.15	17/233 (7.3%)	39/414 (9.4%)	NS
Cord venous pH ≤ 7.25	28/242 (11.6%)	39/431 (9.0%)	NS

Values represent a proportion or mean ± SD.

*Represents p value by Student's t, χ^2, or Fisher's exact test, as appropriate, and *NS* represents $p < 0.10$.

†p Value by linear logistic regression technique controlling for possible confounding variables. In the second comparison (3.8% vs. 9.1%) six patients were excluded because epidural analgesia was given after an abnormal labor pattern was established.

cidence ($p < 0.005$) of cesarean section for dystocia in the epidural group when compared with the nonepidural group. Six cases of cesarean section for dystocia were removed from the epidural group as the epidural anesthetic was placed during or after abnormal labor occurred. There remained a significant increase ($p < 0.005$) in incidence of cesarean section for dystocia in the epidural group (9.1%) when compared with the nonepidural group (3.8%) by multivariate analysis controlling the same factors.

Meconium-stained amniotic fluid during labor occurred in similar frequency ($p > 0.20$) in the nonepidural (20%) and epidural (23%) groups. The cord arterial pH value (mean ± SD) in the nonepidural group (7.24 ± 0.07; $n = 232$) was similar to that of the epidural group (7.24 ± 0.07; $n = 414$). Likewise, the cord venous pH value (mean ± SD) in the nonepidural group (7.32 ± 0.06; $n = 241$) was similar to that of the epidural group (7.32 ± 0.06; $n = 431$). All of the other cord arterial and venous blood gas parameters including P_{CO_2}, P_{O_2}, bicarbonate, and base deficit were also similar in both the nonepidural and epidural groups. Both the nonepidural and epidural groups had similar proportions of 5-minute Apgar scores <7, cord arterial pH values ≤7.15, and cord venous pH values ≤7.25 (Table I).

These four analgesia subgroups were studied separately: patients having no analgesia, patients having narcotic analgesia, patients having epidural analgesia, and finally patients having both epidural and narcotic analgesia (Table II). Comparison of the birth weights in the four analgesia subgroups by analysis of variance resulted in an F test of 5.80 ($p = 0.0006$). Scheffé test demonstrated a significant difference between patients receiving narcotics only and those with epidural analgesia only ($p = 0.02$) and between those receiving narcotics only and those receiving epidural analgesia and narcotics ($p = 0.03$). There was no significant difference in birth weight between those patients receiving no analgesia versus those receiving only epidural analgesia, yet there was a significant increase in cesarean section for dystocia in the latter group (3.5% vs. 10.3%, $p < 0.01$). The incidence of cesarean section for dystocia in the various subgroups with and without macrosomic newborns (≥4000 gm) is depicted in Table II.

Comment

The incidence of cesarean section for dystocia in the epidural group was significantly greater than that in the nonepidural group, despite correction for selection bias and controlling for confounding variables by multivariate analysis. This study suggests that epidural analgesia in labor may increase the risk of cesarean section for dystocia in nulliparous women. This finding is especially significant in our institution since oxytocin is used aggressively in a large proportion of our nulliparous women (55.8% in this study) according to the principles of active management of labor.[5] Active management of labor was introduced to our institution in 1985 and significantly ($p < 0.05$) reduced the total cesarean section rate from 25% to 20%, without altering major perinatal outcome variables.[6] This decrease in the total cesarean section rate was entirely due to the

Table II. Comparison of the analgesia subgroups

	No analgesia	*Narcotic analgesia*	*Epidural analgesia*	*Epidural and narcotic analgesia*
Cesarean section for dystocia*	3.5% (5/142)	4.1% (5/122)	10.3% (39/378)	10.1% (7/69)
Cesarean section for dystocia,† birth weight < 4000 gm	3.0% (4/135)	2.6% (3/117)	9.4% (32/342)	6.7% (4/60)
Birth weight‡ (gm)	3272 ± 450	3237 ± 464	3392 ± 465	3448 ± 472

All values are expressed as proportions or mean ± SD.

*A χ^2 analysis demonstrates a significant difference between the following subgroups: no analgesia versus epidural; no analgesia versus epidural and narcotic; narcotic versus epidural; narcotic versus epidural and narcotic ($p < 0.05$).

†A χ^2 analysis demonstrates a significant difference between the following subgroups: no analgesia versus epidural narcotic versus epidural ($p < 0.05$).

‡Analysis of variance and Scheffé test show a significant difference in birth weight between the following subgroups: narcotic versus epidural ($p = 0.03$); narcotic versus epidural and narcotic ($p = 0.02$). No difference in birth weight was noted between the no analgesia versus the epidural subgroups, yet there was significant increase in cesarean section for dystocia between these subgroups ($p < 0.01$).

decrease in incidence of cesarean section for dystocia in nulliparous women from 15% to 10% ($p < 0.05$). However, the implementation of active management of labor in our institution was only partially successful, as it did not decrease the incidence of cesarean section to the 5% range as it has remained at the National Maternity Hospital in Dublin.[5] There have been many possible explanations for our "partial success." One argument considers the fact that the Dublin population remains essentially homogeneous, in contrast to our multiracial population. However, Turner et al.[8] in England have recently introduced active management of labor in a multiracial population and achieved cesarean section rates approaching those in Dublin.[5] Another argument suggests that lack of adherence to the strict principles of active management of labor may be responsible for the "partial success" in Houston. However, Akoury et al.[9] also achieved cesarean section rates approaching those in Dublin, without the benefit of an "experienced Irish hand," such as we had in Houston (P. C. B.). Approximately 63% of our parturient women received epidural analgesic for labor, in contrast to only 5% in Dublin,[5] 25% in the study of Akoury et al.,[9] and 31% in the study of Turner et al.[8] These observations are consistent with the findings of this study; i.e., epidural analgesia in labor may increase the incidence of cesarean section for dystocia.

There are many mechanisms by which epidural analgesia could increase the incidence of cesarean section for dystocia. A decrease in uterine activity[10, 11] without appropriate intervention with oxytocin could result in more cesarean sections for dystocia. The allegation that oxytocin causes birth asphyxia and other adverse perinatal outcomes is one of the more common medicolegal claims in obstetrics in the United States.[12] Therefore, despite recent data demonstrating the safety of oxytocin,[13] it is often inappropriately withheld because of medicolegal concerns. Relaxation of the pelvic diaphragm could predispose fetuses in a cephalic presentation to minor malpresentations such as occipitoposterior, deflexion, and asynclitism.[10, 14, 15] In addition, epidural analgesia could decrease the maternal stimulus and ability to push during the second stage.[10, 15] Several studies[14] have associated epidural analgesia with increased rates of instrument-assisted vaginal delivery. In the current medicolegal climate, the obstetrician is less likely to intervene with forceps, and therefore an increased incidence of cesarean section for dystocia could result.

The popular belief that epidural analgesia does not increase the incidence of cesarean section is not clearly established in the literature. In fact, Diro and Beydoun[1] found that epidural analgesia was associated with an increased incidence of cesarean section. In their study the authors[1] retrospectively compared 43 parturient women of mixed parity receiving epidural analgesia with 43 matched control women and found a significant increase in the incidence of cesarean section for dystocia in the epidural group. One retrospective analysis[2] suggested that epidural analgesia has no effect on the incidence of cesarean section during a 10-year interval in which the incidence of epidural use rose from 0% to 40%. However, it is not clear how this conclusion was made since the incidence of cesarean section rose from 6.48% to 11.03% during the same time period. McNeill and Wilson[16] noted no increase in total cesarean section rate in 500 consecutive patients receiving epidural analgesia in labor when compared with the total obstetric population. However, their study[16] did not assess the incidence of cesarean section for dystocia and did not investigate an exclusively nulliparous population. Several observational studies reported cesarean section rates in large series of patients with epidural analgesia; however, no comparisons with control groups were made.[17-19] In a controlled study Studd et al.[20] found no effect of epidural analgesia on the

incidence of emergency cesarean section for fetal distress; however, they did not report the incidence of cesarean section for dystocia. Our results are consistent with these findings, demonstrating no effect of epidural analgesia on the incidence of cesarean section for fetal distress.

More recently, two randomized, double-blind, placebo-controlled studies[3, 4] suggested that epidural analgesia, via continuous infusion after 8 cm of dilatation and throughout the second stage, did not increase the incidence of cesarean section. However, both experimental and control groups received epidural anesthetic up to 8 cm of cervical dilatation and then were randomized to continuous infusion of placebo or local anesthetic agent. Therefore these studies[3, 4] assessed the effect of continuous-infusion epidural anesthesia in late labor, not the effect of standard epidural analgesia begun early in active labor. To our knowledge, the present study is the only controlled study specifically analyzing the effect of epidural analgesia on the incidence of cesarean section for dystocia in nulliparous women in labor. If epidural analgesia increases cesarean sections for dystocia in our institution, where active management of labor policies dictate an early aggressive treatment of dysfunctional labor, a much more profound effect would be anticipated in other hospitals.

We recognize potential criticisms of our study, the most significant of which is the selection bias in comparing the nonepidural group with the epidural group. Clearly, some patients who do not initially request epidural analgesia will begin to labor abnormally and then will either request or be advised to have epidural analgesia. As Table I suggests, the nonepidural and epidural groups are different, as evidenced by the somewhat larger birth weights and lesser cervical dilatation on admission in the epidural group. We attempted to address this concern by eliminating from the study all of the patients who had cesarean section for dystocia in the epidural group who received epidural analgesia after abnormal labor was established. Furthermore, we controlled for the significant confounding variables by multivariate analysis. This method of analysis would tend to nullify the difference in cesarean section rates for dystocia between the epidural and nonepidural groups if the cause was related to the confounding variables analyzed. However, there remained a significant difference between the two groups.

One could attempt to explain the difference in cesarean sections for dystocia in the nonepidural and epidural groups by the slightly larger birth weights and higher risk status of the patients in the epidural group. However, analysis of variance and Scheffé test demonstrated no difference in birth weights between those patients having no analgesia and those having only epidural analgesia, yet there was a significant increase in cesarean section for dystocia when these subgroups were compared (Table II). When all the macrosomic newborns were deleted, there also remained significant increases in cesarean section for dystocia in the epidural group. Finally, the outcome data do not support the contention that the epidural group was a significantly higher risk group. Both groups had similar frequencies of cesarean section for fetal distress, similar cord arterial and venous pH values, and similar proportions of low 5-minute Apgar scores and low cord pH values (Table I).

For the reasons listed above, we do not think that the discrepancy in cesarean sections for dystocia between the groups (Table I) or subgroups (Table II) can be fully explained by selection bias. Unless patients are randomly assigned to a nonepidural group before labor, an ideal control group would be impossible to achieve. Ethical considerations and patient drop out will be problems with randomly allocating patients to a nonepidural group before labor.[15] Therefore prospective studies are not the final solution and, unless patients are randomized before the onset of labor, there will also be problems with selection bias. Further study of nulliparous women at term with spontaneous onset of labor, controlling for labor abnormalities and time of epidural placement, is necessary to confirm our findings and prove a cause-effect relationship. If a cause-effect relationship is established, further study to ascertain subgroups of nulliparous women who are at high risk of developing dystocia with epidural analgesia would be clinically useful.

We thank Mr. Kenneth E. Thorp, Sr., for assistance with the data base, and the Obstetrical Anesthesia Department at the University of Texas Medical School–Houston/Hermann Hospital for providing their excellent services.

REFERENCES

1. Diro M, Beydoun SN. Segmental epidural analgesia in labor: a matched control study. J Natl Med Assoc 1985; 78:569-73.
2. Chandler CJ, Davidson BA. The influence of lumbar epidural analgesia in labor on mode of delivery. Int J Gynaecol Obstet 1982;20:353-6.
3. Chestnut DH, Vandewalker GE, Owen CL, Bates JN, Choi WW. The influence of continuous epidural bupivacaine analgesia on the second stage of labor and method of delivery in nulliparous women. Anesthesiology 1987; 66:774-80.
4. Chestnut DH, Bates JN, Won WC. Continuous infusion epidural analgesia with lidocaine: efficacy and influence during the second stage of labor. Obstet Gynecol 1987; 69:323-7.
5. O'Driscoll K, Foley M, MacDonald D. Active management of labor as an alternative to cesarean section for dystocia. Obstet Gynecol 1984;63:485-90.
6. Boylan PC, Frankowski R. Dystocia, parity, and the cesarean problem [Letter]. Am J Obstet Gynecol 1986; 155:455-6.
7. Lee ET. A computer program for linear logistic regression analysis. Comput Programs Biomed 1974;4:80-92.

8. Turner MJ, Brassil M, Gordon H. Active management of labor associated with a decrease in the cesarean section rate in nulliparas. Obstet Gynecol 1988;71:150-8.
9. Akoury HA, Brodie G, Caddick R, McLaughlin VD, Pugh PA. Active management of labor and operative delivery in nulliparous women. AM J OBSTET GYNECOL 1988; 158:255-8.
10. Crawford JS. Principles and practice of obstetric anaesthesia. 5th ed. Boston, Massachusetts: Blackwell Scientific Publications, 1984:242-3.
11. Bromage PR. Epidural analgesia. Philadelphia, Pennsylvania: WB Saunders, 1978:526.
12. Cetrulo CL, Cetrulo LG. Medicolegal dystocia. Clin Obstet Gynecol 1987;30:106-13.
13. Thorp JA, Boylan PC, Parisi VM, Heslin EP. Effects of high-dose oxytocin augmentation on umbilical cord blood gas values in primigravid women. AM J OBSTET GYNECOL 1988;159:670-5.
14. Kaminski HM, Stafl A, Aiman J. The effect of epidural analgesia on the frequency of instrumental obstetric delivery. Obstet Gynecol 1987;69:770-3.
15. Morgan B. Problems in obstetric anesthesia. New York, New York: John Wiley & Sons, 1987:110.
16. McNeill HW, Wilson PL. Obstetric use of continuous epidural blodk in a private community hospital: review of 500 cases. South Med J 1981;74:941-4.
17. Matouskova A, Hanson B, Elmen H. Continuous mini-infusion of bupivacaine into the epidural space during labor. Acta Obstet Gynecol Scand 1979;83(suppl):43-52.
18. Maltau JM, Anderson HT. Continuous epidural anesthesia with a low frequency of instrumental deliveries. Acta Obstet Gynecol Scand 1975;54:401-6.
19. Hollmen A, Jouppila R, Pihlajaniemi R, Karvonen P, Sjostedt E. Selective lumbar epidural block in labour. A clinical analysis. Acta Anesthesiol Scand 1979;21:174-81.
20. Studd JW, Crawford JS, Duignan NM, Rawbotham CJ, Hughes AO. The effect of lumbar epidural analgesia on the rate of cervical dilatation and the outcome of labour of spontaneous onset. Br J Obstet Gynaecol 1980;87: 1015-21.

Make researching easier with the Ten-Year Cumulative Index (1978-1987) to the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY

The Ten-Year Cumulative Index is a complete guide to more than 30,000 pages of original articles, case reports, letters, and editorials published in the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY from 1978 through 1987 (volumes 130 to 157). Numbering more than 800 pages, the Cumulative Index includes both a subject and an author index. This hard-cover volume is $64.50 ($69.00 international). Prices include shipping. *Payment must accompany all orders and must be in U.S. funds, drawn on a U.S. bank.*

Contact The C.V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146 USA; phone (314) 872-8370, ext. 394. In the U.S., call toll-free: 1-800-325-4177, ext. 394. Please allow six weeks for delivery.

Can platelet volume predict progressive hypertensive disease in pregnancy?

James J. Walker, MB, ChB,[a] Alan D. Cameron, MB, ChB,[a] Steingrimur Bjornsson, MD,[a] Charles R. J. Singer, MB, ChB,[b] and Carol Fraser, FMILS[b]

Glasgow, Scotland

Increase in platelet size has been found in conditions with increased platelet destruction and use. This increase in platelet size can be found in patients with moderate or severe hypertension in pregnancy, even in the presence of a normal platelet count. The goal of this study was to investigate whether an increase in platelet size was present before the manifestation of the disease and therefore could be used to predict the progression of the disease before it is clinically apparent. Three hundred normal primigravid pregnancies were studied between 28 and 30 weeks' gestation, and blood was taken to assess platelet count and mean platelet volume. Two hundred sixteen patients had no hypertensive problems during their pregnancy, 84 developed hypertension, 62 had mild hypertension only with diastolic blood pressures between 90 and 99 mm Hg, 13 had moderate hypertension with diastolic blood pressures between 100 and 109, and nine had severe hypertension with diastolic blood pressure above 110. No difference was found between these groups in the parameters measured. Therefore it was felt that platelet volume may not be a useful screening test in a low-risk population. A second study was carried out to investigate the serial changes in these parameters in patients at risk of progressive disease. Thirty-four patients with essential hypertension were followed from 24 weeks with serial blood sampling to study changes in platelet size. An additional 40 patients with mild pregnancy-induced hypertension were also studied. From these patient groups, 20 patients developed severe hypertension. The mean platelet volume increased significantly at least 1 week before the hypertension became clinically apparent. There was no change in platelet count at this time. It is concluded that increasing platelet size can predict which patients are likely to progress to severe disease before it becomes clinically obvious. (AM J OBSTET GYNECOL 1989;161:676-9.)

Key words: Platelets, platelet volume, pregnancy-induced hypertension

In pregnancy-induced hypertension and preeclampsia, studies have shown changes in platelet numbers, platelet survival, and mean platelet volume, which have been interpreted as evidence of increased platelet consumption.[1-5] Some studies have suggested that platelet count falls early in the disease and precede hypertensive renal changes, suggesting that platelet consumption plays an active role in the pathophysiology of this disorder.[3] We have previously shown that an increase in platelet size was found in patients with moderate or severe hypertension in pregnancy, even in the presence of a normal platelet count.[5] Increased mean platelet volume is further evidence of increased platelet turnover in patients with preeclampsia. However, since a decrease in platelet count is not inevitable even at the onset of an eclamptic episode,[6] it may be possible to show increasing platelet consumption in these patients by demonstrating an increase in platelet size, even though platelet count remains within the normal range. Also, since Redman et al.[3] suggest that the fall in platelet count occurs before the rise in serum urate, a change in mean platelet volume occurring before a fall in platelet count may predict the progression of preeclampsia before it is clinically apparent.

From the University Department of Obstetrics,[a] Glasgow Royal Maternity Hospital, and the Department of Haematology,[b] Glasgow Royal Infirmary.

Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.

Reprint requests: J. J. Walker, MRCOG, Department of Obstetrics and Gynaecology, Glasgow Royal Infirmary, 10 Alexandra Parade, Glasgow, Scotland G31 2ER.

6/6/14468

Methods

Patients. This study was conducted in two parts. In the first part 300 normotensive primigravid patients were studied between 28 and 30 weeks of gestation. Blood was taken to assess hematocrit level, platelet count, and mean platelet volume. The results obtained from these patients were compared with the outcome of their pregnancies. In the second part 34 patients with essential hypertension were followed from 24 weeks. A diagnosis of essential hypertension had been made in these patients either before pregnancy or before 20 weeks' gestation in this pregnancy. The group was made up of 26 parous women and eight primigravidas. No patient received any antihypertensive therapy. An additional 40 primigravid patients who had mild pregnancy-induced hypertension at between 28

Table I. Outcome of pregnancies in 300 primigravid patients screened at 28 to 30 weeks (value ± SD)

Outcome	*No.*	*%*	*Hct*	*MPV*	*Pl Ct*
All	300	100	35.5 ± 2.1	8.5 ± 0.5	268 ± 35.8
Normal	216	70	35.5 ± 2.0	8.6 ± 0.5	277 ± 35.7
Mild PIH	62	21	35.6 ± 2.1	8.7 ± 0.9	270 ± 58.9
Moderate PIH	13	7	35.9 ± 3.4	8.7 ± 1.4	270 ± 51.0
Severe	9	3	34.5 ± 2.8	8.8 ± 1.5	246 ± 79.1

Hct, Hematocrit; *MPV*, mean platelet volume; *PlCt*, platelet count; *PIH*, pregnancy-induced hypertension.

Table II. Outcome of 34 patients with essential hypertension sampled serially and changes in mean platelet volume; total numbers for each group are reduced as gestation increases as patients are delivered

Outcome	*No.*	*24 wk*	*28 wk*	*32 wk*	*36 wk*	*Significance (1)**
Stable	20	8.5 ± 1.2	8.6 ± 1.0	8.8 ± 1.4	8.7 ± 1.1	NS
Moderate	8	8.7 ± 1.0	8.7 ± 1.1	8.9 ± 1.3	9.1 ± 1.3	$p < 0.01$
Severe	6	8.6 ± 1.2	9.1 ± 1.4	9.7 ± 1.2	10.2 ± 1.2	$p < 0.005$
Significance (2)†		NS	NS	$p < 0.01$	$p < 0.005$	

*Significance (1): Significance within groups is calculated from the 24-week mean platelet volume against the 36-week value or the last value before delivery if the patient was delivered before 36 weeks. Significance test used was the Wilcoxon signed-rank test for matched pairs.

†Significance (2): Significance between groups was calculated between the severe and stable groups using patients in each group who were undelivered at the gestation stated. Significance test used was the Mann Whitney U test.

and 34 weeks' gestation were also studied from the time the condition was first diagnosed. Both of these groups attended our outpatient day-care monitoring unit on a weekly basis unless admission was required. Serial sampling was carried out weekly in both groups for hematocrit, platelet count, and platelet size. Sampling continued on a weekly basis until delivery. All patients were managed by the hospital hypertension team and routine monitoring followed the protocol for the hospital, which consists of weekly blood pressure monitoring, uric acid estimation, platelet count, urinalysis for protein, nonstress test and ultrasound assessment for fetal weight, and liquor volume. Platelet size data were unavailable to management, and all analysis for this study was carried out after the pregnancy was over.

The protocol for this study was submitted to and passed by our local hospital Ethical Committee.

Mild pregnancy-induced hypertension was diagnosed if blood pressure remained between 90 and 99 mm Hg, moderate if diastolic blood pressure was persistently between 100 and 109 mm Hg, and severe if above 110 mm Hg. For purposes of this study, no other parameters, such as proteinuria or uric acid, were used in the criteria for diagnosis.

Sample handling. Blood samples were obtained from a large vein with a syringe and 19-gauge needle but no tourniquet. Blood was immediately mixed with dipotassium ethylenediaminetetraacetic acid (1.5 mg/ml blood) and maintained at room temperature for 90 to 120 minutes before analysis. Analysis was carried out on a Coulter Counter (Model S-Plus, Coulter Electronics Ltd., Luton, United Kingdom), which was subject to continous quality control. Channel calibration with latex particles (platelet volume calibration latex, Model No. 8.6 fl; Coulter Electronics) was carried daily and later weekly to ensure sample comparability with respect to mean platelet volume and platelet destribution width values generated.

Statistics. Fisher's test, Mann-Whitney U test, and the Wilcoxon signed-rank test for matched pairs were used for statistical analysis in this study.

Results

Of the 300 normal primigravid pregnancies, 216 patients had no hypertensive problems throughout the rest of their pregnancy, and 84 developed hypertension. Although a trend upward could be seen, when all parameters for these patients were compared, there was no significant difference in any of the parameters measured (Table I). Therefore it was felt that platelet volume at 28 to 30 weeks' gestation could not be used as a screening test in this low-risk population. If the mean platelet volume of this group was split into those who had a value above the mean and those who had a value below the mean, it was found that eight of the 154 patients who had a mean platelet volume >8.5 went on to develop severe preeclampsia, whereas only one of the 146 patients with and a mean platelet volume of <8.5 developed severe preeclampsia. Although this was statistically significant ($p < 0.02$), it is of little clinical significance, with a positive predictive value of only 5.19%.

Table III. Outcome of 40 patients with mild pregnancy-induced hypertension sampled serially and changes in mean platelet volume; timing is related to when the diagnosis was made and the first 3 weeks afterward and not to gestation; total numbers for each group are reduced as the weeks increase

Outcome	*No.*	*At diagnosis*	*1 wk*	*2 wk*	*3 wk*	*Significance (1)**
Stable	17	8.5 ± 1.1	8.8 ± 1.2	8.9 ± 1.3	9.0 ± 1.1	NS
Moderate	14	8.8 ± 1.2	9.0 ± 1.2	9.1 ± 1.4	9.2 ± 1.2	NS
Severe	9	8.8 ± 1.3	9.1 ± 1.4	10.0 ± 1.2	11.2 ± 1.2	$p < 0.001$
Significance (2)†		NS	NS	$p < 0.005$	$p < 0.001$	

*Significance (1): Significance within each group was calculated from the mean platelet volume at diagnosis of the mild hypertension compared with the 3-week mean platelet volume or the last mean platelet volume before delivery if patients were delivered before this time; significance test used was the Wilcoxon signed-rank test for matched pairs.

†Significance (2): Significance between groups was calculated between the severe and stable groups at the times indicated using the patients still undelivered at that time in both groups; significance test used was the Mann Whitney U test.

In the serial study of 34 patients with essential hypertension, 14 patients had worsening hypertension (Table II). There was no significant change in the mean platelet volume in the 20 patients who remained stable. A small but significant increase was found in those patients who progressed to moderate disease ($p < 0.01$), and a large increase occurred in those who developed severe hypertension ($p < 0.005$). Between-group differences were seen only between the stable group and the severely affected group between 32 and 36 weeks' gestation. The largest rise in mean platelet volume seen in the stable group was 0.6 fl. If a rise of 1 fl was taken as significant, all patients developing severe disease had a rise >1 fl at least 1 week before a diagnosis of severe hypertension was made by the hypertension team. Onset of severe disease ranged from 32 to 38 weeks. Table II gives values up to 36 weeks, since after this time a significant number of patients in each group had been delivered, and mean values for the groups would be less valid. Similar results were seen for the 40 primigravid patients with mild pregnancy-induced hypertension followed serially (Table III). No change was seen in the mean platelet volume in the patients who remained mildly hypertensive or those who progressed to moderate hypertension, but a significant rise was found in those patients who developed severe disease. Similarly, only the severe group had a rise in mean platelet volume compared with the stable group. All patients with severe hypertension had a rise of >1 fl in mean platelet volume. This rise was seen in this group at least 1 week before a clinical diagnosis of pregnancy-induced hypertension was made by the hypertension team. The development of severe hypertension was between 2 and 4 weeks after the diagnosis of mild disease. Gestational age at the time of diagnosis ranged between 30 and 36 weeks. In 15 patients from both groups who developed severe disease with a rise of 1 fl in the mean platelet volume, seven of the 15 babies were born with a birth-weight under the tenth percentile compared with only 12 of 59 babies born to mothers who remained either stable or progressed only to moderate pregnancy-induced hypertension.

Comment

Hypertension in pregnancy is a very common diagnosis. The vast majority of patients remain in the mild-to-moderate group and do not have any major obstetric problem, and the outcome is good for both mother and baby. However, in a small group of patients, the disease can progress to a more severe form, increasing the maternal risk of seizure and cerebrovascular accident, along with increasing morbidity of severe hypertension and the required medication and hospitalization. For the baby, severe hypertension is associated with increased placental insufficiency and intrauterine growth retardation. Many studies in women with pregnancy-induced hypertension and preeclampsia have shown changes in platelet numbers,[1-3,5] platelet survival,[4,7] and mean platelet volume,[4,5] which have been interpreted as increased consumption. Thrombocytopenia is a common finding and is associated with progressive disease and also disease severity. Platelet lifespan is known to be reduced in pregnancy-induced hypertension and is thought to be caused by intravascular platelet aggregation and increased adhesion to vascular endothelium.[7] Because the signs of this platelet consumption appear to antedate other signs and clinical manifestations of the disease, many investigators believe that platelets are involved in the pathophysiology of this disorder.[3] This is the reasoning behind the low-dose aspirin studies that are now being undertaken in the United States and the United Kingdom. Although the findings of this study were not evidence of a significant increase in platelet consumption remote from the onset of the clinical disease, the fact that mean platelet volume tended to be higher in patients who progressed suggests that there may be early platelet consumption in this group. However, it is of no value as a screening test. Similarly, there was no evidence that an increased hematocrit level was associated with patients who devel-

oped serious hypertension as previously suggested. The second part of the study confirmed that there was increasing platelet consumption in patients who developed moderate and severe pregnancy-induced hypertension and that the changes related to this increased platelet consumption antedated the appearance of clinical signs by at least 1 week. Since other methods of investigation can be used to help monitor patients at risk of developing progressive pregnancy-induced hypertension, it is difficult to know whether mean platelet volume is necessarily any better than monitoring serial platelet counts and uric acid levels. Correlation of apparent intrauterine growth retardation with patients who developed severe disease with rising mean platelet volume may support the evidence that patients with severe disease have increasing platelet consumption and deposition of platelets and fibrin.[8] Such changes within the placenta could partly explain the findings described in previous pathologic studies.[9] These findings support the argument for the use of low-dose aspirin in patients believed to be at risk of severe preeclampsia or intrauterine growth retardation. If this reduces the rate of platelet consumption that occurs, it may well slow or stop progression of the disease to its severe form and reduce the chance of placental insufficiency. A large-scale study of patients at risk with rising mean platelet volume as a marker for intervention may be indicated.

REFERENCES

1. Giles C. The platelet count and mean platelet volume. Br J Haematol 1981;48:31-7.
2. Giles C, Inglis TCM. Thrombocytopenia and macrothrombocytosis in gestational hypertension. Br J Obstet Gynaecol 1981;88:115-9.
3. Redman CWG, Bonnar J, Beilin L. Early platelet consumption in preeclampsia. Br Med J 1978;i:467-9.
4. Wallenburg HCS, Rotmans N. Circulating large platelets and platelet turnover in normotensive and hypertensive pregnancies with insufficient fetal growth. In: Sammour MB, Symonds EM, Zuspan FP, El Tomi N, eds. Pregnancy hypertension. Cairo: University Press, 1980.
5. Singer CRJ, Walker JJ, Cameron A. Platelet studies in normal pregnancy and pregnancy-induced hypertension. Clin Lab Haematol 1986;8:27-32.
6. Pritchard JA, Cunningham FG, Mason RA. Coagulation changes in eclampsia. Their frequency and pathogenesis. Am J Obstet Gynecol 1976;124:855-64.
7. Wallenburg HCS, Van Kessel PH. Platelet life span in normal pregnancy as determined by a non-radioisotopic technique. Br J Obstet Gynaecol 1978;85:33-6.
8. Howie PW, Prentice CRM, McNicol GP. Coagulation fibrinolysis and platelet function in pre-eclampsia, essential hypertension and placental insufficiency. J Obstet Gynaecol Br Common 1971;78:992-1003.
9. Brosens IA. Morphological changes in the utero-placental bed in pregnancy hypertension. Clin Obstet Gynecol 1977;4:3:573-93.

Measurement of cardiac output in pregnancy by thoracic electrical bioimpedance and thermodilution

A preliminary report

Damon I. Masaki, MD, Jeffrey S. Greenspoon, MD, and Joseph G. Ouzounian, BS
Los Angeles, California

Thoracic electrical bioimpedance is a noninvasive, continuous method of obtaining cardiac output that requires no operator skill. However, the most recent thoracic electrical bioimpedance technology has not been validated in pregnancy. We therefore compared two methods of measuring cardiac output in pregnancy, thoracic electrical bioimpedance and thermodilution. We studied 11 patients who required pulmonary artery catheterization for peripartum management and measured cardiac output simultaneously by thoracic electrical bioimpedance and thermodilution. Among eight of nine patients, there was agreement (within ±20%) between the two methods. Bivariate linear regression with these nine cases showed excellent correlation ($r = 0.91$, $p < 0.001$) with a slope of 1.04, which indicated a one-to-one relationship between thoracic electrical bioimpedance and thermodilution. The remaining two cases were removed from analysis because of septic shock in one case (which invalidates thoracic electrical bioimpedance) and 4+ tricuspid regurgitation in another case (which invalidates thermodilution). These data support that thoracic electrical bioimpedance measurement of cardiac output may be valid in most peripartum patients. (AM J OBSTET GYNECOL 1989;161:680-4.)

Key words: Pregnancy, cardiac output, thoracic electrical bioimpedance

The estimation of cardiac output is an integral part of the management of pregnant women who have underlying cardiovascular disorders or who develop serious medical complications. The most commonly employed clinical method of measuring cardiac output is thermodilution, which requires the insertion of a pulmonary artery catheter. The invasive nature precludes the use of thermodilution to assess cardiac output in the majority of patients. Although thermodilution to assess cardiac output has gained wide acceptance and is considered the "gold standard" in clinical settings, it has an overall error of 15% to 20%[1] and overestimates cardiac output in low-flow states.[2] A noninvasive alternative to thermodilution that provides continuous updating of cardiac output is desirable.

Measurement of cardiac output by Doppler techniques is noninvasive and has been validated in pregnancy.[3, 4] However, these methods require significant technical skill and are intermittent rather than continuous.

Thoracic electrical bioimpedance, also referred to as impedance cardiography, is a noninvasive method of estimating stroke volume and, therefore cardiac output. In addition, thoracic electrical bioimpedance provides continuous beat-to-beat data on cardiac output and requires no technical skill provided the electrodes are correctly placed. Earlier studies of cardiac output by thoracic electrical bioimpedance during pregnancy have been reported with older thoracic electrical bioimpedance technology.[5-7] The early studies used cumbersome tetrapolar band electrodes and were based on the Kubicek equation, which had a tendency to overestimate cardiac output.[8] Recent improvements in measurements of cardiac output by thoracic electrical bioimpedance involved the modification of the Kubicek equation by Sramek and Bernstein,[9] and incorporation of the calculations into a computer microprocessor called the Noninvasive Continuous Cardiac Output Monitor, model 3 (NCCOM-3). Studies with the NCCOM-3 system indicate that measurement of cardiac output by thoracic electrical bioimpedance is reliable in nonpregnant adults[10-12] and pediatric patients[13] but has not been validated in pregnancy.

The NCCOM-3 system has been revised several times.[14] The revision 4 model (available in 1985) was unreliable when the heart rate exceeded 150 beats/min. The studies previously cited used the revision 4 system.[10-13] Our study used revision 6 of the NCCOM-3, which has been available since 1986. Revision 6 was modified to permit measurement of cardiac output at heart rates up to 199 beats/min, and adjusted the Sramek stroke volume equation for weight.[14]

From the Department of Obstetrics and Gynecology, University of Southern California School of Medicine.
Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.
Reprint requests: Damon I. Masaki, MD, Women's Hospital, Room 5K-40, 1240 North Mission Road, Los Angeles, CA 90033.
6/6/13895

Table I. Patient data including simultaneous cardiac output estimates by thermodilution and thoracic electrical bioimpedance

Patient no.	*Indication for pulmonary artery catheter*	*Thermodilution estimate of cardiac output*		*Thoracic electrical bioimpedance estimate of cardiac output*		*Difference (%)*
		Mean	*Range*	*Mean*	*Range*	
1	Class III MS	5.9	4.9-6.4	5.5	5.3-5.6	−6.8
2	Class III MS	4.2	3.2-4.9	4.0	3.4-4.2	−4.8
3	MS with mild PIH	4.2	3.8-4.4	3.6	3.4-3.7	−14.3
4	Pancreatitis ARDS	7.7	6.9-8.6	6.2	5.6-6.5	−19.8
5	Severe PIH Oliguria	7.4	7.1-7.5	6.0	5.1-6.9	−19.1
6	Severe PIH Oliguria	8.9	6.8-10.7	9.4	8.4-10.7	+5.6
7	Severe PIH Oliguria	9.8	9.1-10.5	8.7	8.1-8.9	−11.2
8	Severe IH Oliguria	9.9	8.6-10.7	10.4	10.1-10.6	+5.1
9	Severe PIH Oliguria	7.2	6.8-7.6	4.6	4.6-4.7	−35.6
10	Class III* 4+ TR 1+ MR	3.4	3.0-3.8	5.2	5.0-5.5	+53.0
11	Septic† shock	7.9	7.8-8.3	5.3	5.1-5.7	−32.4

ARDS, Adult respiratory distress syndrome; *PIH*, pregnancy-induced hypertension; *MS*, mitral stenosis; *TR*, tricuspid regurgitation; *MR*, mitral regurgitation.

Difference (%) = Thoracic electrical bioimpedance − Thermodilution/Thermodilution × 100.

Cardiac output reported is mean and range of five measurements expressed in liters per minute.

*Patient 10 not included in agreement or regression analysis because thermodilution is not reliable in measuring cardiac output in patients with 4+ tricuspid regurgitation.

†Patient 11 not included in the agreement or regression analysis because the estimation of cardiac output by thoracic electrical bioimpedance has not been reliable in septic shock.

We present the first study of peripartum patients undergoing pulmonary artery catheterization in whom simultaneous thermodilution to assess cardiac output and measurement of cardiac output by thoracic electrical bioimpedance, using revision 6 of the NCCOM-3 model, were performed to compare the two methods.

Material and methods

Eleven peripartum patients were studied at the Los Angeles County–University of Southern California Medical Center during a 15-month period from November 1987 to January 1989. Patients were included if they required pulmonary artery catheterization for peripartum management and consented to thoracic electrical bioimpedance.

The pulmonary artery catheters (model 93A-831-7.5F, American Edwards Laboratories, Irvine, Calif.) were inserted percutaneously via the right internal jugular vein in all cases. The medical conditions requiring pulmonary artery catheterization are shown in Table I. The correct position of the pulmonary artery catheter was confirmed by pressure waveform and chest radiograph. Pressure transducers (model T12AD-R, Gould Inc., Oxnard, Calif.) were calibrated to a zero reference at the midaxillary line. All measurements were made with the patient in the supine position with a left lateral tilt of approximately 25 degrees. A 10 ml injectate of 5% dextrose in water at room temperature (23° to 25° C) was used for obtaining thermodilution to assess cardiac output. Five consecutive thermodilution measurements were made randomly with respect to the respiratory cycle, regardless of whether the patient had spontaneous respirations or required mechanical ventilation; the five readings were then averaged. Values obtained by thermodilution to assess cardiac output were determined on a cardiac output computer (model 9520A, American Edwards Laboratories).

Determination of cardiac output by thoracic electrical bioimpedance was obtained with the NCCOM-3, revision 6 (NCCOM-3, rev. 6, Bomed Medical Manufacturing Ltd., Irvine, Calif.). Eight electrodes (Silvon ECG electrodes, NDM Corp., Dayton, Ohio) were applied as follows (see Fig. 1). Two sensing electrodes were placed 180 degrees apart from each other at the root of the neck, and two sensing electrodes were placed 180 degrees from each other at the level of the xiphoid

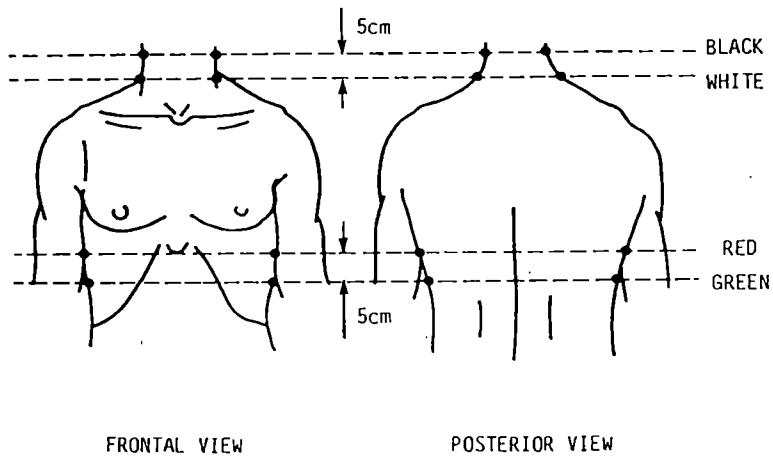


Fig. 1. Diagram of electrode placement. Electrodes are color coded to ensure correct application. *Black* and *white* (root of neck) electrodes are 180 degrees apart. *Red* (level of xiphoid) and *green* electrodes are at midaxillary line. Black and green electrodes are transmitting electrodes; white and red ones are sensing electrodes. (From Bomed Manufacturing Ltd.)

process at the midaxillary line. Two pairs of transmitting electrodes were placed adjacent to the sensing electrodes in the same plane so that the electrode centers were 5 cm apart. The cardiac output by thoracic electrical bioimpedance for each heartbeat for the next 12-beat interval (which is the usual interval required for a cardiac output measurement determined by thermodilution) is averaged by the microprocessor in the NCCOM-3, rev. 6, and displayed.

At the beginning of the five measurements of cardiac output by thermodilution, a cardiac output by thoracic electrical bioimpedance reading was begun simultaneously. A total of five simultaneous measurements were performed on each patient and averaged.

Statistical analysis to assess agreement between the measurement of cardiac output by thermodilution and thoracic electrical bioimpedance was performed as described by Bland and Altman.[15] Thermodilution to assess cardiac output and cardiac output by thoracic electrical bioimpedance data were also analyzed by bivariate linear regression with MR-TOD (Medical Research–Time Oriented Database, Retriever Data Systems, Seattle, Wash.).

Patients gave written informed consent before pulmonary artery catheterization for medical management and before cardiac output by thoracic electrical bioimpedance studies. The use of thoracic electrical bioimpedance to determine cardiac output was approved by the Human Research Review Committee at the University of Southern California–Los Angeles County Medical Center.

Results

Data obtained from the individual patients are shown in Table I. The percent difference between the two methods is also calculated. Two patients were excluded from the analysis for agreement and bivariate linear regression because both had a condition in which cardiac output estimation is inaccurate with one of the methods. Patient No. 10 had tricuspid regurgitation (4+), rendering thermodilution assessment of cardiac output invalid. Patient 11 had septic shock, a condition in which determination of cardiac output by thoracic electrical bioimpedance has been unreliable in nonpregnant patients.

The cardiac output data for nine patients are presented graphically as recommended by Bland and Altman[15] in Fig. 2. Bivariate linear regression analysis between thermodilution to assess cardiac output and cardiac output by thoracic electrical bioimpedance, in which thermodilution to assess cardiac output was designated as the independent variable was also performed. The results of this analysis are: Cardiac output by thoracic electrical bioimpedance $= -1.03 \pm 1.04 \times$ (Cardiac output by thermodilution); $r = 0.91$ ($p < 0.001$). The standard error of estimate was equal to 1.07.

Comment

Thoracic electrical bioimpedance is a noninvasive blood flow technology that estimates left ventricular stroke volume on a continuous beat-to-beat basis. With each ventricular contraction, there is an increase in the electrical conductance of the thoracic cavity and results in the bioimpedance waveform. The NCCOM-3 computer microprocessor senses and records these impedance changes through its eight-lead montage and uses them to calculate stroke volume and thus cardiac output. The Sramek-Bernstein equation used to calculate stroke volume (SV) is:

$$SV = L^3/4.2 \times T_{LVE} \times (dz/dt)_{max}/Z_0$$

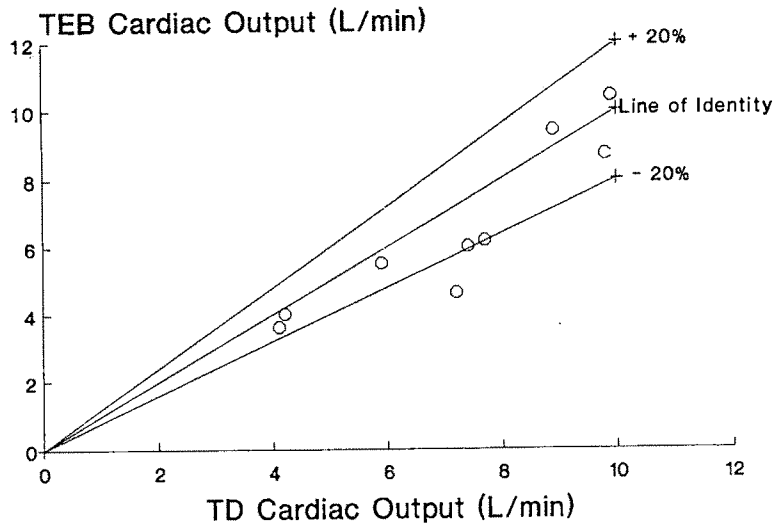


Fig. 2. Cardiac output by thoracic electrical bioimpedance for the first nine patients described in Table I. *Line of identity* designates perfect agreement between the two methods. The lines for ±20% of cardiac output indicate accepted accuracy limits in measurement of cardiac output.[1]

The derivation of this equation was reviewed by Bernstein.[9] In the equation, T_{LVE} is left ventricular ejection time (in seconds, displayed as ventricular ejection time), $(dZ/dt)_{max}$ is the maximum rate of impedance change (in ohms per second, displayed as ejection velocity index), and Z_0 is the basal thoracic impedance (in ohms, displayed as the thoracic fluid index). The L value is the thoracic length, which is used to calculate the thoracic volume. Note that the L value is the only value not determined by the NCCOM-3 directly from the bioimpedance waveform. The accuracy of the L value is critical because it appears in the third power in the stroke volume equation. An error of 1 cm in this value can alter the cardiac output by as much as 10%.[9] The L value is derived from a nomogram that uses the patient's height and weight and adjusts for deviations from ideal body weight. This nomogram was derived from nonpregnant subjects. Although cardiac output by thoracic electrical bioimpedance estimates by the NCCOM-3 are reliable in most nonpregnant adults, the cardiac output by thoracic electrical bioimpedance method has not been validated in pregnancy.

The cardiac output by thoracic electrical bioimpedance estimate was less than thermodilution to assess cardiac output by 35.6% in patient No. 9. One possible explanation for this discrepancy between the method is that the L value for short subjects may be incorrect. A study of cardiac output by thoracic electrical bioimpedance in short (pediatric) patients suggested that the L value is underestimated with respect to height in short (pediatric) patients, therefore cardiac output is underestimated by thoracic electrical bioimpedance.[13] This patient's height was 144 cm (4 feet 9 inches), which is near the lower limit (145 cm) of the nomogram for the determination of L. These potential errors in L may account for the observed differences in cardiac output.

Previous studies in nonpregnant adults demonstrated that cardiac output by thoracic electrical bioimpedance consistently underestimates cardiac output measured by thermodilution and is unreliable during sepsis with high cardiac output.[8] This is probably caused by augmented blood flow to the skin from intense peripheral vasodilation. The thorax thus behaves more like a "shell conductor than a volume conductor."[8] The $(dZ/dt)_{max}$ and ultimately cardiac output by thoracic electrical bioimpedance are therefore underestimated during this condition. The cardiac output by thoracic electrical bioimpedance underestimated cardiac output by thermodilution by 32.4% in patient no. 11 (during septic shock), which confirms this observation. This patient was excluded a priori from the analysis comparing the two methods because she had a clinical condition in which measurement of cardiac output by thoracic electrical bioimpedance is known to be invalid. Whether the vasodilator properties of drugs such as hydralazine and magnesium sulfate have an effect on the cardiac output by thoracic electrical bioimpedance similar to that of sepsis was not studied. This possibility should be considered in patients with pregnancy-induced hypertension (patients no. 5 to 9). All five of these patients received hydralazine and magnesium sulfate at the time of this study. Complex dysrhythmias, electrocautery, and motion artifact may interfere with cardiac output by thoracic electrical bioimpedance estimates[8] but were not encountered in this series.

The comparison of thermodilution to assess cardiac output and cardiac output by thoracic electrical bio-

impedance requires that both methods be done with accuracy in addition to proper statistical analysis. The use of thermodilution to assess cardiac output measures right ventricular cardiac output at the point where the thermistor on the tip of the pulmonary artery catheter is located. Thermodilution to assess cardiac output is sensitive to changes in preload and can vary up to 50% during different phases of ventilation.[16] Thermodilution to assess cardiac output performed exclusively at end-exhalation will be more reproducible, which is useful for determining trends in the clinical setting but will be less accurate in terms of representing cardiac output during the entire respiratory cycle.[17] We therefore chose to perform thermodilution to assess cardiac output randomly in relation to the respiratory cycle. The volume (10 ml) and temperature (23° to 25° C) of the injectate selected simplifies the technique without loss of accuracy.[18] Accurate measurement of cardiac output by thoracic electrical bioimpedance requires careful determination of the thoracic length and correct electrode placement.

The statistical comparison of thermodilution to assess cardiac output with assessment of cardiac output by thoracic electrical bioimpedance was performed only after all five cardiac output values by each method were averaged. The large effect of ventilation on thermodilution to assess cardiac output variability and the lack of effect on cardiac output by thoracic electrical bioimpedance would make comparisons between single thermodilution to assess cardiac output and cardiac output by thoracic electrical bioimpedance estimates misleading. Fig. 2 graphically displays the comparison between thermodilution to assess cardiac output and assessment of cardiac output by thoracic electrical bioimpedance. If perfect agreement existed between the two methods of measuring cardiac output, then all points would fall on the line of identity. The ±20% cardiac output lines on Fig. 2 show the accepted accuracy limits of thermodilution to assess cardiac output.[1] The estimate of cardiac output by thoracic electrical bioimpedance is within the ±20% limits in eight of nine cases.

Regression analysis also was performed, which requires that each data point be comprised of independent observations. Therefore one observation from each patient was analyzed for correlation. An excellent correlation of $r = 0.91$, $p < 0.001$ was established.

In summary, these results indicate that the NCCOM-3, rev. 6, estimate of cardiac output by thoracic electrical bioimpedance is similar to the use of thermodilution to assess cardiac output in most cases during the peripartum period in our study. Measurement of cardiac output by thoracic electrical bioimpedance is noninvasive, continuous, and requires no operator skill. Nevertheless, even if measurement of cardiac output by thoracic electrical bioimpedance is confirmed to be accurate in larger studies of pregnant patients, it must only be used as an adjunct to good clinical judgment.

REFERENCES

1. Levett JM, Replogle RL. Thermodilution cardiac output: a critical analysis and review of the literature. J Surg Res 1979;27:392-404.
2. van Grondelle A, Ditchey RV, Groves BM, et al. Thermodilution methods overestimate low cardiac output in humans. Am J Physiol 1983;245:H690.
3. Easterling TR, Watts DH, Schmucker BC, Benedetti TJ. Measurement of cardiac output during pregnancy: validation of Doppler technique and clinical observations in preeclampsia. Obstet Gynecol 1987;69:845-50.
4. Lee W, Rokey R, Cotton DB. Noninvasive maternal stroke volume and cardiac output determinations by pulsed Doppler echocardiography. AM J OBSTET GYNECOL 1988; 158:505-10.
5. Milsom I, Forssman L, Sivertsson R, Dottori O. Measurement of cardiac stroke volume by impedance cardiography in the last trimester of pregnancy. Acta Obstet Gynecol Scand 1983;62:473-9.
6. Davies P, Francis RI, Docker MF, Watt JM, Crawford JS. Analysis of impedance cardiography longitudinally applied in pregnancy. Br J Obstet Gynaecol 1986;93:717-20.
7. De Swiet M, Talbert DG. The measurement of cardiac output by electrical impedance plethysmography in pregnancy. Are the assumptions valid? Br J Obstet Gynaecol 1986;93:721-6.
8. Bernstein DP. Noninvasive cardiac output measurement. In: Shoemaker WC, Thompson WL, Holbrook PR, eds. Textbook of critical care. Philadelphia: WB Saunders, 1989:159-85.
9. Bernstein DP. A new stroke volume equation for thoracic electrical bioimpedance: theory and rationale. Crit Care Med 1986;14:904-9.
10. Appel PL, Kram HB, Macabee J, et al. Comparison of measurements of cardiac output by bioimpedance and thermodilution in severely ill surgical patients. Crit Care Med 1986;14:933-5.
11. Bernstein DP. Continuous noninvasive real-time monitoring of stroke volume and cardiac output by thoracic electrical bioimpedance. Crit Care Med 1986;14:898-900.
12. Salandin V, Zussa C, Risica G, et al. Comparison of cardiac output estimation by thoracic electrical bioimpedance, thermodilution, and Fick methods. Crit Care Med 1988; 16:1157-8.
13. Introna RPS, Pruett JK, Crumrine RC, Cuadrado AR. Use of transthoracic bioimpedance to determine cardiac output in pediatric patients. Crit Care Med 1988;16: 1101-5.
14. Masaki DI, Greenspoon JS, Ouzounian JG. Noninvasive determination of cardiac output by thoracic electrical bioimpedance (letter). Crit Care Med (in press)
15. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986;1:307-10.
16. Synder JV, Powner DJ. Effects of mechanical ventilation on the measurement of cardiac output by thermodilution. Crit Care Med 1982;10:677-82.
17. Stevens JH, Raffin TA, Mihm FG, Rosenthal MH, Stetz CW. Thermodilution cardiac output measurement: effects of the respiratory cycle on its reproducibility. JAMA 1985;253:2240-2.
18. Elkayam U, Berkley R, Azen S, Weber L, Geva B, Henry WL. Cardiac output by thermodilution technique: effect of injectate's volume and temperature on accuracy and reproducibility in the critically ill patient. Chest 1983; 84:418-22.

In utero cocaine exposure: Observations of fetal behavioral state may predict neonatal outcome

Roderick F. Hume, Jr., MD, Maj, MC, USA,[a] Karen J. O'Donnell, PhD,[b,c] Cathi L. Stanger, RN,[a] Allen P. Killam, MD,[a] and Jeannine L. Gingras, MD[b]
Durham, North Carolina

Components of fetal behavioral state organization reflect the successful integration of the central nervous system, have a specific developmental timetable, and can be studied with fetal ultrasonographic techniques. To test the hypothesis that evaluation of state organization is a marker of abnormal central nervous system maturation and a predictor of risk, we studied 20 fetuses and newborns exposed to cocaine in utero. Fetal assessments were accomplished by serial ultrasonographic examination, videotaped, and scored by a scheme developed by the authors to assess organization and regulation of behavioral states. Newborn neurobehavioral assessments also emphasized organization and regulation of behavioral state. Abnormal or delayed state behavior was identified in 13 of 20 fetuses. State organization was evaluated as suspect or abnormal for 16 of the 20 exposed newborns. Disorganized behavioral state in the fetus successfully predicted abnormal newborn behavior. These findings support the concepts that cocaine exposure disrupts central nervous system development and that fetal assessment of state is predictive of neonatal outcome. (Am J Obstet Gynecol 1989;161:685-90.)

Key words: Cocaine, fetal behavioral state, state organization, neonatal state

Cocaine abuse is a major problem in our society. Its increasing use as the recreational drug of choice among the poor and the affluent has been responsible for an explosion of awareness of its potential for devastating addiction and medical catastrophe.[1] Cocaine use is also increasing among the reproductively active in our society. Cocaine use in pregnancy is associated with poor perinatal outcome: increased incidence of premature labor and delivery, intrauterine growth retardation, abruptioplacentae, stillbirth, maternal and neonatal intracranial hemorrhage, fetal distress, and altered neonatal behavior.[2-5] Also alarming is the possibility of an increased incidence of sudden unexplained infant death in the offspring of cocaine-abusing women.[2]

Neonates born to mothers who abuse cocaine during pregnancy have been shown to exhibit altered behavioral state organization in concert with poor perinatal outcome.[2,3,5] We have made the additional observation of a similar disorganization of fetal state-related behavior. State organization is seen as reflecting proper integration of the central nervous system, and we theorized that state organization or disorganization would be an early fetal marker of neurobehavioral risk. The present study investigated two hypotheses: (1) fetuses exposed to cocaine in utero would demonstrate altered behavioral state organization and (2) altered fetal behavioral state would be an early marker of the integrity of central nervous system maturation. This manuscript reports the observations on a cohort of perinates whose mothers abused cocaine during gestation and who were serially observed in utero with ultrasonography. The infants were examined in the neonatal period.

Material and methods

Duke University Medical Center provides the primary obstetric service for urban Durham and the surrounding rural counties. During the year of this study, there were 2245 deliveries at the medical center. Twenty women identified as cocaine users through clinic referral or self-referral volunteered and were enrolled in this institutionally approved study. The history of maternal substance abuse was obtained by the primary author (R. F. H.) serially and concurrently throughout prenatal care. The prospective, nonpunitive ascertainment of drug use allowed more information to be gathered relative to the timing and pattern of maternal substance abuse. The pattern of maternal substance abuse was obtained by interview and recorded for all of our subjects during their prenatal care and at delivery. Systematic drug screening was not an aspect of this study design. Serial ultrasonographic evaluations of fetal growth, well-being, and state-related behavior were recorded as a component of their

From the Departments of Obstetrics and Gynecology,[a] Pediatrics,[b] and Psychiatry,[c] Duke University Medical Center.
Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.
The opinions or assertions contained herein are those of the authors and are not to be construed as official or reflecting the views of the U.S. Army or the Department of Defense.
Reprint requests: J. L. Gingras, MD, Duke University Medical Center, P. O. Box 3179, Durham, NC 27710.
6/6/14471

Table I. Distribution of maternal pattern of abuse*.

Duration of use	*Mode of cocaine use*			*Alcohol use*	*Narcotic use*
	Intravenous	*Nasal*	*Free base*		
1st Trimester only (4/20)	1/4	3/4	0/4	3/4	0/4
1st + 2nd Trimester (7/20)	5/7	1/7	1/7	7/7	3/7
1st-3rd Trimester (9/20)	4/9	4/9	1/9	9/9	2/9
TOTAL (N = 20) (%)	50	40	10	95	25

*This includes the cumulative duration and mode of cocaine abuse and other substance abuse.

Table II. Medical-obstetric complications for cocaine-exposed group compared with control population at Duke University Medical Center

	PCSG (%)	*DUMC/LCHC (%)*
Anemia	25	22.0
Premature labor	25	24.0
Spontaneous premature rupture of membranes	20	2.0*
Preeclampsia	0	4.6
Small for gestational age–intrauterine growth retardation	30	14.0
Abruptioplacentae	5	1.1
Birth defects	5	5.7
Premature delivery	25	16.7
Fetal distress in labor	20	11.3
Cesarean section	20	20.0

PCSG, Perinatal Cocaine Study Group; *DUMC/LCHC*, Duke University Medical Center/Lincoln Community Health Center.
*$p < 0.01$.

high-risk obstetric care. Subjects only identified at delivery as using cocaine were excluded from this report. This study reports on the observations of 20 fetuses serially evaluated in utero and followed through the neonatal period. No subjects were lost to follow-up. One infant delivered at term, who was examined in utero and after birth, died at home at age 21 days of *Klebsiella* sepsis.

Routine ultrasonographic evaluations included standard morphometric assessments of fetal weight, growth,[6] and well-being.[7] Our assessment of fetal behavioral state was adapted from techniques previously described by Nijhuis et al.[8] and Arduini et al.[9] Components of fetal behavior assessed included fetal heart rate variability, breathing motion, body tone and movements, and chin, mouth, and eye movements. Organization and regulation were defined as the coordination of related components of fetal behavior in a stable yet responsive state. Our modification, "Fetal Neurobehavioral Profile" (in preparation), emphasizes behavior state differentiation, organization, and regulation through systematic observations of unperturbed fetal behavior, effect of vibroacoustic perturbation, and the fetus' attempt at self-regulation after perturbation. The ultrasonographic technique requires an image of the fetal face, chin, and chest to allow coincidental evaluation of fetal eye, mouth, breathing, and heart motion. We used a GE RT 2600 device with 3.5 and 5 MHz linear array transducers to obtain a split-screen, real-time B-mode and M-mode image of fetal behavior, which was recorded on standard VHS format for later review. The stable association of rapid eye movements, chin motion, breathing, and reactive heart rate pattern is scored as active sleep. The stable association of no eye movements, chin motion, or breathing with a nonreactive heart rate pattern is scored as quiet sleep. After a 10- to 15-minute period of observation of unperturbed fetal behavior, vibroacoustic stimulation was applied in either of these stable behavioral states during direct ultrasonographic imaging with a brief (<1 second) stimulus from a Bell Laboratory artificial larynx. The fetal stimulus-response pattern was repeated to evaluate fetal habituation to sound perturbation. Fetal behavior was scored from video tape recorded ultrasonographic observations. Rating of fetal neurobehavioral profile was initially made at each ultrasonographic evaluation and recorded prospectively (R. F. H., C. L. S.). Prenatal data, including pattern of drug use, were recorded prospectively and stored in files coded to protect subject confidentiality.

Maternal and neonatal charts were reviewed to record obstetric and neonatal complications and outcome (C. L. S.). Gestational age and growth were evaluated to determine the appropriateness of growth for gestational age. Neonatal neurobehavioral assessment was accomplished between days 1 and 3 and before dis-

Table III. Maternal pattern of cocaine use and fetal effects

Duration of use	*Intrauterine growth retardation*	*Acute intoxication*	*Disorganized fetal state*
1st Trimester only (4/20)	0/4	0/4	1/4
1st + 2nd Trimester (7/20)	2/7	2/7	5/7
1st-3rd Trimester (9/20)	4/9	1/9	7/9
TOTAL (%)	30	15	65

charge by an examiner (K. O. D., J. L. G.) who was blinded to pattern of maternal substance abuse, fetal behavioral profile results, intrapartum course, and drug screen results, if obtained. Because a history of cocaine exposure is an indication for referral for neonatal neurobehavioral testing, these examiners were not blinded to cocaine exposure. The neonates were assessed with methods described by others[10, 11] in the following areas: autonomic stability, motor status, reflex profile, state organization, and attention to auditory and visual stimuli. Overall neurobehavioral status was scored for these analyses as normal, suspect, or abnormal. Newborn state organization scores, classified as normal or abnormal for gestational age, were also analyzed. The reliability of fetal assessment to predict newborn assessment was accomplished by nonparametric means (χ^2). Other data are reported by frequency and proportion scores.

Results

Subjects. In our obstetric community 35 of 2245 deliveries (1.6%) were recorded as being complicated by maternal substance abuse. No systematic study for urine toxicology was done to obtain true prevalence during this period. Ten of these 35 (28%) received no prenatal care or received prenatal care outside of our clinic system. Additionally, five (18%) received prenatal care within our clinic system but were not able to be enrolled in our study because of logistic problems. Therefore 20 of the 35 women (54%) participated in this study. The timing of gestation at cocaine exposure, the use of other agents, and the mode of cocaine use are summarized in Table I. All subjects were exposed during the first trimester. Most attempted to discontinue their cocaine use on discovery of their pregnancy.

Table II lists the medical and obstetric complications experienced by our total cocaine-exposed cohort and compares them with the complications in a population of 2210 mothers not known to abuse cocaine who were delivered during the study period. Comparisons of these proportions indicated that significantly more women who abused cocaine had spontaneous premature rupture of the membranes ($p < 0.05$). The 20 infants evaluated in this study were born between 37 and 40 weeks of gestational age.

Fetal behavioral state organization. Abnormal fetal state-related behavior was identified in 13 of 20 neonates (65%) exposed to cocaine in utero who survived beyond midgestation. Our observations included both short-term (during maternal cocaine intoxication) and long-term (during maternal abstinence) effects on fetal behavior. This information is presented in Table III.

Manifestations of acute fetal intoxication were observed in three fetuses and included hyperflexion, irritability, prolonged periods of scanning eye movements during vigorous, almost continuous sucking, and excessive fetal motion, especially in response to stimulation. Fetal tachycardia and extreme baseline heart rate variability were most often evident on fetal heart rate testing. Of these three fetuses, two were also observed during acute maternal cocaine withdrawal. The fetal withdrawal effects were similar to the maternal symptoms of cocaine withdrawal with recurrent-repetitive yawning and problems of arousal most apparent. The fetus appears to experience concordant physiologic and behavioral effects along with its mother in response to cocaine.

Long-term or delayed effects of cocaine exposure were defined as delay or disruption of state organization, occurring in 13 of the 20 (65%), or as unusual fetal behavior, such as recurrent-repetitive yawning seen in three fetuses. A deep sustained, regular, hyperpneic (deep inspiratory cycle = expiratory cycle) fetal breathing pattern was seen in three fetuses. This latter pattern resembled the central neurogenic hyperventilation described in adults. Both the recurrent yawning and sustained hyperpneic breathing patterns persisted between behavioral states.

Six fetuses (30%) were assessed as growth-retarded on fetal ultrasonographic evaluation. Additionally, disorganization of fetal behavior appeared to be associated with the magnitude of maternal cocaine abuse (see Table III), although the relationships were not statistically significant.

Neonatal outcome. Newborn infants were assessed for overall neurobehavioral well-being in the areas of autonomic stability, motor and reflex status, state organization, and attentional capacities. The most frequent indicators of autonomic instability included excessive tremulousness in extremities, unexplained tachypnea, or both. Disrupted state organization was evidenced by hyperresponsiveness and by difficulty in

Table IV. Fetal state organization as predictive of newborn neurobehavioral well-being

	Newborn			
	Normal	*Suspect*	*Abnormal*	*Total*
Fetal				
Normal	1	5	1	7
Abnormal	0	4	9	13
TOTAL	1	9	10	20

$\chi^2 = 6.2; p < 0.05$.

Table V. Fetal state organization as predictive of newborn state organization

	Newborn		
	Organized	*Disorganized*	*Total*
Fetal			
Organized	4	3	7
Disorganized	0	13	13
TOTAL	4	16	20

$\chi^2 = 9.2; p < 0.01$.

arousal. These infants often were seen as "all or nothing," that is, difficult to arouse, inconsolable when aroused, and having brief or no periods of quiet alertness. Deficits in attentional capacities (response to auditory and visual stimuli) usually were interpreted as being secondary to disrupted behavioral state regulation.

Overall neurobehavioral status was scored as normal, suspect, or abnormal (Table IV). Ten infants (50%) were neurobehaviorally abnormal, with nine scored as suspect and one scored as normal. It is important that fetal state organization successfully predicted overall newborn behavioral well-being ($\chi^2 = 6.2$; $p < 0.05$). For example, of the 13 fetuses seen as exhibiting abnormal state behavior, nine were abnormal and none were scored as normal newborns.

Newborn state organization, rated as organized (normal) or disorganized (abnormal), was abnormal in 16 of the 20 infants, again successfully predicted by the fetal state assessment ($\chi^2 = 9.2; p < 0.01$) (Table V). All 13 fetuses who had abnormal fetal state behavior were abnormal in state organization as newborns. Additionally, disorganization of neonatal behavior appeared to be associated with the magnitude of maternal cocaine abuse, although the relationships were not statistically significant. However, we did detect fetal and neonatal behavioral abnormalities even if exposure was limited to the first trimester.

Comment

These observations suggest that maternal cocaine usage results in both short-term (immediate) fetal and long-term postnatal effects. Short-term effects include manifestations of fetal intoxication (hyperflexion, irritability, excessive fetal movements, and sucking with fetal tachycardia and extreme baseline heart rate variability) and withdrawal (recurrent yawning and sustained hyperpnea). Long-term effects include intrauterine growth retardation and fetal neurodevelopmental delay or disruption as defined by altered fetal behavioral state organization and regulation. Since altered fetal behavioral state is associated with adverse perinatal outcome, our data strongly support a role for cocaine as a neurodevelopmental teratogen. The purpose of the current study was to test the hypothesis that altered fetal behavior could be detected ultrasonographically and that such abnormal fetal behavior would predict subsequent abnormal neonatal behavioral testing in a cohort of cocaine-exposed pregnancies. Prospective longitudinal study of these infants and matched controls is required to determine the significance of the delay or disruption of state organization identified with the fetal neurobehavioral profile.

Fetal behavioral states show a normal ontogeny with progressive coincidence of components of behavior becoming organized into clearly defined and stable patterns with maturity. This maturation has been described by Nijhuis et al.[8] and correlates with the behavioral states of the newborn. The progressive organizational, integrative, and interactive ability of the fetus with advancing gestational age serves as the foundation of newborn neurobehavioral examinations.[10, 11] Similarly, the correlation of fetal behavioral state with fetal biophysical parameters such as breathing, heart

rate variability patterns, Doppler flow in umbilical vessels, and echocardiographic measures of hemodynamics reveals a progressive integration of function of numerous organ systems. This concerted coordination of normal physiologic action is under the control or modulation of the central nervous system.[12] Thus the application of fetal behavioral state assessment may serve as a useful screening test for appropriate neurodevelopment. Indeed, altered fetal behavior has been described in animal models after exposure to narcotics and other agents.[13] Delayed development of the integration of fetal behavioral states has also been reported in human fetuses suffering intrauterine growth retardation.[14, 15]

Animal models of altered fetal environments that are associated with fetal growth retardation often include rather marked elevations of endogenous catecholamines, especially norepinephrine.[16] This biogenic amine has also been identified as responsible for the acceleration of pulmonary maturation. The peripheral nervous system and autonomic nervous system in these fetuses also exhibit advanced maturity when compared with normal fetuses. However, the development of the fetal central nervous system is disturbed and predominantly delayed in its ontogeny.[17] Cocaine may have similar effects on the developing fetus that are secondary to altered norepinephrine metabolism. This discordance of developmental achievement between the central nervous system–autonomic nervous system may be operative in the inappropriate response of these fetuses to environmental perturbation: fetal distress, stillbirth, neonatal transitional difficulties, and sudden unexplained infant death.

We have observed a similar disruption (disorganization) of fetal behavioral state organization and regulation associated with maternal cocaine use. Alterations in fetal behavior were manifested by dissociation or lack of coincidence of components of behavioral state, as well as an apparent delay in the onset of integration. Both short- and long-term neurodevelopmental effects of maternal cocaine abuse were observed. Immediate drug-induced responses included hyperrigidity in extreme flexion, hyperactivity with violent total body movements in response to stimulation, scanning eye movements to the exclusion of the normally predominant rapid eye motion, and marked alteration in fetal breathing patterns and fetal heart rate variability. Additionally, the short-term response appeared to correlate with the known kinetics of cocaine metabolism and degradation. We also noted a return to a more normal fetal biophysical profile and behavior the day or two after maternal cocaine ingestion. Although the most significantly impaired fetus was that of a chronic, multiple agent intravenous drug abuser who continued her daily use up to delivery, we discovered similar atypical behavior in the episodic user whose use was confined to the first trimester.

The explanation for persistent abnormalities in the episodic user may relate to the direct central nervous system–autonomic nervous system actions of cocaine, or a metabolite, on catecholamine uptake kinetics.[18] Such neurotransmitter effects could alter the ontogeny of neurotransmitter distribution or interfere with the normal development of the central nervous system. Alternatively, the hemodynamic effects of cocaine, mediated through norepinephrine, may be operative as a cause of growth retardation and delayed neurodevelopment.[19, 20] Further studies are necessary to evaluate these potential pathophysiologic mechanisms.

The dramatically increased incidence of cocaine-associated perinatal morbidity and mortality (impaired intrauterine growth, neonatal transitional dysfunction, and disorganization of behavioral states) reported in the literature is supported by our results. Additionally, we have observed altered behavior in those fetuses exposed to cocaine in utero. These data are in concert with the concept that cocaine alters central nervous system development. Fetal neurobehavioral assessment may serve as a useful screening test for intact neurodevelopment.

We thank Mrs. Mary Wade and Ms. Judi Goss for their assistance in the preparation of this manuscript.

REFERENCES

1. Cregler LL, Mark H. Medical complications of cocaine abuse. N Engl J Med 1986;315:1495-500.
2. Chasnoff IJ, Burns KA, Burns WJ. Cocaine use in pregnancy. Perinatal morbidity and mortality. Neurotoxicol Teratol 1987;9:291-3.
3. Oro AS, Dixon SD. Perinatal cocaine and methamphetamine exposure: maternal and neonatal correlates. J Pediatr 1987;111:571-8.
4. Little BB, Snell LM, Palmore MK, Gilstrap LC. Cocaine use in pregnant women in a large public hospital. Am J Perinatol 1988;5:206-7.
5. MacGregor SN, Keith LG, Chasnoff IJ, et al. Cocaine use during pregnancy: adverse perinatal outcome. Am J Obstet Gynecol 1987;157:686-90.
6. DeVore GR, Platt LD. Diagnosis of intrauterine growth retardation: the use of sequential measurements of fetal growth parameters. Clin Obstet Gynecol 1987;30:968-84.
7. Manning FA, Morrison I, Lange IR, Harman CR, Chamberlain PF. Fetal assessment based on fetal biophysical profile scoring: experience in 12,620 referred high-risk pregnancies. Am J Obstet Gynecol 1985;151:343-50.
8. Nijhuis JG, Prechtl HFR, Martin CB, Bots RSGM. Are there behavioral states in the human fetus? Early Hum Dev 1982;6:177-95.
9. Arduini D, Rizzo G, Giorlandino C, et al. The fetal behavioral states: an ultrasonic study. Prenat Diagn 1985; 4:269-76.
10. Brazelton TB. Neonatal behavioral assessment scale. Spastics Int 1968;2:63-4.
11. Dubowitz V, Dubowitz L, Goldberg S. Clinical assessment of gestational age. J Pediatr 1970;77:1-10.
12. Arduini D, Rizzo G, Pennestri F, Romanini C. Modulation of echocardiographic parameters by fetal behavior. Prenat Diagn 1987;7:179-87.

13. Hofmeyr GJ, Bamford OS, Howlett T, Parkes MJ. Methionine-enkephalin and the arrest of fetal breathing. In: The physiological development of the fetus and neonate. London: Academic Press, 1985:653-7.
14. van Vliet MAT, Martin CB, Nijhuis JG, Prechtl HFR. Behavioral states in growth-retarded human fetuses. Early Hum Dev 1985;12:183-97.
15. Rizzo G, Arduini D, Pennesttri F, Romanini C, Mancuso S. Fetal behaviour in growth retardation: its relationship to fetal blood flow. Prenat Diagn 1987;7:229-38.
16. Jones CT. Biogenic Amines in Development. Amsterdam: Elsevier Biomedical Press, 1980:63-86.
17. Slotkin TA, Weigel SJ, Barnes GA, Whitmore WL, Seidler FJ. Alterations in the development of catecholamine turnover induced by perinatal methadone: differences in central vs peripheral sympathetic nervous system. Life Sci 1981;29:2519-25.
18. Pitts DK, Marwah J. Cocaine modulation of central monoaminergic neurotransmission. Pharmacol Biochem Behav 1987;26:453-61.
19. Moore TR, Sorg J, Miller L, Key TC, Resnik R. Hemodynamic effects of intravenous cocaine on the pregnant ewe and fetus. AM J OBSTET GYNECOL 1986;155:889-93.
20. Woods JR, Plessinger MA, Clark KE. Effects of cocaine on uterine blood flow and fetal oxygenation. JAMA 1987;257:957-61.

In utero diagnosis of benign fetal macrocephaly

Robert DeRosa, MD,[a] Roger R. Lenke, MD,[a] Thaddeus W. Kurczynski, MD,[b] Wayne H. Persutte, BS, RDMS,[a] and Joanne M. Nemes, RNC, BSN[a]

Toledo, Ohio

Benign familial macrocephaly is an autosomal dominant disorder associated with a large absolute circumference of the head. In this disorder serial growth demonstrates a proportional rather than an excessive rate of growth. To date, we are not aware of any published case reports that confirm the diagnosis prenatally. We report a case of benign familial macrocephaly diagnosed in utero by ultrasonographic evaluation. This case report points out the necessity of combining appropriate family history and physical examination in cases of prenatally detected anomalies. (AM J OBSTET GYNECOL 1989;161:690-2.)

Key words: Prenatal diagnosis, benign familial macrocephaly

Macrocephaly is defined as an occipitofrontal head circumference >2 SDs above the mean or above the 98th percentile for chronologic age.[1, 2] Macrocephaly may be associated with hydrocephalus; neurocutaneous syndromes such as neurofibromatosis, multiple lipomas, and hemangiomas (Bannayan-Zonana syndrome), multiple hemangiomas (Riley-Smith syndrome); or benign familial macrocephaly. Benign familial macrocephaly is a common autosomal dominant trait in which the absolute circumference of the head is large, but serial measurements show a proportional rather than an excessive rate of growth.[3] It often occurs in normal individuals without demonstrable neurologic abnormalities.[1] We report a case of benign familial macrocephaly diagnosed in utero by a combination of prenatal ultrasonographic evaluations and family history.

From the Departments of Obstetrics and Gynecology[a] and Pediatrics[b] Medical College of Ohio.
Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.
Reprint requests: Robert DeRosa, MD, Department of Obstetrics and Gynecology, Medical College of Ohio, P.O. Box 10008, Toledo, OH 43699-0008.
6/6/14149

Case report

The patient was a 21-year-old white woman, gravida 2, para 0, living children 0. Her previous laboratory evaluations included a blood type of AB, rhesus negative, a normal maternal serum α-fetoprotein level at 16 weeks' gestation (0.94 multiples of the median) and normal glucose tolerance test results at 30 weeks' gestation. Pregnancy dating was confirmed by an accurate last menstrual period and early pelvic examination at 12 weeks' gestation. Correct gestational age was also confirmed by an ultrasonographic evaluation at 21 weeks' gestation. This initial examination showed no discrepancy between head circumference (190 mm for 21.3 weeks' gestation), biparietal diameter (52 mm for 21.8 weeks' gestation), abdominal circumference (166 mm for 21.6 weeks' gestation), femur length (34 mm for 20.7 weeks' gestation), and humeral length (33 mm for 21.3 weeks' gestation). An ultrasonographic evaluation at 32 weeks' gestation was obtained because of a history of vaginal bleeding. Fetal measurements were as follows: biparietal diameter 96 mm (39 weeks' gestation); abdominal circumference 341 mm (38 weeks' gestation); femur length 64 mm (33 weeks' gestation); tibial length 58 mm (34 weeks' gestation). There was no evidence of fetal ascites or skin edema. The fetal lateral ventricle/hemispheric ratio was nor-

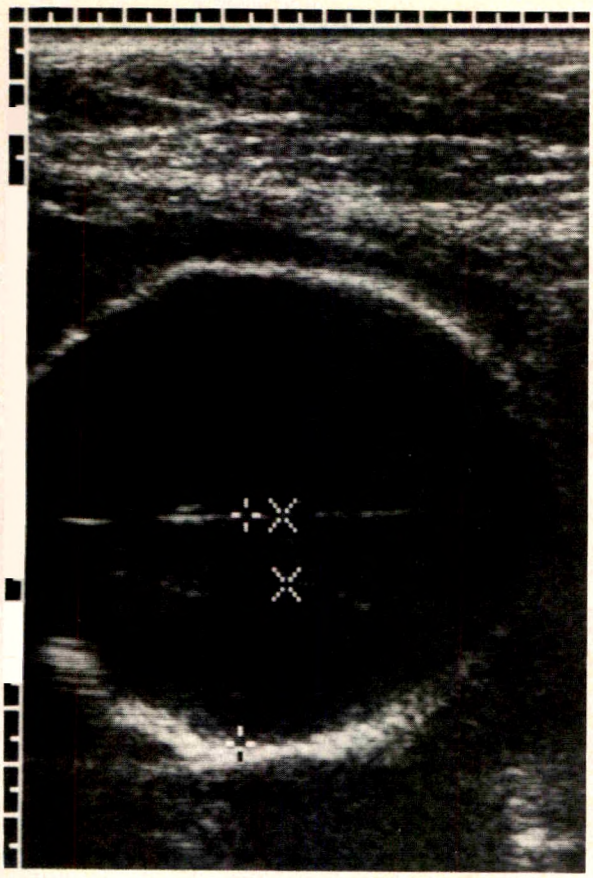


Fig. 1. Transverse view of fetal head at 33 weeks' gestation. No visible structural anomaly. (Lateral ventricle/hemispheric ratio is 31%.)

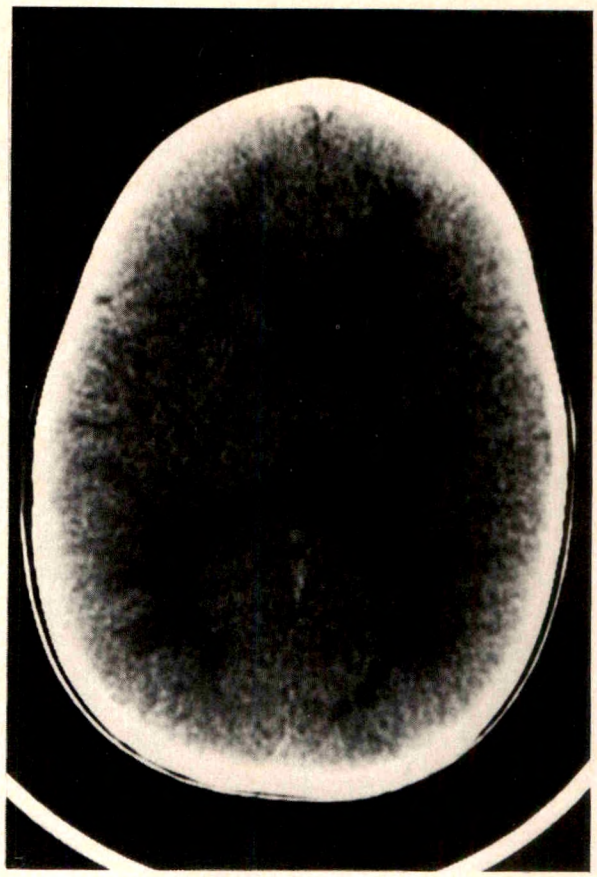


Fig. 2. Computerized axial tomography scan of infant's head at 8 months of age. No visible intracranial anomaly.

mal (31%) (Fig. 1). No other fetal abnormalities were identified. There was no evidence of placenta previa or abruptio placentae.

Physical examination of the patient showed a maternal height of 4 feet 11 inches and weight of 211 pounds. Maternal head circumference was 61 cm (>98th percentile). No other abnormalities were noted on physical examination. Review of the patient's family history revealed several maternal half-sisters of approximately the same height who were also reported to have "large heads." Physical examination of the patient's mother also revealed a head circumference of 61.2 cm (>98th percentile). Unfortunately, no other relatives were available for physical examination.

On the basis of this information, a diagnosis of benign familial macrocephaly was made. A follow-up ultrasonographic evaluation at 36 weeks' gestation showed a biparietal diameter of 102 mm (>40 weeks' gestation); head circumference of 369 mm (>40 weeks' gestation); abdominal circumference of 368 mm (>40 weeks' gestation); femur length of 72 mm (37 weeks' gestation); and fetal intracranial anatomy was normal with a lateral ventricle/hemispheric ratio of 29%.

The patient had suspicious antepartum test results at 39 weeks' gestation and an oxytocin induction was performed. The patient had a normal spontaneous vaginal delivery of a 4190 gm male infant with Apgar scores of 9 at both 1 and 5 minutes.

Evaluation of the infant at birth showed a head circumference of 37.5 cm (98th percentile) and a body length of 53 cm (90th percentile). No other physical abnormalities were detected. Follow-up evaluation of the infant at 6 months of age showed a normal neurologic evaluation but persistent macrocephaly. The head circumference was at the 98th percentile, and body length was at the 75th percentile with weight at the 90th percentile. A computerized axial tomography scan performed at 8 months of age did not show abnormalities (Fig. 2).

Comment

Our patient was initially referred to our facility with a diagnosis of possible fetal hydrocephaly. Our ultrasound evaluation confirmed an enlarged fetal head but found a normal relationship between the fetal ventricles and head size. The enlarged fetal head raised the possibilities of fetal macrocephaly versus fetal macrosomia, or possibly a combination of both.

In the obstetric literature, macrosomia is often de-

fined as a birth weight >4000 gm.[5] The in utero diaganosis is often made on the basis of a disproportionately large fetal abdominal circumference. In the present case, the infant had an abdominal circumference that was consistently large for dates. The birth weight was also >4000 gm. Thus, by definition, the infant was macrosomic. Previous publications, though, have not shown macrosomia to be associated with macrocephaly.[6] There was also a discrepancy between the fetal head size and long-bone measurements. Because this is the first reported case of prenatally diagnosed benign familial macrocephaly, one cannot say that infants with this condition will or will not be macrosomic.

There have been several case reports of benign familial macrocephaly published, but in all these previous cases the diagnosis was made after delivery.[1, 2, 4] To our knowledge, this is the first reported case of prenatally diagnosed benign familial macrocephaly. The benign nature of this diagnosis points out the necessity of obtaining an appropriate family history when ultrasonographic evaluation shows possible fetal abnormalities. The presence of known benign familial macrocephaly in either parent should alert the obstetrician and the ultrasonographer to look for this finding in the fetus. Only careful follow-up examinations will serve to distinguish this condition from other more serious disorders. If one does not include this benign condition in the initial differential diagnosis of an enlarged head, then one may cause undue anxiety for the patient.

REFERENCES

1. Asch AJ, Myers GL. Benign familial megalencephaly. Pediatrics 1976;57:535.
2. Day RE, Schutt WH. Normal children with large heads—benign familial megalencephaly. Arch Dis Child 1979; 54:512.
3. Milhorst TH. Neurosurgery of the newborn. In: Avery GB, ed. Neonatology, pathophysiology and management of the newborn. 3rd ed. Philadelphia: Lippincott, 1987: 1133-5.
4. Schreier H, Rapin I, Davis J. Familial megalencephaly or hydrocephalus? Neurology 1974;24:232.
5. Cunningham FG, MacDonald PC, Gant NF. Dystocia due to abnormalities in presentation, position, or development of the fetus. In: Williams' obstetrics. Norwalk, Connecticut: Appleton and Lange, 1989:365.
6. Wladimiroff JW, Bloemsma CA, Wallenburg HCS. Ultrasonic diagnosis of the large for dates infant. Obstet Gynecol 1978;52:285.

Rapid determination of fetal sex by deoxyribonucleic acid amplification of Y chromosome–specific sequences

Thomas L. Pinckert, MD, Roger V. Lebo, PhD, and Mitchell S. Golbus, MD
San Francisco, California

Rapid fetal sex tests use either dot blot hybridization to Y chromosome–specific (male) repeat sequences or polymerase chain reaction deoxyribonucleic acid amplification of these Y-specific sequences. We have performed 35 fetal sex determinations, 16 by dot blot alone, 13 by polymerase chain reaction alone, and 6 by dot blot and polymerase chain reaction on samples of fetal blood, amniotic fluid, and chorionic villi. All results have been confirmed by karyotyping. Dot blots have given false-positive "male" results three times. In contrast, the polymerase chain reaction has correctly determined fetal sex in every case, even when the dot blot was in error. The Y-specific polymerase chain reaction has been applied to fetal deoxyribonucleic DNA with a chromosome 15; Y translocation to identify the origin of the translocated material. Thus the polymerase chain reaction appears to be a reliable method to rapidly determine fetal sex that also can be used diagnostically to identify translocated Y-chromosomal material. (Am J Obstet Gynecol 1989; 161:693-8.)

Key words: Y chromosome, DNA amplification, polymerase chain reaction

Prenatal determination of fetal sex is extremely important in the management of X-linked disorders.[1] Duchenne and Becker muscular dystrophy, hemophilia A and B, and the Lesch-Nyhan syndrome are among the 139 X-linked phenotypes in which fetal sex is an important factor in counseling. Previously, fetal sex determination relied on karyotyping amniocytes or chorionic villus cells. Standard karyotyping depends on obtaining multiple high-quality metaphases from cells cultured for days to weeks before analysis. Because karyotyping without tissue culture is not always definitive, efforts have been made to develop a prenatal sex test that is rapid, specific, and can be performed early in gestation.

The dot blot test developed by Lau[2] was used by our laboratory as an adjunct to karyotyping as part of our protocol to prenatally diagnose fetuses at risk for X-linked diseases. We used the dot blot protocol to test for repetitive sequences on the terminal portion of the long arm of chromosome Y. Repeated sequences are very useful to test small quantities of deoxyribonucleic acid (DNA) because the signal is amplified the number of times the sequence is repeated. Because the cloned 3.4 kb *Hae* III sequence hybridizes to approximately 2000 homologous sequences on the Y chromosome, small quantities of DNA can be tested without purification. A cloned *Alu* sequence hybridizes to approximately 300,000 homologous *Alu* sequences located ubiquitously throughout the genome. *Alu* is used as a control to establish that DNA has been recovered and applied to each dot (Fig. 1).

Subsequently DNA amplification of Y-chromosome–specific sequences was developed by Kogan et al.[3] The polymerase chain reaction protocol repeats a series of DNA denaturing, annealing, and elongation cycles 35 times (Fig. 2). This amplification synthesizes thousands of unique length copies of each original strand. This procedure has been used to diagnose sickle cell anemia[4] and β-thalassemia[5] with the use of primers that recognize and amplify unique gene sequences. The protocol has also been used to detect human immunodeficiency virus in peripheral blood lymphocytes[6] and is being applied to the detection of other viruses. We tested both the dot blot and polymerase chain reaction protocols on a series of pregnancies at risk for a fetus with an X-linked disease.

Chromosomal translocations in fetal cells have different implications depending on which translocated chromosomal regions remain and where the breakpoints occur. If extra chromosomal material results from a translocation, these regions generally carry active genes that make the cell aneuploid for those genes. Reciprocal translocations themselves carry a low but real risk because the correct number of genes is maintained and breaking and rejoining usually occur between active gene regions but may occur within genes

From the Department of Obstetrics, Gynecology and Reproductive Sciences and the Department of Pediatrics, University of California, San Francisco.
This work was supported in part by Grant 2-203 from the March of Dimes Birth Defects Foundation to M. S. G.
Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.
Reprint requests: Mitchell S. Golbus, MD, Department of Obstetrics, Gynecology and Reproductive Sciences, U-262, University of California, San Francisco, San Francisco, CA 94143-0720.
6/6/14295

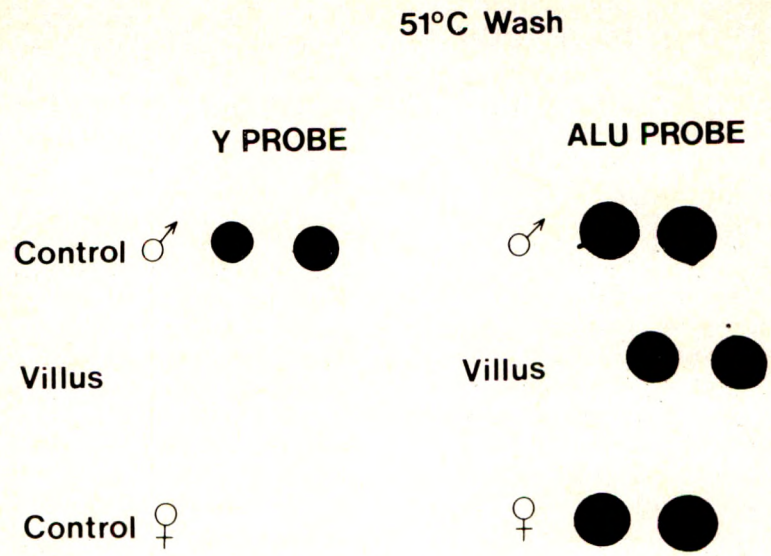


Fig. 1. Dot-blot analysis of Y-specific repeats. Male DNA hybridizes specifically to the probe *(top)* while female DNA hybridizes weakly *(bottom)*. Unknown villus DNA is female. *Alu* probe hybridizes to ubiquitous DNA repeats and acts as a control to demonstrate the presence of DNA in each sample. The *dot* in the lower left was used to orient the autoradiograph.

and alter gene function. Ideally, the precise rearrangement is identified by chromosome banding or by gene mapping to determine as accurately as possible the risk to the fetus. Herein we describe testing fetal DNA from an ambiguous karyotype for the presence of Y-repetitive chromosomal material to clarify the risk to the fetus.

Material and methods

Dot blot analysis. Cells from 1.0 ml of amniotic fluid or two chorionic villi are centrifuged and resuspended in 20 μl 0.1N sodium hydroxide–2 mol/L sodium chloride and 0.5% sodium dodecyl sulfate to disrupt the cells. After vortex mixing, the pellet is boiled 2 minutes at 100° C to complete DNA denaturation. Cellular debris is separated by Eppendorf centrifugation for 2 minutes. The supernatant is dotted onto nitrocellulose filter paper, dried, baked, prehybridized, and then hybridized to cloned Y-specific 3.4 kb *Hae* III fragments or ubiquitous repetitive *Alu* DNA probe overnight at 41° C in 50% formamide. The next morning the filters were washed and autoradiographed for 2 hours according to Lau[2] (see Fig. 1 for results).

Polymerase chain reaction amplification. DNA is prepared and denatured from amniocytes or chorionic villi as above. Samples of approximately 0.5 μg male and female DNA are included as controls. The reaction mix consists of denatured DNA, 25 pmol each of two Y-specific oligonucleotide primers,[2] 1.5 mmol/L of each deoxynucleotide triphosphate, 5 U *Taq* polymerase, 67 mmol/L Tris hydrochloride (pH 8.8), 16.6 mmol/L ammonium sulfate, 6.7 mmol/L magnesium chloride, 6.7 mmol/L ethylenediaminetetraacetic acid, and 170 μg/ml bovine serum albumin.[3] A control reaction mix is included in which 25 pmol each of both *Alu* oligonucleotide primers has been substituted for the Y-specific primers. Samples are amplified for 35 cycles on a Perkin-Elmer/Cetus instrument. Samples are denatured initially for 2 minutes at 94° C. The 35 cycles each include a 30-second denaturation step at 92° C, a 25-second annealing step at 40° C, and a 25-second elongation step at 65° C. The amplified reaction mix is loaded on 12% polyacrylamide gel and electrophoresed for approximately 2 hours at 100 V. The separated DNA fragments are visualized by staining with ethidium bromide and destaining before photography (see Fig. 2).

Biquinacrine staining. Mounted G-banded chromosome slides were recovered by dissolving the mounting medium in xylene. Slides were then stained in 0.002% biquinacrine hydrochloride for 3 minutes and destained in running distilled water 4 minutes.

Results

The dot blot protocol was applied to 22 samples. Usually male DNA hybridizes more intensely to the 3.4 kb Y probe than control female DNA to unambiguously establish the sex of the DNA tested. However, in 3 of 22 cases the homologous repetitive sequences on the autosomes hybridized sufficiently to the repetitive Y-chromosomal DNA probe to give a "false-positive" or apparent male signal in fetal female DNA.

In the polymerase chain reaction test DNA is amplified with primers that synthesize male-specific 150 bp

fragments, different length autosomal fragments (Fig. 3, left), and repeated multiple length *Alu* repetitive sequences in the control reaction (Fig. 3, right).[3] We have used this protocol to analyze six samples tested by dot blot including the three that gave false-positive results, as well as 13 additional samples tested by the polymerase chain reaction alone. In each case the polymerase chain reaction gave the correct result. All results were verified by karyotyping. The polymerase chain reaction method routinely gave results in 1 or 2 working days and required less effort than the dot-blot method.

We have also applied this technique to identify the chromosomal origin of an extra chromosome fragment translocated to the short arm of chromosome 15 in an XX fetus (Fig. 4). Giemsa banding showed that this chromosome segment was heterochromatic and probably derived from chromosome 1, 9, 16, or Y. The portion of chromosome 15 deleted by the translocation carries ribosomal genes that are duplicated in the other nine acrocentric chromosomes so that this deletion would not be detrimental. DNA from the cultured cells was extracted and tested by the polymerase chain reaction. An amplified 150 bp Y-specific DNA fragment was identified in the fetal DNA (data not shown). Dot blot analysis was consistent with this finding. This result established that the extra chromosomal fragment was from the Y-chromosome heterochromatin, a reassuring finding. To further confirm this result, we used biquinacrine stain to restain chromosomes that had been banded with Giemsa. Enhancement of the repetitive Y-chromosome region fluorescence is several

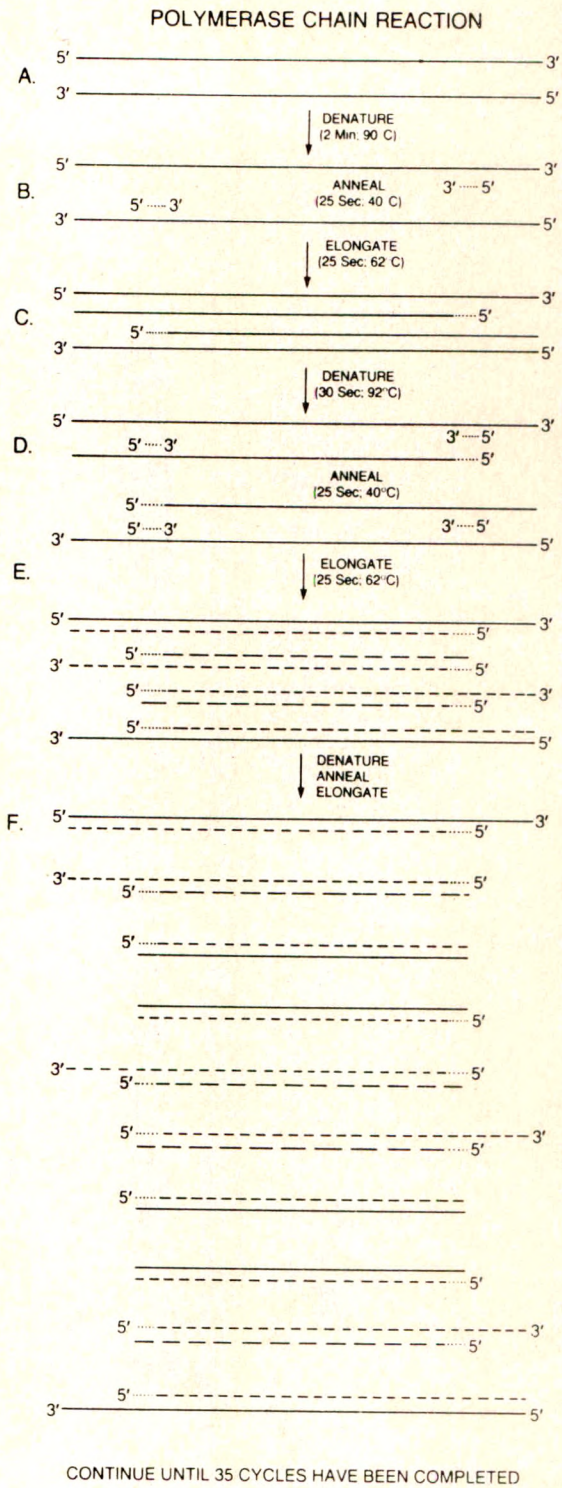


Fig. 2. For legend see left-hand column.

Fig. 2. Polymerase chain reaction scheme. $A \rightarrow B$, Double-stranded DNA is denatured by high temperature (90° C for 2 minutes) into two single strands. *B*, The temperature is lowered to allow sequence-specific primers to stick (anneal) to highly homologous DNA segments. *C*, The original denatured DNA strands act as the elongation templates to extend the primers. Nucleotides and DNA polymerase are added to the primers to synthesize complementary DNA strands. Two primers are required to direct DNA synthesis because the DNA polymerase moves only in a $5' \rightarrow 3'$ direction and both strands must be primed. This step synthesizes two copies of the chromosomal region of interest. The duration of elongation determines the length of the synthesized fragments. $D \rightarrow E$, In turn, these two double-stranded daughter copies are denatured, reannealed, and elongated to form four new copies of double-stranded DNA (again only in the chromosomal region of interest). Note that these include the two original gene strands, four longer amplified copies that extend beyond the reverse primer recognition site, and two unique length amplified copies. $E \rightarrow F$, By repeating this process the next cycle yields the two original gene strands, six longer amplified copies, and eight unique length amplified copies. This process is repeated multiple times to logarithmically increase the number of unique length sequences.

times greater with biquinicrine than with quinacrine. As expected, the Y-chromosome–specific region translocated to the short arm of 15 stained very brightly (Fig. 5). This result further confirmed the polymerase chain reaction result that repetitive Y-chromosome sequences were carried by this patient. The gene for the

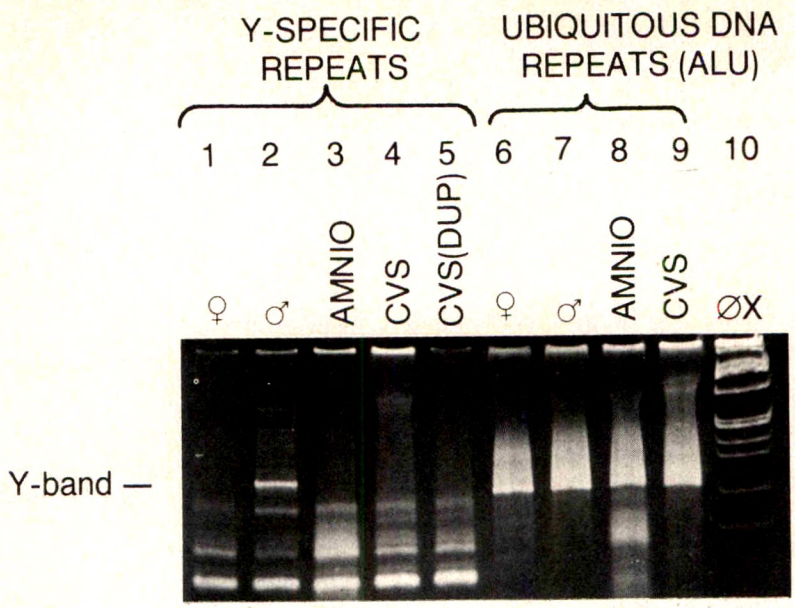


Fig. 3. Photograph of polyacrylamide gel electrophoresis illustrating the polymerase chain reaction protocol. Lanes *1* to *5* were amplified with Y-chromosome repeat primer and lanes *6* to *10* with ubiquitous repetitive *Alu* primer to verify DNA amplification in each sample. Lane *1* is control female DNA from lymphocytes, lane *2* is control male DNA from lymphocytes, lane *3* is unknown amniocyte DNA (female), and lanes *4* and *5* were a chorionic villus DNA sample (female) tested in duplicate. Note the bright Y-specific band identified in lane *2*.

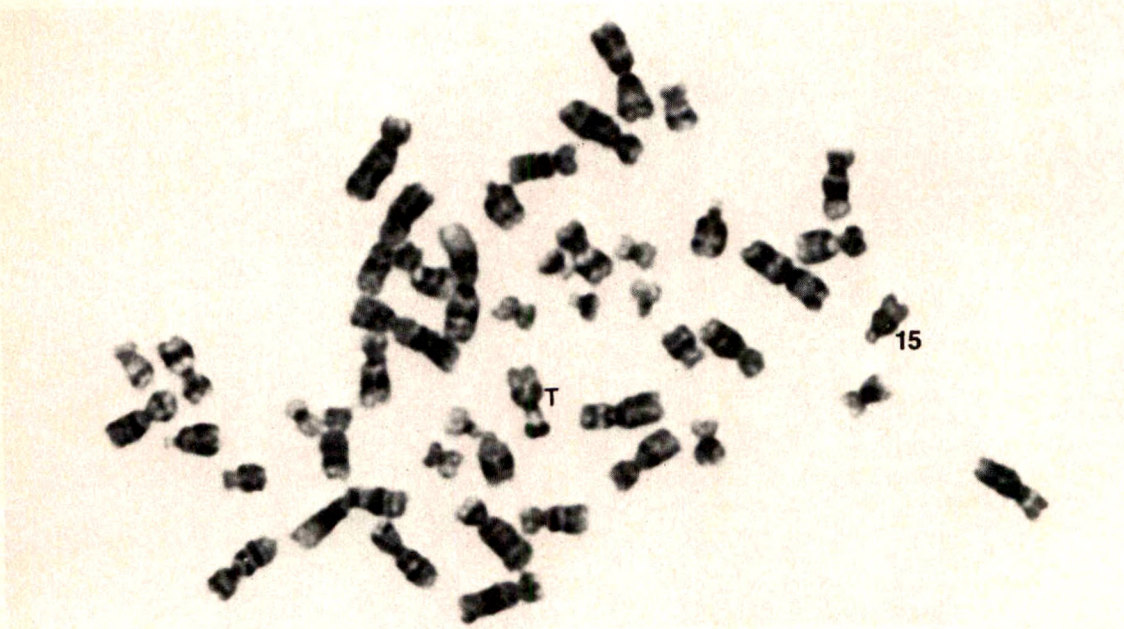


Fig. 4. Giemsa-banded chromosome analysis. One translocated chromosome 15 *(T)* has a G-positive segment translocated to the short arm while the other chromosome 15 *(15)* is normal. Since Giemsa banding does not distinguish the origin of this extra chromosomal material, this karyotype designation is 46,XX, − 15, + t(15;?)(p13;?).

testis-determining factor has been reported to be on the short arm of the Y chromosome. A sonogram of the fetus was consistent with female genitalia. We interpret these data to indicate that the extra Y-chromosome material in this female fetus represents a minimal phenotypic risk.

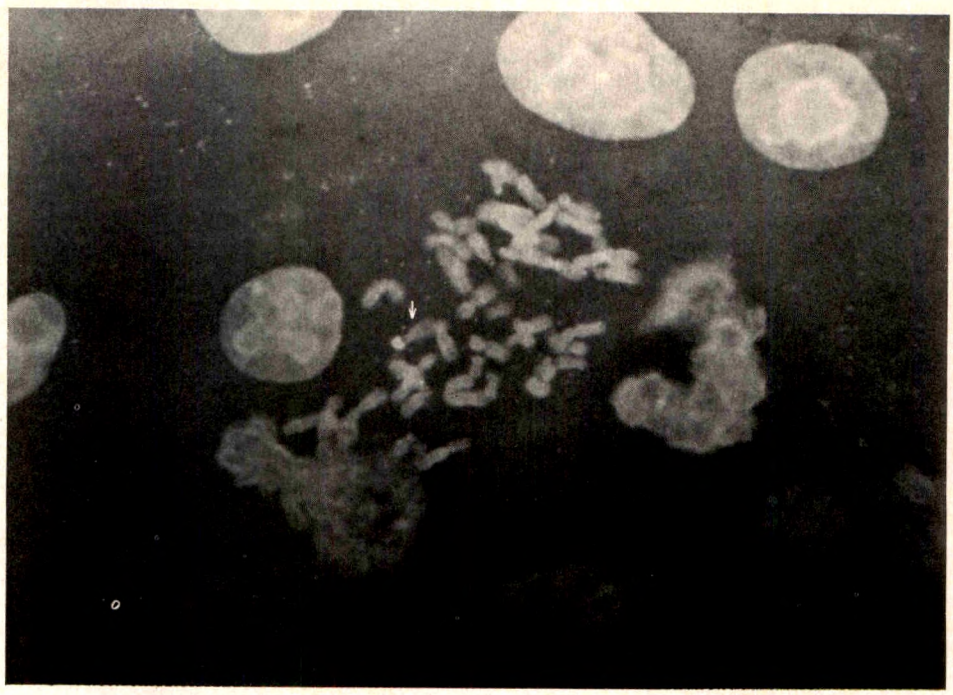


Fig. 5. Biquinacrine-banded chromosome analysis. The translocated chromosome 15 indicated by the *arrow* carries a brightly staining chromosome region typical of the inactive repetitive region of the Y chromosome. This result confirms that the karyotype is 46,XX,−15,+t(15;Y)(p13;q12).

Comment

Polymerase chain reaction is a rapid and sensitive method to analyze a specific DNA sequence. Sequence-specific oligonucleotide primers are extended by *Taq* polymerase isolated from *Thermus aquaticus*. Because this bacterium grows well in hot springs, all of its enzymes have adapted to become heat resistant. Thus DNA can be denatured repeatedly at high temperature in the presence of *Taq* polymerase without destroying polymerase activity. In addition, improved fidelity is maintained during elongation at higher temperatures. (See Fig. 2 for further details.) Polymerase chain reaction has been applied to analyze the DNA contained in as little as 10 μl of amniotic fluid,[2] a single chorionic villus (our laboratory), or a single cell equivalent[7] (our laboratory). The amount of DNA needed for polymerase chain reaction is small enough that an amniocentesis sample or chorionic villus sample is sufficient for both polymerase chain reaction and any other cytogenetic or biochemical test required. The test is rapid because the DNA polymerase generates thousands of duplicate fragments in hours. The amplification is not only quite efficient (50% to 90% duplication per cycle) but highly specific as it amplifies the sequences representing only 0.04% of the total DNA added to the reaction mix.

In contrast to the specificity of polymerase chain reaction, the dot blot test originally used for fetal sex determination gave false-positive results on three occasions. The dot blot assay depends on the quantitative hybridization of the Y-repetitive probe to Y-specific sequences. However, cross hybridization to autosomal sequences in female DNA decreases the ability to differentiate a male-specific signal. Repeat sequences located immediately beneath the centromere on chromosome 15 have been found to hybridize to the Y-repetitive probe at the assay conditions used. The size of this chromosome 15 region has been demonstrated to be normally variable by DIPI [4′,6-*bis*(2′-imidazolinyl-4′-5′H)-2-phenylindole] staining of distamycin A–stained chromosomes[8] and confirmed by flow analysis of chromosomes.[9] The heterochromatic region of chromosome Y is also known to vary substantially in size in normal men. Differentiating two large chromosome 15 repeat sequence regions in a female from a small heterochromatic Y-chromosome region can be difficult. These normal variations in size probably account for the false-positive results.

The application of polymerase chain reaction is uncomplicated and in most cases generates a result in 4 to 6 hours. The utilization of heat-stable *Taq* polymerase and a programmable polymerase chain reaction machine minimizes laboratory effort and makes this assay routine. The ability to obtain a rapid, accurate sex determination allows families at risk for an X-linked disease more time to make decisions regarding the fetus. It also reduces pressure on laboratory personnel with a heavy workload who need to prioritize diagnostic

tests. These characteristics make polymerase chain reaction sex testing an ideal tool for prenatal studies. Future applications of this technique will be limited only by the number of genes cloned and sequenced and by the imagination of the investigators studying them.

We thank Robert Flandermeyer for biquinacrine staining, Dr. Steven Schonberg for Giemsa-banded karyotypes, and Drs. James Goldberg and Robert Anderson for patient's samples. We thank C. C. Lin for his generous gift of biquinacrine hydrochloride.

REFERENCES

1. McKusick VA. Mendelian inheritance in man. Baltimore: Johns Hopkins, 1988:11.
2. Lay YF. Organization of the human Y-specific HaeIII 3.4-kb repeat sequences and their application in clinical diagnosis. In: The Y chromosome. Part A. Basic characteristics of the Y chromosome. New York: Alan R Liss, 1985:177-92.
3. Kogan S, Doherty M, Gitschier J. An improved method for prenatal diagnosis of genetic diseases by analysis of amplified DNA sequences. N Engl J Med 1987;317:985-90.
4. Saiki RK, Scharf S, Faloona F, et al. Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science 1985;230: 1350-4.
5. Wong C, Dowling CE, Saiki RK, Higuchi RS, Erlich HA, Kazazian HH Jr. Direct genomic sequencing of amplified single copy DNA: rapid characterization of unknown β-thalassemia mutations. Nature 1987;330:384-6.
6. Ou C-Y, Kwok S, Mitchell SW, et al. DNA amplification for direct detection of HIV-1 in DNA of peripheral blood mononuclear cells. Science 1988;239:295-7.
7. Honghua L, Gyllensten UB, Cui X, Saiki RK, Erlich HA, Arnheim N. Amplification and analysis of DNA sequences in single human sperm and diploid cells. Nature 1988; 335:414-7.
8. Schweizer D. Counterstain-enhanced chromosome banding. Hum Genet 1981;57:1-14.
9. Lebo RV, Golbus MS, Cheung M-C. Detecting abnormal human chromosome constitutions by dual laser flow cytogenetics. Am J Med Genet 1986;25:519-29.

"Possibly" de novo translocations: Prenatal risk counseling

E. Robert Wassman, MD, Debra L. Cheyovich, DrPH, and Yasunori Nakahara, MD

Pasadena, California

De novo rearrangements incidentally found at prenatal diagnosis may carry an increased risk of abnormal outcome. Situations in which the paternal chromosomes cannot be analyzed (e.g., artificial insemination, nonpaternity, deceased or otherwise unavailable father) are only "possibly" de novo. In our series of 23,495 prenatal diagnoses we unexpectedly found 67 balanced translocations. Of these, 26 were Robertsonian and 41 were reciprocal. A total of 45 (67%) of these translocations were inherited, and 19 (28%) were apparently de novo. Inherited translocations were maternal in 25 cases (55%) and paternal in 20 cases (45%). Follow-up was available in 87% overall, and revealed abnormal outcomes in 2/15 de novo cases, and 2/38 inherited cases. We suggest that 10% may be a reasonable estimate of risk for de novo translocations found at prenatal diagnosis. Given the observed distributions of inherited cases, a lower risk of abnormal outcome should be counseled in "possibly" de novo cases. (Am J Obstet Gynecol 1989;161:698-702.)

Key words: De novo translocations, prenatal diagnosis, paternity

The occurrence of mental retardation or malformations in persons with apparently balanced translocations has been well documented.[1,2] The excess of such translocations that are de novo in populations of the mentally retarded,[3,4,5] as compared with general newborn surveys[6,7] has suggested that such noninherited rearrangements are the cause of the impairment in those individuals. Submicroscopic chromosome loss, position effect, and breakpoint site mutations have been suggested as plausible hypotheses to explain this observation.[1]

The widespread use of prenatal cytogenetic diagnosis for advanced maternal age and other indications[8,9] has predictably lead to numerous cases of incidental detection of apparently balanced translocations in the fetus. This finding requires parental chromosome analysis and accurate genetic counseling. Generally, translocations inherited from a normal parent have been considered to carry little or no inherent risk of abnormal outcome. However, on the basis of the surveys

From The Genetics Institute.
Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.
Reprint requests: E. Robert Wassman, MD, Medical Director, ALFIGEN–The Genetics Institute, 11 W. Del Mar, Pasadena, CA 91105.
6/6/14096

cited previously, de novo rearrangements have been considered to carry an increased risk that remains poorly defined despite Warburton's (1984)[10] collaborative study of such cases.

Situations in which the paternal chromosomes cannot be analyzed can further confound the genetic counseling. Artificial insemination, nonpaternity, death of the father, parental separation, non-pair-bonded coupling, and extended travel may all be potential causes for the problem of the "possibly" de novo translocation. Experience with several such cases and the belief that they should be counseled at a lower risk than that for established de novo cases prompted us to review our experience in a private prenatal diagnostic center.

We have used detailed information on the 67 apparently balanced translocations found incidentally among 23,495 consecutive prenatal cytogenetic studies at The Genetics Institute between 1981 and 1987, and conducted follow-up surveys on those cases. These data are compiled with other similar series to attempt to obtain a reasonable calculation of the risk of abnormal outcome.

Material and methods

The Genetics Institute is a private practice of medical genetics including prenatal diagnosis and other clinical and laboratory services. All records of The Institute's cytogenetic laboratory in Pasadena, California, were systematically reviewed and compiled from its founding in 1981 through the end of 1987.

Approximately 25% of referrals use the Institute as a reference laboratory, whereas the remainder represent cases from our clinics. Cases are routinely analyzed with quinacrine dihydrochloride fluorescence banding by established methods.[11] A maternal blood sample is cultured along with each case and analyzed when needed.[12] For this study, all cases in which a nonmosaic apparently balanced translocation was found were reviewed in detail. Cases in which an unbalanced rearrangement was found were not considered, nor were cases in which the indication for amniocentesis was for a known familial translocation. A total of 67 cases of such incidentally found balanced translocations were encountered among the 23,495 prenatal diagnoses reviewed. The parental chromosome analyses were similarly reviewed and the cases were divided into three categories—inherited, de novo, and unknown.

No infants or abortuses were examined by the authors in this process. Systematic telephone follow-up with regard to malformations and developmental milestones with either the delivering obstetrician, subsequent pediatrician, or patient was attempted in all cases in addition to our routine postnatal follow-up form requested from referring physicians. Cases were considered for outcome classification based on the status at the point of longest follow-up. Therefore in a case in which difficulties were encountered at term but the infant subsequently demonstrated normal growth and development and was without malformations noted, the outcome was considered normal.

Table I. Distribution of balanced translocations incidentally found at amniocenteses

	Maternal	*Paternal*	*De novo*	*Unknown*	*Total*
Reciprocal	15	11	13	2	41
Robertsonian	10	9	6	1	26
TOTAL	25	20	19	3	67

Results

In our series of 23,495 prenatal diagnoses (mean age = 36.6 years), 67 balanced nonmosaic translocations were incidentally found. Only 12 additional instances of balanced translocations were found in patients referred because of a known familial balanced translocation, and these were excluded from the present study. The primary indication for amniocentesis (47/67) was advanced maternal age. The mean maternal age for inherited and de novo cases were 34.7 years old and 35.5 years old, respectively. Five patients were referred because of abnormal maternal serum α-fetoprotein results—four high and one low. Three cases were from twin gestations; one of which was dizygotic based on discordance of the translocation finding.

Of the 67 translocations studied, 26 were Robertsonian and 41 were reciprocal. A total of 45 (67%) of the translocations were inherited from a parent and 19 (20%) were presumed to be de novo on the basis of parental chromosome studies (Table I). Inherited translocations were from the mother in 25 instances (55%) and from the father in 20 instances (45%).

The Robertsonian translocations were predominantly (73%) 13q14q, of which 26% were de novo. The next most frequent Robertsonian translocations were 14q21q (three cases) and 14q15q (two cases). Only the 13q22q and 15q21q possible Robertsonian combinations were not observed. No X-autosome translocations were observed in this series. Other cytogenetic abnormalities were seen in five cases: two cases of pericentric inversion of chromosome 9; two of trisomy 21; and one of trisomy 18.

Follow-up information was available in 87% of all cases (58/67), and 95% of de novo cases (18/19). In the inherited group, aside from two cases terminated because of a concomitant trisomy found, no elective terminations of pregnancy occurred. Of the remainder of the familial cases, one infant who was apparently

Table II. Follow-up: De novo and unknown cases

De novo	*Outcome*	*Unknown*	*Outcome*
t(10;18)	Normal 3 yr female	t(14q15q)	Normal newborn 6 lb 15 oz male
t(8;15)	Normal 3 yr female	t(13;14)	No data
t(3;16)	Normal 8 mo male	t(15;20)	Normal 8 yr female
t(15;17)	Elective abortion because of trisomy 21		
t(2;14)	Normal 7 mo male; placental defect, abruptio placentae, disseminated intravascular coagulation at birth		
t(3;20)	Normal 6 mo male; mild micrognathia at birth		
t(6;22)	Normal newborn male		
t(15;19)	Normal newborn 6 lb 13 oz male		
t(4;8)	Elective abortion		
t(4;16)	Normal 3 yr female		
t(1;11)	Normal newborn male		
t(1;13)	Normal newborn 8 lb female		
t(6;8)	No data		
t(13q14q)	Normal 3 ½ yr male		
t(13q14q)	Elective abortion because of neural tube defect		
t(13q14q)	Elective abortion		
t(14q21q)	Normal newborn 8 lb 6 oz male		
t(13q14q)	Normal 7 mo male		
t(13q14q)	Fetal death; Rh factor sensitization(?)		

normal at birth subsequently had developmental delay, and another had ascites detected prenatally and was born prematurely with intrauterine growth retardation, hepatosplenomegaly, and ascites, presumably all of viral etiology.

In the de novo group (Table II), two patients elected to terminate their pregnancies without abnormalities noted by ultrasonography. Fetal examinations were not possible in these patients. One patient terminated the pregnancy because of a concomitant finding of trisomy 21. Another patient underwent an elective abortion because of a neural tube defect detected by ultrasonography and α-fetoprotein analysis. A single fetal death occurred that reportedly was a result of rhesus factor sensitization. Of infants carried to term, we have specific follow-up data for ≥3 years in four cases and 6 months to 1 year in four more cases, without any abnormal outcomes noted. One case with a placental defect detected by ultrasonography and abruptio placentae disseminated intravascular coagulation at term, has subsequently done well, and another with mild micrognathia at birth has similarly developed normally.

Normal follow-up results were available on two thirds of the "possibly" de novo cases.

Comment

The incidental detection of an apparently balanced chromosomal rearrangement at prenatal diagnosis can be extremely discomforting to a patient who expects "clear cut" normal results versus abnormal results. The reassuring value of finding the same structural aberration in a parent is immeasurable. Uncertainty clearly remains in cases of de novo rearrangements and in situations in which the father or rarely both parents cannot be studied. Unavoidable circumstances, social situations in present-day society, and increased use of artificial insemination donors may be expected to increase the number of such "possibly" de novo cases, and it is necessary to provide a reasonable approach to counseling in these situations. We therefore conducted this follow-up survey from a single very large center and compiled it with comparable data from the literature to attempt to provide such information. We focused solely on translocations; however, similar problems arise with other types of chromosomal rearrangements and rare variants.

In our prenatal diagnosis population we found a rate of presumed de novo translocations of 0.8:1000. This is slightly higher than the previously reported rates from amniocenteses.[13-15] This may reflect the more complete ascertainment of parental carriers than is possible in collaborative studies,[14, 15] and the larger numbers in our series than in previous single center studies.[13] This rate is twofold to threefold higher than that observed in newborn surveys.[6, 7] Inasmuch as the mean age of our de novo group (35.5 years) is advanced and comparable to our prenatal diagnosis population (36.6 years), this may support a previous suggestion that the rate of de novo translocations is maternal age–dependent.[14]

It is of interest that none of our patients was referred because of a primary indication of exposure to potential teratogens or mutagens. This category was represented in excess in the series reviewed by Hook et al.[14] However, four cases of our 67 patients were referred because of elevated maternal serum α-fetoprotein values.

Table III. Clinical outcomes of incidentally discovered balanced translocations

Reference	Total cases	*Inherited*		*De novo*	
		Normal	*Abnormal*	*Normal*	*Abnormal*
Present study	23,495	37	2	13	2
Vejerslev and Friedrich[15] (1984)	5,547	14	0	2	1
Nielsen and Krag-Olsen[7] (1931)	11,148	24	3	5	0
Warburton[10] (1984)	76,952	—	—	53	4
TOTAL	117,142	75	5	73	7

Overall, the number of referrals because of abnormal maternal serum α-fetoprotein values are increasing as this test and ultrasonography are used as noninvasive indices of fetal abnormality and replace some of the older indications as the primary reason for referral.[16] In 1987, 14% (3/22) of our incidental balanced translocations were referred because of high maternal serum α-fetoprotein values, whereas only 3.9% of the total referrals in that year were because of this reason. Only one of our four cases was a de novo translocation, which corresponds with the percentage of de novo cases in our total population (28%).

Although the numbers are too small for meaningful statistical analysis, we observed a possible excess of balanced translocations in trisomic fetuses. Two cases of trisomy 21 were observed and one case of trisomy 18. One of the cases was concomitant with a de novo reciprocal translocation, whereas the other two cases were with a maternally derived reciprocal translocation and a paternally derived Robertsonian translocation, respectively. Two cases were also seen in which a pericentric inversion of chromosome 9 was found; however, in neither case was this variant inherited from the same parent as the translocation.

Our distribution of translocation types (Robertsonian/reciprocal = 0.63) is intermediate between previous amniocentesis series (Robertsonian/reciprocal = 0.25)[13-15] and newborn surveys (Robertsonian/reciprocal = 1.03).[6,7] Reasons for the differences are unclear.

Our series reflects a slight excess of maternally derived rearrangements among the inherited translocations, which is more so within the reciprocal rearrangements (mat/pat = 1.36) than Robertsonian (mat/pat = 1.11). This is also comparable to previous observations.[6,7,13,14] All but one of our reciprocal translocations would be expected to generally segregate 2:2, and the slight skew toward maternal carriers here is very close to that observed by Daniel et al.[17] in relation to unbalanced progeny.

The mutation rate for Robertsonian rearrangements was lower (23% versus 38%) than that observed by Hook et al.,[14] but our rate for reciprocals matched theirs closely (31% versus 29%, respectively).

Follow-up data on balanced translocations found at prenatal diagnosis have been difficult to obtain. The presumption is often made that familial rearrangements should not differ from the general population. Warburton[10] used a collaborative series to evaluate the outcome of cases of de novo structural rearrangements found at amniocenteses. An excess of abnormalities was suggested by Warburton's study but not on the order of magnitude seen in selective population surveys.[3-5] This evaluation was limited by the still relatively small numbers involved and the limited duration of follow-up. An upper limit of risk of 14% for any type of balanced structural rearrangement was suggested by Warburton.[10] We have been able to obtain specific follow-up data on 95% of our de novo balanced translocations, of which nearly one third were ≥3 years old and another one third were ≥6 months old. Combination of our follow-up data with that on translocations only from Warburton's study already cited,[10] a smaller single-center prenatal study,[13] and a consecutive newborn series from Denmark[7] allowed analysis of 158 translocations culled from 93,647 cases (Table III). Only five abnormal outcomes were noted among 80 inherited translocations, and only seven among 80 de novo cases. These rates are not significantly different from one another by χ^2 analysis or from a conservative estimate of the expected frequency of abnormality in the general population of 3% to 4%.

It appears that with careful follow-up the majority of cases of balanced translocations incidentally found at amniocentesis can be determined to be either inherited or de novo. Despite this, delays in the procurement of samples can be as problematic as total nonavailability of samples. To preclude the problems discussed herein, we strongly urge cytogenetic and other genetic screening in proposed anonymous artificial insemination donors.[18]

The pooled data suggest that for known de novo translocations, the risk for abnormal outcome is not significantly greater than that of the inherited group, nor the general population; however, complete long-term developmental follow-up of such cases remains limited. Certainly, the experience with population stud-

ies of the retarded[8-5] suggest caution in counseling of these seemingly nonsignificant risks. It remains possible that some additional biasing factor is responsible for the differences observed, and that a subgroup of prenatally detected cases may have comparable risk levels. After careful ultrasonographic evaluation has proved normal, we recommend that a maximum risk of 10% be used for such cases, and that the risk is roughly one half of that figure for "possibly" de novo cases because of paternal nonavailability. Given the similarity between outcome in inherited and de novo cases we do not recommend further pursuit of possibly false paternity in de novo cases.

We thank the entire staff of The Genetics Institute, its referring physicians, Helen Hamaguchi, RN, Sonia Martinez, MD, and Anita Yousoofian for their assistance in the study and preparation of this manuscript.

REFERENCES

1. Tharapel AT, Summitt RL, Wilroy RS, Martens P. Apparently balanced de novo translocations in patients with abnormal phenotypes: report of 6 cases. Clin Genet 1979;11:255-69.
2. Funderburk SJ, Spence MA, Sparkes RS. Mental retardation associated with "balanced" chromosome rearrangements. Am J Hum Genet 1977;29:136-41.
3. Coco R, Penchaszadeh VB. Cytogenetic findings in 200 children with mental retardation and multiple congenital anomalies of unknown cause. Am J Med Genet 1982; 12:155-73.
4. Faed MJW, Robertson J, Field MAS, Mellon JP. A chromosome survey of a hospital for the mentally subnormal. Clin Genet 1979;16:191-204.
5. Li S-Y, Tsai C, Chou M, Lin J. A cytogenetic study of mentally retarded school children in Taiwan with special reference to the fragile X chromosome. Hum Genet 1988;79:292-6.
6. Jacobs P. Mutation rates of structural chromosome rearrangements in man. Am J Hum Genet 1981;33:44-54.
7. Nielsen J, Krag-Olsen B. Follow-up of 32 children with autosomal translocations found among 11,148 consecutively newborn children from 1969 to 1974. Clin Genet 1981;20:48-54.
8. Benn PA, Hsu LYF, Carlson A, Tannenbaum HL. The centralized prenatal genetics screening program of New York City III: the first 7,000 cases. Am J Med Genet 1985;20:369-84.
9. Terzian E, Boreham J, Cuckle HS, Wald NJ. A survey of diagnostic amniocenteses in Oxford from 1974-1981. Prenat Diagn 1985;5:401-13.
10. Warburton D. Outcome of cases of de novo structural rearrangements diagnosed at amniocentesis. Prenat Diagn 1984;4:19-80.
11. Alfi OS, Donnell GN, Derencsenyi A. Quinacrine fluoromicroscopy in the identification of human mitotic chromosomes. Pediatrics 1971;48:423-5.
12. Alfi OS, Derenscenyi A, Donnell GN. Chromosome polymorphism and prenatal diagnosis. Lancet 1975;1:1253.
13. Vejerslev LO, Friedrich U. Experiences with unexpected structural chromosome aberrations in prenatal diagnosis in a Danish series. Prenat Diagn 1984;4:181-6.
14. Hook EB, Schreinemachers DM, Willey AM, Cross PK. Rates of mutant structural chromosome rearrangements in human fetuses: data from prenatal cytogenetic studies and associations with maternal age and parental mutagen exposure. Am J Hum Genet 1983;35:96-109.
15. Fergusen-Smith MA, Yates JRW. Maternal age specific rates for chromosome aberrations and factors influencing them: report of a collaborative European study on 52,965 amniocentesis. Prenat Diagn 1984;4:5-44.
16. Platt LD, De Vore GR, Alfi O, Falk R. The role of amniocentesis in ultrasound-detected fetal malformations. Obstet Gynecol 1986;68:153-5.
17. Daniel A, Hook EB, Wulf G. Risk of unbalanced progeny at amniocentesis to carriers of chromosome rearrangements. Data from European, U.S.A. and Canadian laboratories. Am J Hum Genet 1988;43:A230;918.
18. Fraser FC, Forse RA. On genetic screening of donors for artificial insemination. Am J Med Genet 1981;10:399-405.

Amniotic fluid acetylcholinesterase ratios in prenatal diagnosis of fetal abnormalities

JoAnn C. Kelly, PhD, Elizabeth Petrocik, and E. Robert Wassman, MD
Pasadena, California

This study examined 141 pregnancies with acetylcholinesterase in the amniotic fluid to determine if an acetylcholinesterase/pseudocholinesterase ratio cutoff could be set to distinguish between neural tube defects, ventral wall defects, other fetal defects, and pregnancy problems. Amniotic fluid specimens with positive or faintly positive acetylcholinesterase bands were studied. They were scanned and analyzed for the acetylcholinesterase/pseudocholinesterase ratio. In 62 of 65 fluid samples from open neural tube defects, the acetylcholinesterase/pseudocholinesterase ratios were >0.13. All cases with ventral wall defects (n = 29), fetal blood contamination (n = 16), or fetal ascites (n = 2) had acetylcholinesterase/pseudocholinesterase ratios <0.13. Samples of cystic hygroma fluid (n = 10) had acetylcholinesterase/pseudocholinesterase ratios >0.13. Two patients with fetal teratomas had intermediate values. All patients with normal outcomes but positive acetylcholinesterase had ratios of <0.12. Acetylcholinesterase/pseudocholinesterase ratios are a valuable part of a prenatal program to accurately diagnose fetal abnormalities. (AM J OBSTET GYNECOL 1989;161:703-5.)

Key words: Acetylcholinesterase, AChE, neural tube defects

Gel electrophoresis acetylcholinesterase has proved useful in differentiating false-positive amniotic fluid α-fetoprotein values from those indicating true fetal defects.[1-3] Detection of acetylcholinesterase not only provides biochemical evidence for the presence of a neural tube defect[4] but also reduces the incidence of false-positive diagnoses that are based on α-fetoprotein determinations.[5] The presence of acetylcholinesterase, however, is not specific for neural tube defects. It can be found in the presence of other fetal defects and pregnancy complications, including ventral wall defects and fetal blood contamination.[6, 7] Earlier studies have shown that neural tube defects can usually be distinguished from ventral wall defects by using a densitometric scan ratio of acetylcholinesterase to pseudocholinesterase.[6, 8, 9]

In this study 141 pregnancies with known outcomes and with acetylcholinesterase in the amniotic fluid were examined to determine a cutoff for the acetylcholinesterase/pseudocholinesterase ratio that could distinguish among neural tube defects, ventral wall defects, other fetal defects, and pregnancy problems.

Material and methods

One hundred forty-one amniotic fluid specimens with positive or faintly positive acetylcholinesterase bands were collected between January 1, 1986, and January 1, 1989, and stored at −20° C. They were retrieved from storage, electrophoresed in duplicate a second time, scanned, and analyzed for the acetylcholinesterase/pseudocholinesterase ratio. Ratios reported here were the average of two determinations. The pregnancy outcomes were known in all cases. All samples included in the study had elevated or "high normal" amniotic fluid α-fetoprotein levels of >1.7 multiples of the median. All samples negative for acetylcholinesterase, regardless of α-fetoprotein level, were excluded from the study. Samples obviously contaminated with blood were checked for fetal cells with a modified Kleihauer-Betke stain.[10]

Polyacrylamide slab gel electrophoresis was performed with a minislab gel electrophoresis apparatus.[2, 6, 8] The acetylcholinesterase-specific inhibitor BW284c51 was used to confirm the identity of the diagnostic band. The gels were stained with dithiooxamide to amplify the cholinesterase band densities. The gels were scanned on a Helena Laboratory Cliniscan 2 densitometer. The pseudocholinesterase and acetylcholinesterase bands were denoted as those in positions 1 and 2 by Smith et al.[1]

Results

There were 65 pregnancies in which there was an open neural tube defect in the fetus: 11 with anencephaly, 49 with open spina bifida, and 5 with open encephalocele. Of the 65 open neural tube defect pregnancies, 62 had acetylcholinesterase/pseudocholinesterase ratios >0.13 (see Table I). All three patients with

From The Genetics Institute.
Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.
Reprint requests: Dr. JoAnn C. Kelly, The Genetics Institute, 11 West Del Mar Blvd., Pasadena, CA 91105.
6/6/13893

Table I. Acetylcholinesterase/pseudocholinesterase ratios in amniotic fluid samples

	n	*Acetylcholinesterase/pseudocholinesterase ratio*	
		Mean ± 1 SD	*Range*
Neural tube defect			
Anencephaly	11	0.27 ± 0.11	0.12-0.46
Open spina bifida	49	0.48 ± 0.16	0.11-0.94
Encephalocele	5	0.27 ± 0.15	0.07-0.43
Ventral wall defect			
Gastroschisis	19	0.06 ± 0.02	0.03-0.11
Omphalocele	10	0.06 ± 0.02	0.02-0.09
Cystic hygroma fluid	10	0.34 ± 0.11	0.16-0.49
Fetal ascites	2		0.04, 0.12
Bloody fluid	16	0.05 ± 0.03	0.02-0.08
False positive results	6	0.05 ± 0.04	0.01-0.12

lower acetylcholinesterase/pseudocholinesterase ratios had confirmed open defects (anencephaly, spina bifida, or encephalocele). One additional pregnancy with a membrane-covered spina bifida cystica had a ratio of 0.03. All 29 of the fluids from fetuses with ventral wall defects had acetylcholinesterase/pseudocholinesterase ratios that were <0.11 (Table I).

Twelve pregnancies with abnormal fetal fluid accumulations were studied. The 10 specimens in which fluid was obtained from cystic hygromas had acetylcholinesterase/pseudocholinesterase ratios >0.16 (Table I). Two pregnancies with fetal ascites fluid had relatively low ratios (0.04 and 0.12) in the amniotic fluid.

There were 16 bloody amniotic fluid specimens in which fetal blood cells were identified. All of these samples had acetylcholinesterase/pseudocholinesterase ratios <0.08 (Table I). In addition, there were six samples with acetylcholinesterase present in the amniotic fluid but ultrasonographic examinations, karyotypes, and outcomes were normal. These six cases represent true false-positive results, but in no case was the acetylcholinesterase/pseudocholinesterase ratio >0.12 (Table I).

Twelve other cases of miscellaneous fetal and pregnancy problems also had acetylcholinesterase in the amniotic fluid and were examined for the acetylcholinesterase/pseudocholinesterase ratios (see Table II). Three cases of chromosomal abnormalities were found that had elevated α-fetoprotein levels with acetylcholinesterase in the amniotic fluid. These cases included two of trisomy 13 without scalp defects and one of trisomy 21. There were no open fetal defects observed in these cases. There were five cases involving death of one or more fetuses (Table II). In each of these cases the acetylcholinesterase/pseudocholinesterase ratio was determined. In four of the five cases ratios were in the range seen with neural tube defects; however, the migratory pattern was usually smeared and visibly different. Two cases of fetal teratomas studied had acetylcholinesterase/pseudocholinesterase ratios of 0.18 and 0.08. One case of fetal hydronephrosis had a very light band with a ratio of 0.01. Finally, one case in which the fluid was drawn from a renal cyst had an intermediate acetylcholinesterase/pseudocholinesterase ratio of 0.14.

Table II. Acetylcholinesterase/pseudocholinesterase ratios in amniotic fluid from pregnancies with chromosomal and other fetal problems

Defect	*Acetylcholinesterase/pseudocholinesterase ratio*
Chromosomal abnormality	
Trisomy 13	0.10
Trisomy 13, fetal death	0.02
Trisomy 21	0.08
Fetal death	
1 Fetus of triplets	0.18
Both twins	0.32
Both twins	0.37
After amniocentesis	0.09
1 Fetus of twins	0.27
Teratoma	
Case 1	0.18
Case 2	0.08
Miscellaneous	
Renal cyst fluid	0.14
Fetal hydronephrosis	0.01

Comment

The results from this study confirm and extend earlier reports on the usefulness of acetylcholinesterase electrophoresis as a secondary test in the prenatal diagnosis of neural tube defects and other fetal and pregnancy problems.[1-3] Acetylcholinesterase testing enhances the specificity of prenatal testing for neural tube defects.[4,5] The presence of acetylcholinesterase in the amniotic fluid increases the odds of finding a neural tube defect when the amniotic fluid α-fetoprotein level is elevated. However, positive acetylcholinesterase results have also been noted with ventral wall defects and other conditions.[6,7] Densitometric scanning of acetylcholinesterase electrophoresis gels is useful in the differential diagnosis of fetal defects. In this study we scanned gels of amniotic fluid samples that contained acetylcholinesterase from pregnancies with various problems to determine whether the acetylcholinesterase/pseudocholinesterase ratios would be consistent and useful in the interpretation of the results. Our results in the pregnancies with neural tube defects and ventral wall defects were in accord with those of previous reports.[6,9] In those smaller studies a ratio of 0.15 was used to differentiate neural tube defects and ventral wall defects. In our laboratory we found that a comparable cutoff value of 0.13 gave the most consistent interpretation.

This study also included amniotic fluid from other

fetal and pregnancy conditions. It was determined that fluid from cystic hygromas was similar to fluid from pregnancies with neural tube defects. Fetal ascitic fluid, however, yielded lower ratios. Bloody specimens that contain fetal blood cells can have acetylcholinesterase present in the amniotic fluid and, as such, can yield conflicting results. In this study it was observed that all such bloody fluid samples had ratios lower than those seen with neural tube defects.

Several other conditions yielded positive acetylcholinesterase results. There were three pregnancies with fetal aneuploidy without the presence of neural tube defects that were positive. In each of these cases the acetylcholinesterase/pseudocholinesterase ratio was less than the cutoff of 0.13. The presence of fetal death also can cause positive acetylcholinesterase results. In these cases the gel pattern frequently appears somewhat different, although the scanning results were variable. Fetal teratomas, renal cysts, and hydronephrosis can also cause acetylcholinesterase to be present in the amniotic fluid. Ultrasonographic examination is useful in the interpretation of the results in such cases.

There were six actual false-positive specimens in this study that had elevated α-fetoprotein levels and also were positive for acetylcholinesterase. These six specimens all had ratios that were lower than those seen with neural tube defects. High-resolution ultrasonography is often helpful and should always be offered to patients with elevated amniotic fluid α-fetoprotein levels and positive acetylcholinesterase electrophoresis. Patients with normal ultrasonograms, normal karyotypes, and normal outcomes had ratios of <0.09. Overall, significant exceptions to the findings occurred in <4% of all cases. Acetylcholinesterase/pseudocholinesterase ratios are a valuable part of a prenatal program to accurately diagnose fetal abnormalities.

REFERENCES

1. Smith AD, Wald NJ, Cuckle HS, Stirrat GM, Bobrow M. Lagercrantz H. Amniotic fluid acetylcholinesterase as a possible diagnostic test for neural tube defects in early pregnancy. Lancet 1979;1:685.
2. Barlow RD, Cuckle HS, Wald NJ. A simple method for amniotic fluid gel-acetylcholinesterase determination, suitable for routine use in the antenatal diagnosis of open neural tube defects. Clin Chim Acta 1982;119:137.
3. Report of the Collaborative Acetylcholinesterase Study. Amniotic fluid acetylcholinesterase electrophoresis as a secondary test in the diagnosis of anencephaly and open spina bifida in early pregnancy. Lancet 1981;2:32.
4. Brock DJH, Hayward C. Gel electrophoresis of amniotic fluid acetylcholinesterase as an aid to the prenatal diagnosis of fetal defects. Clin Chim Acta 1980;108:135.
5. Haddow JE, Morin ME, Holman MS, Miller WA. Acetylcholinesterase and fetal malformations: modified qualitative technique for diagnosis of neural tube defects. Clin Chem 1981;27:61.
6. Wald NJ, Barlow RD, Cuckle HS, Turnbull AC, Goldfine C, Haddow JE. Ratio of amniotic fluid acetylcholinesterase to pseudocholinesterase as an antenatal diagnostic test for exomphalos and gastroschisis. Br J Obstet Gynaecol 1984;91:882.
7. Barlow RD, Cuckle HS, Wald NJ, Rodeck CH. False positive gel-acetylcholinesterase results in blood-stained amniotic fluids. Br J Obstet Gynaecol 1982;89:821.
8. Goldfine C, Miller WA, Haddow JE. Amniotic fluid cholinesterase density ratios in fetal open defects of the neural tube and ventral wall. Br J Obstet Gynaecol 1983;90:238.
9. Burton BK. Positive amniotic fluid acetylcholinesterase: distinguishing between open spina bifida and ventral wall defects. Am J Obstet Gynecol 1986;155:984.
10. Clayton EM Jr, Foster EB, Clayton EP. New stain for fetal erythrocytes in peripheral blood smears. Obstet Gynecol 1970;35:642.

Effect of diagnostic and therapeutic cordocentesis on maternal serum α-fetoprotein concentration

Carl Weiner, MD,[a] Stanley Grant, RN,[a] Jerry Hudson, PhD,[b] Roger Williamson, MD,[a] and Katharine Wenstrom, MD[a]

Iowa City, Iowa

We investigated the affect of cordocentesis (n = 36) and intravascular transfusion (n = 14) performed with a fixed needle guide on maternal serum α-fetoprotein levels. In 50% of the procedures, the placenta was anterior and punctured. For all patients, maternal serum α-fetoprotein levels rose 70.8% ± 28%. The magnitude of the rise was unrelated to the number of attempts necessary, gestational age, or the initial maternal serum α-fetoprotein levels concentration. The location of the placenta was the sole identified variable related to the rise. A total of 44% of the patients had a significant increase in maternal serum α-fetoprotein level when the placenta was anterior. In contrast, only 4% of the patients had a significant rise in maternal serum α-fetoprotein level when the placenta was other than anterior. There was no difference in the frequency of a significant rise in maternal serum α-fetoprotein level concentration between the patients who underwent cordocentesis and those who underwent intravascular transfusion. On the basis of these findings, we recommend that when maternal isoimmunization is a potential concern and the placenta is anterior, the umbilical cord should be approached either through a window or laterally from the placenta. (AM J OBSTET GYNECOL 1989;161:706-8.)

Key words: α-Fetoprotein, cordocentesis, pregnancy, placenta

Cordocentesis is both safe and efficient in experienced hands.[1, 2] It has become the method of choice to obtain a fetal blood sample. Amniocentesis is associated with a transient increase in maternal serum α-fetoprotein concentration.[3-7] Variables that affect the magnitude of the increase include needle size, the number of punctures necessary, and whether the needle traverses the placenta. It is likely the increase results from the leakage of either fetal blood, plasma, or both into the maternal circulation. One of the major indications for cordocentesis is the diagnosis and treatment of fetal hemolytic disease.[1] It has been opined at several scientific meetings that whereas cordocentesis has the advantage of supplying direct and accurate information compared with amniocentesis, it enhances maternal sensitization more than does amniocentesis. Should this be true, it will affect the indications and methods used for cordocentesis. A randomized trial that compares the efficacy of amniocentesis with cordocentesis for the evaluation of fetal hemolytic disease has not been, and for multiple reasons probably will not be, performed. This investigation has taken a different approach. We sought to determine prospectively the risk of maternal contamination with fetal blood-plasma during or shortly after cordocentesis by measuring the concentration of maternal serum α-fetoprotein.

Material and methods

Fifty consecutive women who underwent either diagnostic (n = 36) or therapeutic (intravascular transfusion, n = 14) cordocentesis for clinical indications were studied. This investigation was approved by the University of Iowa Human Subjects Committee and consent was obtained from each patient.

A specimen of maternal blood was obtained immediately before and 25 to 35 minutes after the procedure. All maternal serum α-fetoprotein measurements were performed by the State of Iowa Hygienics Laboratory, which participates in the Foundation for Blood Research's (Scarborough, Me.) external quality control program. The sample pairs were measured in duplicate by radioimmunassay (Amersham, Arlington Heights, Ill.) during the same run to eliminate interassay variation. Intra-assay variation was <10%.

Our method for cordocentesis has been described in detail elsewhere.[1] All procedures were performed with a 22-gauge spinal needle (Beck-Dickinson, Rutherford, N.J.) and a 5 mHz sector transducer (General Electric 3600, Rancho Mirage, Calif.) with an attached needle

From the Fetal Diagnosis and Treatment Unit, Division of Maternal Fetal Medicine, Department of Obstetrics and Gynecology, University of Iowa School of Medicine,[a] and State Hygienics Laboratory.[b]

Supported in part by Grant No. HD 24494.

Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.

Reprint requests: Carl Weiner, MD, Fetal Diagnosis and Treatment Unit, Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of Iowa School of Medicine, Iowa City, IA 52242.

6/6/13671

Table I. Placental location and increases in maternal serum α-fetoprotein levels

	N	*Percent with maternal serum α-fetoprotein rise >25 μg/ml*	*Percent with maternal serum α-fetoprotein rise > 40%*
Total by placental location	50	30	24
Anterior	25	52*	44†
Other	25	8*	4†

*$p = 0.003$.[5]
†$p < 0.05$.[3]

guide (CIVCO, Kalona, Iowa). No attempt was made to avoid the placenta.

Statistical analyses used subroutines from ABstat, rel 5.02 (Anderson-Bell, Parker, Colo.). The combined group and the diagnostic and therapeutic procedures were individually used as data sets. An independent Student *t* test was used to compare ordinal means, χ^2 was used with Yates correction to compare percentages, and linear regression was used to examine the relationship between maternal serum α-fetoprotein and the change in maternal serum α-fetoprotein level, gestational age, and the number of needle insertions. A *p* value ≤0.05 was considered significant.

Results

The planned procedure was successful in all patients and there were no complications. The mean (±SEM) gestational age was 27 weeks' gestation ± 0.7 weeks (range, 19 to 37 weeks). The placenta was located anteriorly in 50% of both diagnostic (18:36) and therapeutic (7:14) procedures. Placental location was unrelated to the number of attempts necessary. The maternal skin was punctured ≤three times in 94% (47:50) of the patients.

The initial maternal serum α-fetoprotein level correlated with gestational age ($r = 0.384, p = 0.006$). Maternal serum α-fetoprotein concentration rose a mean of 70.8% ± 28%, (range, −14.3% to 1058%) after the procedure. The number of attempts necessary to obtain the blood sample was unrelated to the percentage the maternal serum α-fetoprotein level rose. Further, there was no correlation between the percentage the maternal serum α-fetoprotein level rose and either gestational age ($r = 0.18$, $p = 0.22$) or the initial maternal serum α-fetoprotein concentration ($r = 0.08$, $p = 0.56$).

Placental location was the sole identified determinant of the rise. Maternal serum α-fetoprotein concentration rose 133% (52%) when the placenta was anterior (and therefore punctured) compared with 8.6% ± 8% when it was not ($p = 0.02$, two-tailed Student *t* test).

The results were assessed with two previously published criteria[3,5] for a significant rise in maternal serum α-fetoprotein levels after amniocentesis with stratification by placental location (Table I). The percentage of women who had a significant rise was greater when the placenta was anterior with both methods.

The above analyses was repeated when the participants were stratified by the type of procedure (diagnostic or therapeutic). The results were virtually identical and are not shown.

Comment

The number of centers that perform cordocentesis has grown rapidly. Because a major indication for cordocentesis is the diagnosis and treatment of fetal hemolytic disease, concern that cordocentesis might lead to enhanced maternal sensitization is understandable inasmuch as the placenta is often punctured and the needle position is often manipulated when a free-hand technique is used. We have observed the occasional patient who had a marked increase in the indirect Coombs titer after cordocentesis. However, many of these patients have had a history of severely affected offspring or high antibody titer at the outset.

The present study clearly documented a rise in the maternal serum α-fetoprotein concentration after both cordocentesis and intravascular transfusion. It does not address the risk of a "slow leak" nor does it attempt to discern between the leakage of fetal plasma versus whole blood. However, the study convincingly demonstrates that the risk of contamination is limited mostly to those women with an anterior placenta in which placental puncture was routine. With the use of previously published figures[3-7] (with correction for the distribution of placental locations), there is no obvious difference for the risk of a significant rise in maternal serum α-fetoprotein levels between amniocentesis and cordocentesis as performed in our institution with a fixed needle guide. We observed that the number of attempts required to obtain the needed sample was unrelated to the rise in maternal serum α-fetoprotein concentration. This may seem illogical. However, most patients with an anterior placenta required only one attempt. A larger number of patients with an anterior placenta who required multiple attempts might reveal a positive relationship between the number of attempts and the rise in maternal serum α-fetoprotein levels.

Fetal α-fetoprotein concentration declines with advancing gestation. Thus it is possible that a large leak relatively late in gestation might have minimal impact on the maternal serum α-fetoprotein level. A control group of patients who underwent amniocentesis across a range of gestational ages is not feasible in our institution. However, the lack of a correlation between gestational age and the magnitude the maternal serum α-fetoprotein level rose argues against this possibility.

In conclusion, cordocentesis is associated with a significant increase in maternal serum α-fetoprotein levels when the placenta is punctured. On the basis of these findings, we recommend that when maternal isoimmunization is a potential concern and the placenta is anterior, the umbilical cord should be approached either through a window or laterally from the placenta. If the woman has an Rh-negative blood type and is unsensitized, the fetal blood type should be determined. If the fetus has an Rh-positive blood type, anti-D immunoglobulin should be administered, as is the practice for amniocentesis.

REFERENCES

1. Weiner CP. Cordocentesis for diagnostic indications: two years' experience. Obstet Gynecol 1987;70:664-8.
2. Daffos F, Capella-Pavlovsky M, Forestier F. Fetal blood sampling during pregnancy with use of a needle guided by ultrasound: a study of 606 consecutive cases. Am J Obstet Gynecol 1985;153:655.
3. Lachman E, Hingley SM, Bates G, et al. Detection and measurement of fetomaternal haemorrhage: serum alpha-fetoprotein. Br Med J 1977;1:1377.
4. Lee AS, Carmody PJ, Hurd ME, O'Leary JA. Fetomaternal bleeding following diagnostic amniocentesis. Obstet Gynecol 1982;60:60.
5. Mennuti MT, DeGaetano A, McDonnell A, Cohen AW, Liston RM. Fetal-maternal bleeding associated with genetic amniocentesis: real-time versus static ultrasound. Obstet Gynecol 1983;62:26-30.
6. Tabor A, Bang J, Norgaard-Pedersen B. Feto-maternal haemorrhage associated with genetic amniocentesis: results of a randomized trial. Br J Obstet Gynaecol 1987;94:528-34.
7. Thomsen SG, Isager-Sally L, Lange AP, Saurbrey N, Gronvall S, Schioler V. Elevated maternal serum alpha-fetoprotein caused by midtrimester amniocentesis: a prognostic factor. Obstet Gynecol 1983;62:297-300.

Two hundred intrauterine exchange transfusions in severe blood incompatibilities

Marie-Hélène Poissonnier, MD,[a] Yves Brossard, MD,[b] Nicole Demedeiros, MD,[a] Juliana Vassileva, MD, Françoise Parnet, MD,[b] Marc Larsen, MD,[b] Monique Gosset,[b] Jacques Chavinie, MD,[a] and Jacques Huchet, MD[b]

Paris, France

Two hundred intrauterine exchange transfusions were performed under local anesthesia in 107 cases of blood incompatibilities (60 fetuses with severe anemia and 47 with hydrops). Under sonographic guidance, depending on fetal and placental position, an optimal puncturing site was selected along the umbilical vein: placental insertion, fetal insertion, or fetal intraabdominal segment. Tests were immediately performed to confirm fetal origin of blood obtained and estimate hemoglobin level. Blood used for exchange transfusion was compatible with maternal blood and had a hematocrit value of 75%. Exchange transfusion was continued until a hemoglobin level of 16 gm/dl was reached. This procedure was first associated with intraperitoneal transfusions and was subsequently used independently once a month to maintain an adequate hemoglobin level. In 4 fetuses with hydrops, antenatal regression of this sign was observed in 33 cases (70.2%). Overall outcome of 107 fetuses after exchanges was 84 living neonates (78.5%), 15 deaths in utero, and eight neonatal deaths. The survival rate was 91.6% for fetuses without hydrops and 61.7% for those with hydrops. The advantage of exchange transfusion appears to be rapid and efficient correction of anemia with elimination of incompatible fetal red blood cells. (Am J Obstet Gynecol 1989;161:709-13.)

Key words: Hydrops, fetal therapy, intrauterine fetal exchange transfusion, isoimmunization, fetal blood sampling

Severe fetal anemia and fetal hydrops caused by blood incompatibilities are generally associated with poor perinatal outcome, especially if hydropic signs develop during the second trimester. Although intraperitoneal transfusion has been used efficiently to treat fetuses without hydrops, its results for treatment of hydropic fetuses have been disappointing.[1-3] Rodeck et al.[4] reported intravascular transfusions first under fetoscopy and then under sonographic guidance.[6] Others have subsequently used the latter technique successfully.[6-12] More recently, Jacquetin et al.[13] and Grannum et al.[14] have performed intrauterine fetal exchange transfusions.

The purpose of this study was to evaluate the efficiency of intravenous fetal exchange transfusion for severe immunizations.

From Service de Gynécologie Obstétrique,[a] Hôpital Saint-Vincent-De-Paul, and Centre d'Hémobiologie Périnatale,[b] Hôpital Saint-Antoine.

Supported in part by a grant from the Ordre de Malte.

Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.

Reprint requests: M. H. Poissonnier, MD, Service de Gynécologie Obstétrique, Hôpital Saint-Vincent-De-Paul, 74 Avenue Denfert Rochereau, 75014 Paris, France.

6/6/14313

Material and methods

Patient evaluation. Since 1985, of the severely isoimmunized pregnant patients referred to us from France and other countries, 107 were selected for intravenous fetal exchange transfusion for severe fetal anemia with or without hydrops. At the beginning of the series, only fetuses with hydrops were treated with intravenous fetal exchange transfusion and the others with intraperitoneal transfusion. Starting in August 1987, severe anemia cases were also treated with intravenous fetal exchange transfusion. The fetuses treated had anti-D (level between 12.5 and 150 IU/ml), anti-Kell, or anti-c immunizations.

Intravenous fetal exchange transfusion was chosen after diagnosis of severe fetal hemolysis by either optical density measurements of amniotic fluid at an optical density of 450 nm, hemoglobin level by percutaneous umbilical blood sampling <8 gm/dl, or ultrasound evidence of hydrops (ascites alone or associated with pericardial or pleural effusions, subcutaneous edema, placentomegaly, and hydramnios).

Intrauterine fetal exchange transfusions

Instruments. Intrauterine partial exchange transfusions were carried out under sonographic guidance by means of a 15 cm long, 18- or 19-gauge needle that was introduced through the maternal abdominal wall

and uterus and an 18 cm long, 20- to 22-gauge puncturing needle according to gestational age.

Vena puncture. The intravenous fetal exchange transfusion procedure was performed under local anesthesia in an operating room after sedation of the mother with intramuscular diazepam and atropine. Neither tocolytic agents nor antibiotics were used. An optimal puncturing site along the umbilical vein was chosen. The preferred puncturing site was the placental insertion; when not easily accessible (e.g., when the placenta was posterior behind the fetus), another site was chosen, that is, fetal insertion or fetal intraabdominal segment.

To ensure correct needle location in the fetal vein, we used the following criteria: bright red color characteristic of umbilical venous blood, bevel position under sonograhpic guidance, and scintillating aspect of blood flow.

Fetal origin of blood was immediately checked by a panel of antibodies, including small i. The Kleihauer-Betke test was achieved secondarily. All fetuses were paralyzed with vecuronium bromide (0.1 mg/kg) once intravascular access was achieved.[15]

Transfusion technique. Irradiated O-negative packed erythrocytes with a 75% hematocrit level were used. Withdrawn aliquots were calculated on the basis of 5% of total blood volume, which was considered 16 ml/100 gm of estimated fetal weight. Ten to 20 uninterrupted cycles of injections and withdrawals of 1 to 4 ml were performed, depending on the initial hematocrit value and gestational age. During intravenous fetal exchange transfusion, fetal hematocrit and hemoglobin values were periodically checked. Exchange transfusion was continued until the hemoglobin level reached 16 gm/dl. The oxygenated aspect of withdrawn blood and fetal heart rate monitored under sonography served as criteria of fetal health. Average duration of intravenous fetal exchange transfusion was 45 minutes (range, 30 to 90 minutes). The main difficulty throughout the procedure was to avoid needle displacement. In most cases the umbilical vein had to be punctured two or three times either at the same site or at other sites along the tract. The procedure was discontinued when the desired hemoglobin level was attained, where severe fetal heart rate deceleration occurred, or in rare cases of difficulty in repuncturing the cord. The procedure was similar irrespective of gestational age; however, before 20 weeks it was more delicate to perform because of vein size and fragility. Exchange transfusions were repeated once a week until the hydropic state resolved. Pregnancies were followed by daily fetal heart rate monitoring and weekly sonography. Until August 1987, after hydrops reversal, intraperitoneal transfusions were carried out once a week until delivery. After August 1987, intravenous fetal exchange transfusion was used alone once a month because it appeared to be safe and had a more prolonged effect than did intraperitoneal transfusion.

Criteria for delivery were lung maturity checked by the lecithin/sphingomyelin ratio and if possible gestational age of 35 weeks, except of in cases of earlier complication. This led us to perform intravenous fetal exchange transfusion as late as 33 weeks, since the risks associated with the procedure were less than the risk of premature birth.

Results

From March 1985 to September 1988, 107 patients with severe blood incompatibilities were treated by 200 intravenous fetal exchange transfusions. Forty-seven fetuses showed signs of hydrops, and 60 fetuses had severe anemia without hydrops, that is, hemoglobin ≤8 gm/dl. Hydrops was rarely observed before 20 weeks with hemoglobin levels as low as 2 gm. At a later stage, hydrops was usually found associated with hemoglobin levels between 2 and 8 gm/dl. Patients were immunized against D in 91 cases, c in nine cases, Kell in six cases, and Miltenberger in one case. The first intravenous fetal exchange transfusion was performed between 17 and 32 weeks, with a mean gestational age of 26.7 weeks. Until August 1987 intravenous fetal exchange transfusions (one to seven) were used after the first treatment, and thereafter intravenous fetal exchange transfusion (one to four) were used alone.

Reversal of intrauterine hydrops. At the time of the first intravenous fetal exchange transfusion, 47 fetuses already had severe hydrops. Thirty-three (70.2%) showed evidence of reversal of hydrops after one (22) or two (11) intravenous fetal exchange transfusions. Mean hemoglobin levels before and after intravenous fetal exchange transfusion were 5.6 gm/dl (range, 1.7 to 6.7) and 16.2 gm/dl (range, 9 to 17.4), respectively. Reversal of hydrops was correlated with hemoglobin level.

Fetal death. Of 107 fetuses, there were 15 antenatal deaths, 10 of which were in the hydrops group (mean gestational age 26 weeks; range, 24 to 33) and five in the anemia group (mean gestational age 20.4 weeks; range, 18 to 24). Complications possibly resulting from the technique were one cord hematoma and four premature rupture of membranes, which occurred within a week after the procedure at 20, 23, 32, and 33 weeks of gestation. In nine patients severe hydrops did not resolve despite treatment. In one patient hydrops appeared despite intravenous fetal exchange transfusion (Table I).

Outcome of 47 hydropic fetuses. Thirty-seven fetuses were born alive. Eight died during the first week after birth: five were born hydropic, and two died of prematurity complications after premature rupture of membranes. In one case during intraperitoneal trans-

Table I. Results of 200 intauterine fetal exchange transfusions in 107 fetuses

	Severe anemia (n = 60)	*Hydrops (n = 47)*	*Total (N = 107)*
Healthy newborn	55 (91.7%)	29 (61.7%)	84 (78.5%)
Intrauterine deaths	5 (8.3%) (2 PROM, 1 hydrops, 1 cardiac, 1 unexplained)	10 (21.3%) (1 hematoma of cord, 9 severe hydrops)	15 (14%)
Neonatal deaths	—	8 (17%) (1 acute fetal distress, 2 PROM, 5 severe hydrops)	8 (7.5%)

PROM, Premature rupture of membranes.

fusion, an intraperitoneal hemorrhage was seen under sonography and the infant born at 31 weeks died after intraventricular hemorrhage on the third day. Twenty-nine infants were in good neonatal state with a mean birth weight of 2772 gm (range, 1200 to 3330) at a mean gestational age of 33.8 weeks (range, 28.7 to 36.5). The mean hemoglobin level at birth was 11.9 gm/dl (range, 8.7t o 17.2). Sixteen newborns required no exchange transfusion, eight newborns were given one, and five were given two.

Outcome of 60 nonhydropic fetuses. Five fetuses died in utero, but no neonatal deaths occurred among the 55 newborns. The mean gestational age was 33.7 weeks (range, 30 to 39). At birth the mean weight was 2498 gm (range, 1910 to 3200) and the mean hemoglobin level was 10.7 gm/dl (range, 3.7 to 18.2). Twelve newborns required no exchange transfusion, 24 were given one, and 19 were given two to four. No infant was icteric.

Case reports

Case 1. Mrs. T, Rh-negative, anti-D + Duffy, gravida 8, para 3, had a past history of two spontaneous abortions, one neonatal death, and two in utero deaths of hydropic fetuses at 39 and 23 weeks. She was referred to our institution in 1986 for severe maternal isoimmunization. Ten intraperitoneal transfusions were administered between 20 and 33 weeks of gestation. A healthy infant, weighing 2420 gm, was delivered at 34 weeks. The next pregnancy was treated with intravenous fetal exchange transfusion. The antibody level was 10 μg/ml. Although the hemoglobin level was 3.7 gm/dl at 21 weeks, ultrasound studies revealed that the fetus was not hydropic. The hemoglobin level was 17 gm/dl after the first intravenous fetal exchange transfusion. We performed four other intravenous fetal exchange transfusions at 25, 29, 32, and 34 weeks. The last two intervals were shorter because optimal hemoglobin levels were not reached. The fetus was delivered at 36 weeks and weighed 220 gm. Use of intravenous fetal exchange transfusion enabled us to reduce the number of procedures and obtain better maturity.

Case 2. Mrs. B, Rh-negative, anti-D + C, gravida 4, para 3, had a very high antibody level (52 μg/ml). This patient was referred to us with hydrops at 28 weeks of gestation, and the first intravenous fetal exchange transfusion was performed. The initial hemoglobin level was 7.9 gm/dl, with severe thrombopenia (platelet count 23000/ml). The exchange was discontinued at 16.8 gm/dl. Although the hemoglobin level was corrected, hydrops was not resolved. We performed the second intravenous fetal exchange transfusion at 31 weeks. The hemoglobin level was 9 gm/dl at the beginning of 16.10 gm/dl at the end of the procedure, and there was still severe thrombopenia. The fetus died shortly after the intravenous fetal exchange transfusion. Its weight was 2900 gm with hydrops. The placenta weighed 1490 gm.

Case 3. Mrs. L, Rh-negative, anti-D + C, gravida 7, para 6, had a past history of three children and two antenatal deaths at 32 and 24 weeks' gestation. During the index pregnancy, the fetus showed signs of severe hydrops at 21 weeks with peritoneal, pericardial, and pleural effusions. Hemoglobin level was 1.4 gm/dl. There was no thrombopenia or hypoxia. Intravenous fetal exchange transfusion was started with aliquots of 1 ml. At the end of exchange transfusion, the hemoglobin level was 15.7 gm. Hydrops was resolved after 48 hours. Three other intravenous fetal exchange transfusions were performed at 23, 29, and 33 weeks. At 36 weeks a healthy newborn weighing 3000 gm was delivered by cesarean section.

Comment

The three therapeutic procedures currently used for severe isoimmunizations, namely, intraperitoneal, intravascular, and intravascular exchange transfusions, are compared.

Intraperitoneal in utero transfusion has been found to be effective in cases of fetal anemia without hydrops.[3] In hydropic cases, a poor survival rate (28.5%) is reported by Frigoletto et al.[3] The good results (75%) of Harmann et al.[2] are an exception. Because anemia was only partially corrected, it was necessary in most cases to repeat the procedure once a week.[6] Between 1977 and 1986, we treated 228 cases of severe isoimmunizations with 901 intraperitoneal transfusions. An average of 3.9 intrauterine transfusions were performed, with a 76.3% survival rate. For hydrops appearing before 28 weeks, the rate was 24% (unpublished data).

Table II. Comparison of survival rates after straight intravascular transfusions in six studies

	No. of cases	*Overall survival (%)*	*Survival of hydrops (N)*
Rodeck et al.[5]	19	84	11/13
De Crespigny et al.[7]	4	75	1/1
Grannum et al.[14]	26	82	16/20
Berkowitz et al.[19]	16	76	—
Brass et al.[12]	23	85.7	5/6
Mackenzie et al.[18]	10	10	—

Intravascular transfusion. Percutaneous intravascular access to the fetus was first attempted by Daffos et al.[15, 16] in 1983 with a fetal death rate of 1.1%. Rodeck et al.[4] described intravascular transfusion under fetoscopy. Rodeck et al.[5] and others[7, 11, 12, 14] also described percutaneous intravascular transfusion under sonographic guidance. The survival rate was between 10% and 85.7%. Good results were obtained in hydropic cases (Table II).

Intravascular transfusion has the benefit of correcting fetal anemia rapidly.[7, 15, 17] However, large quantities of blood must be transfused, and consequently, cardiovascular decompensation might possibly occur in hydropic fetuses. On the other hand, where simple transfusion is used, there is no purge of incompatible fetal erythrocytes and therefore these can be hemolyzed.

Intrauterine exchange transfusion. Overall survival rates for intravascular and exchange transfusions appear to be similar (84% for Rodeck et al. and 75% for Grannum et al.). However, intravenous fetal exchange transfusion may have some of the following advantages: (1) The theoretic risk of cardiac decompensation in severe hydropic cases might be reduced.[18] (2) The interval between procedures is longer (4 weeks except in rare cases in which the desired hemoglobin level of 16 gm/dl is not obtained). (3) This longer interval is made possible by the slow decrease of the hemoglobin level (2 gm 1 wk) compared with the rate of hematocrit decrease of 1 point per day in the study of Rodeck et al.[5] of simple transfusions. (4) Blood removal during exchange transfusion allows the hemoglobin level to be controlled periodically, giving more accurate indications of the blood volume to be exchanged. (5) Therefore it is unnecessary to calculate as Rodeck et al.[4] suggested the blood volume to be transfused. (6) The bright red color of the oxygenated blood removed gives further indication of fetal health. (7) In most cases, intravenous fetal exchange transfusion enabled delivery to be delayed until fetal maturity. (8) Possibly because of complete clearance of incompatible red cells and anemia correction, only a few newborns required exchange transfusions during the neonatal period. We also observed that persistence of hydrops was very rare in newborns, and complications caused by this state during the neonatal period were reduced.

However, exchange transfusion has some disadvantages compared with intravascular transfusion: the procedure is longer, and in theory the risk of infection is increased. There is also an increased risk of accidental needle displacement from the vessel, even when care is used to reduce fetal movements. This risk may be reduced by the introduction of a catheter, as described by Jacquetin et al.[13] in the anterior placenta.

From our study, we made the following observations regarding prognostic factors: (1) Hydrops per se as revealed by sonography is a well-known criterion of poor prognosis. (2) For nonhydropic fetuses, the hemoglobin level is much more accurate for prognosis than the bilirubin level in the amniotic fluid. (3) In hydropic cases thrombocytopenia and a high transaminase level are signs of poor prognosis regarding reversal of hydrops, even if anemia can be corrected. However, we found no significant decrease in platelet counts despite the removal of clotting factors during the exchange transfusion. Berkowitz et al.[19] have suggested another criterion for prognosis, namely, protein level in fetal blood.

In conclusion, intravenous fetal exchange transfusion appears to be safe procedure if performed by a multidisciplinary team with experience in ultrasound-guided invasive fetal procedures. It enables anemia to be corrected efficiently, a large proportion of cases of hydrops to be reversed, and in most cases leads to delivery of a mature healthy newborn requiring minimal treatment during the neonatal period.

We gratefully acknowledge A. Rodger, C. George, and E. Couvé for editorial assistance.

REFERENCES

1. Chavinié J, Brossard Y, Poissonnier M-H. Traitement prénatal des incompatibilités sanguines foeto-maternelles graves. REV Practicien 1984;34:3309-15.
2. Harman CR, Manning FA, Bowman JM, Lange IR. Severe Rh disease. Poor outcome is not inevitable. AM J OBSTET GYNECOL 1983;145:823-9.
3. Frigoletto FD, Umansky J, Birnholz J, et al. Intrauterine

fetal transfusion in 365 fetuses during fifteen years. AM J OBSTET GYNECOL 1981;139:781-90.
4. Rodeck CH, Nicolaides KH, Warsof SL, Fysh WJ, Gamsu HR, Kemp JR. The management of severe rhesus isoimmunization by fetoscopic intravascular transfusions. AM J OBSTET GYNECOL 1984;150:769-74.
5. Rodeck CH, Holman CA, Karnicki J, Kemp Jr, Whitmore DN, Austin MA. Direction intravascular fetal blood transfusion by fetoscopy in severe rhesus isoimmunization. Lancet 1981;1:625-8.
6. Queenan J. Fetal transfusion. Fetal Ther 1986;1:59-64.
7. De Crespigny LCh, Robinson HP, Quinn M, Doyle ChBL, Ross A, Cauchi M. Ultrasound-guided fetal blood transfusion for severe rhesus isoimmunization. Obstet Gynecol 1985;66:529-32.
8. Seeds JW, Bowes WA. Ultrasound-guided fetal intravascular transfusion in severe rhesus immunization. AM J OBSTET GYNECOL 1986;154:1105-7.
9. Berkowitz RL, Chitkara U, Goldberg JD, Wilkins I, Chervenak FA. Intravascular transfusion in utero: the percutaneous approach. AM J OBSTET GYNECOL 1986;154: 622-3.
10. Socol ML, MacGregor SN, Pielet BW, Tamura RK, Sabbagha RE. Percutaneous umbilical transfusion in severe rhesus isoimmunization: resolution of fetal hydrops. AM J OBSTET GYNECOL 1987;157:1369-75.
11. MacKenzie IZ, Bowell PJ, Castle BM, Selinger M, Ferguson JF. Serial fetal blood sampling for the management of pregnancies complicated by severe rhesus (D) isoimmunization. Br J Obstet Gynaecol 1988;95:753-8.
12. Barss V, Beryl R, Benacessraf Frigoletto FD, et al. Management of isoimmunized pregnancy by use of intravascular techniques. AM J OBSTET GYNECOL 1988;159: 932-7.
13. Jacquetin B, Meyer JL, Furbain M, Jouve P, Vanlieferinchen R. Intrauterine exchange transfusion of the fetus under ultrasound guidance: first successful report. Fetal Ther 1981;1:181-5.
14. Grannum PA, Copel JA, Plakes SC, Scioscia AL, Hobbins JC. In utero exchange transfusion by direct intravascular injection in severe erythroblastosis fetalis. N Engl J Med 1986;314:1431-4.
15. Daffos F, Capella-Pavlovsky M, Forestier F. Fetal blood sampling during pregnancy with use of a needle guided by ultrasound: a study of 606 consecutive cases. AM J OBSTET GYNECOL 1985;153:655-60.
16. Daffos F, Capella-Pavlovsky M, Forestier F. A new procedure for fetal blood sampling in utero: preliminary result of fifty-three cases. AM J OBSTET GYNECOL 1983; 146:985-7.
17. Parer JT. Severe rh isoimmunization—current methods of in utero diagnosis and treatment. AM J OBSTET GYNECOL 1988;158:1323-9.
18. Mackenzie IZ, Bowell PJ, Ferguson J, Castle BM, Entewistle CC. In utero intravascular transfusion of the fetus for the management of severe rhesus isoimmunization a reapraisal. Br J Obstet Gynaecol 1987;94:1068-73.
19. Berkowitz RL, Chitkara U, Wilkins IA, Lynch L, Plosker H, Bernstein HH. Intravascular monitoring and management of erythroblastosis fetalis. AM J OBSTET GYNECOL 1988;158:783-95.

Normal values for human umbilical venous and amniotic fluid pressures and their alteration by fetal disease

Carl P. Weiner, MD, Joni Heilskov, RN, Gay Pelzer, RN, Stanley Grant, RN, Katharine Wenstrom, MD, and Roger A. Williamson, MD

Iowa City, Iowa

Umbilical venous and amniotic fluid pressures were measured in 68 human pregnancies at the time that cordocentesis was performed. Normal umbilical venous pressure was unrelated to gestational age and remained within a tight range (5.3 ± 2.3 mm Hg, mean ± SD). Fetuses with an elevated umbilical venous pressure had disorders consistent with either hepatomegaly or congestive heart failure. Umbilical venous pressure was significantly increased before treatment in two fetuses with immune hydrops; it rapidly declined with treatment. Neither gestational age nor umbilical venous pressure was significantly different in the groups that received and did not receive pancuronium. There was a strong relationship between amniotic fluid pressure and gestational age in normal pregnancy ($r = 0.54$, $p < 0.0001$). Women with hydramnios had amniotc fluid pressures greater than control subjects ($p = 0.0007$). This investigation documents normal human amniotic fluid and fetal umbilical venous pressures. These measurements are altered by disease and may prove to be of diagnostic and therapeutic value in the future. (Am J Obstet Gynecol 1989;161:714-7.)

Key words: Fetus, cordocentesis, amniotic fluid pressure, umbilical venous pressure, hydrops fetalis

The development of cordocentesis has greatly simplified access to the circulation of the human fetus. The umbilical vein is the vessel that is most commonly punctured.[1] Umbilical venous pressure should approximate the central venous pressure as much as pressure in the fetal inferior vena cava. Because of the parallel fetal circultory system and the preferential streaming of umbilical venous blood to the left atrium,[2] umbilical venous pressure measurements may even be more sensitive to left ventricular abnormalities than the inferior vena cava. Neither umbilical venous pressure nor amniotic fluid pressure appears to have been systematically investigated throughout pregnancy. To our knowledge, prior studies of amniotic fluid pressure have focused on uterine activity and have not compared resting tone across a wide range in gestation. We hypothesized that human fetal pathologic conditions could alter umbilical venous pressure and amniotic fluid pressure. The purpose of this investigation was to prospectively test this hypothesis by measuring umbilical venous pressure and amniotic fluid pressure in fetuses undergoing diagnostic cordocentesis for a variety of clinical indications.

From the Fetal Diagnosis and Treatment Unit, Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of Iowa College of Medicine.

Supported in part by grant no. HD24494 from the National Institute of Child Health and Human Development.

Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.

Reprint requests: Carl Weiner, MD, Department of Obstetrics and Gynecology, University of Iowa Hospitals, Iowa City, IA 52242.

6/6/13716

Material and methods

Sixty-eight cordocenteses were performed for a variety of indications (Table I). Eight fetuses had nonimmune hydrops fetalis and two had immune hydrops fetalis. Our method for cordocentesis has been described previously.[1] Each procedure used a 22-gauge spinal needle and a 5 mHz sector transducer (General Electric 3600, Rancho Mirage, Ga.) with an attached needle guide (Civco, Kalona, Iowa). Neuromuscular blockade was produced with pancuronium (0.3 mg/kg of estimated fetal weight intravenously) when deemed necessary by the operator. After the indicated blood specimen had been obtained, the needle was connected to a tube filled with saline solution that was attached to a solid state pressure transducer (Sorenson Transpac II, #46862-04, supplied by Abbott Critical Care Systems, North Chicago) and pressure monitor (Hewlett-Packard #78833B, Houston). The transducer was meticulously placed at the level of the fetal heart as determined during the preceding ultrasonographic examination. The needle was purged and the pressure read at its nadir. The identity of the vessel punctured was confirmed as the umbilical vein by both observing the flow direction of the injected saline solution and the lack of a pulsatile pressure waveform on the pressure monitor screen. The needle was then withdrawn

Table I. Indications for cordocentesis*

	No. of cases
Hemolytic disease	44
Rapid karyotype	17
Rule out infection	13
Intrauterine growth retardation	7
Drug therapy	2
Arrhythmia	2

*Some subjects had more than one indication.

from the vein into the amniotic cavity. After a brief purge, the nadir pressure was recorded. The time required to obtain both umbilical venous and amniotic fluid pressures was approximately 30 seconds. There was no maternal perception or ultrasonographic evidence of uterine activity during the time the final pressure measurements were recorded. The observation that the nadir pressure was stable also decreased the likelihood of unrecognized uterine activity. The reported umbilical venous pressure reflects subtraction of the amniotic fluid pressure from the measured umbilical venous pressure.

A complete blood count and respiratory blood gas measurements were performed on all fetal blood samples. The complete blood count was performed on blood anticoagulated with 1% ethylenediaminetetraacetic acid on a Technicon H-1 analyzer (Tarreytown, N.Y.). Respiratory blood gas measurements were performed on blood anticoagulated with heparin on automated equipment (Instrumentation Laboratory Blood Gas Manager model 1312, Lexington, Mass.) The pH, P_{CO_2}, and P_{O_2} were measured directly. The base excess, bicarbonate, and oxygen saturation were calculated with standard nomograms.

The group of fetuses termed "control" was comprised predominantly of subjects evaluated for hemolytic disease who ultimately were shown to be either unaffected or to have a normal hematocrit and reticulocyte count. The cause of fetal hydrops was listed when known. Oligohydramnios was considered present when the largest amniotic fluid pocket was <2 cm in all dimensions. The diagnosis of hydramnios required a gross increase in amniotic fluid associated with a size greater than dates–fundal height discrepancy and was made before the pressure measurement.

The results were presented as the mean ± 1 SD. Analyses used subroutines from ABstat rel. 5.10 software (Anderson-Bell, Parker, Colo.) and included least-squares multiple linear regression and independent *t* tests.

Results

The mean gestational age of the fetuses studied was 28.2 ± 5 weeks (range, 19 to 39). Both the amniotic

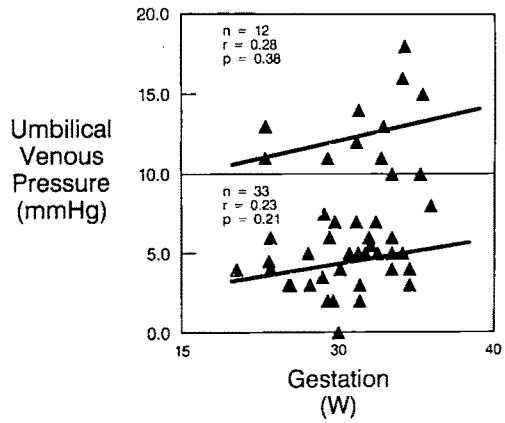


Fig. 1. Scattergram of the 45 fetuses in whom umbilical venous and amniotic fluid pressure was measured. Two distinct populations are obvious. (n = 12 refers to those fetuses with a umbilical venous pressure >2 SD above the norm; n = 33 refers to all others.)

fluid pressure and the umbilical venous pressure measurements were available from 45 subjects. Regression analysis with umbilical venous pressure used as the dependent variable suggested a weak positive relationship with gestational age ($r = 0.28, p = 0.07$). However, this was artifactual. Examination of the scattergram revealed two clear populations of similar gestational age—one whose umbilical venous pressure was <10 mm Hg and one whose umbilical venous pressure was >10 mm Hg (Fig. 1). Of the 14 fetuses assigned to the control group (mean gestational age, 27.4 ± 3 weeks), the mean umbilical venous pressure was 5.3 ± 2.3 mm Hg (range, 2 to 11). There was no relationship between gestational age and umbilical venous pressure in this group ($r = 0.30, p = 0.29$) nor a group made up of all fetuses whose umbilical venous pressure was within 2 SD of the norm.

Each fetus with an umbilical venous pressure >10 mm Hg had a disorder consistent with either congestive heart failure or hepatomegaly, except one fetus with a neural tube defect (Table II). Elevated pressures considered to be consistent with hepatomegaly were observed in two fetuses with hemolytic disease in need of transfusion and two fetuses who had previously been transfused but who had not yet demonstrated complete suppression of erythropoiesis. The two fetuses studied with immune fetal hydrops had an elevated umbilical venous pressure. In each instance the umbilical venous pressure declined rapidly to a normal level during or after intravascular transfusion. An example is shown in Fig. 2. One fetus with a massive cervical teratoma initially had a normal umbilical venous pressure that rose with the development of hydrops. In another fetus with anasarca associated with an asymmetric bilateral chylothorax, the fetal heart was compressed snugly

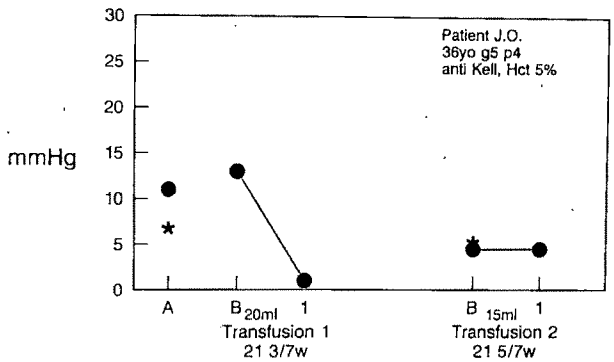


Fig. 2. Umbilical venous pressure *(UVP)* was initially elevated in fetuses with immune hydrops. It declined into the normal range during transfusion. *Asterisk,* Amniotic fluid prssure. *Closed circle* signifies the corrected umbilical venous pressure at each sampling point. The second transfusion occurred 48 hours after the first.

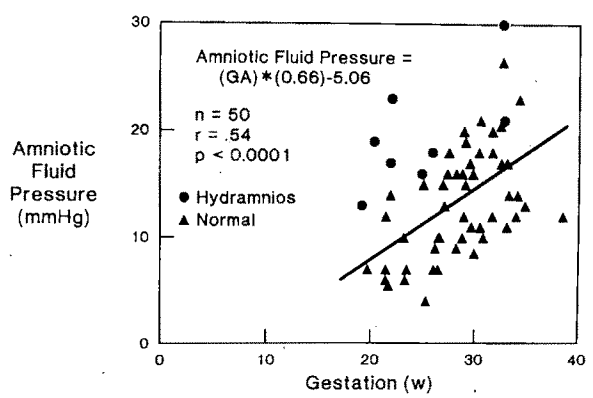


Fig. 3. Amniotic fluid pressure tone rose progressively as gestational age increased. Women with hydramnios had increased amniotic fluid pressure. Pregnancies complicated by anhydramnios (n = 10) excluded.

along the left side of the chest wall. The umbilical artery blood pressure was 18/4 torr. This pressure measurement contrasts markedly with the only other fetuses whose umbilical artery pressure was measured (n = 3): 63/40, 31/26, and 39/19 mm Hg. Bilateral thoracoamniotic shunts were placed, and the hydrops resolved rapidly after the heart returned to its normal position.

Forty of the 45 fetuses (89%) received pancuronium. The gestational age and umbilical venous pressure measurements were not significantly different in the group that received pancuronium compared with the group that did not receive pancuronium.

The mean amniotic fluid pressure for all subjects was 11.8 ± 4 mm Hg. There was a positive relationship between amniotic fluid pressure and gestational age (r = 0.44, p = 0.0006). This relationship increased further when patients with either hydramnios or oligohydramnios were eliminated from the data set

Table II. Disorders associated with an elevated umbilical vein pressure*

Fetal disorder	*n*	*Umbilical vein pressure (mm Hg)*
Immune hydrops	2	11
(1 fetus, two measurements 6 hr apart)		13
		12
Nonhydropic hemolytic disease in need of transfusion	2	15
		11
Previously transfused	2	10
		10
Atrial flutter with hydrops (two measurements 1 day apart)	1	16
		18
Cystic adenomatoid malformation with hydrops	1	13
Congenital hepatitis (drug related)	1	11
Meningomyelocele	1	14

*≥10 mm Hg.

(r = 0.54; p < 0.0001; Fig. 3). Women with obvious hydramnios (n = 8) had a mean amniotic fluid pressure significantly greater than the other patients (19 ± 5 vs. 13.3 ± 5 mm Hg, p = 0.0007) despite being slightly earlier in gestation (27.1 ± 6 vs. 28.6 ± 4 weeks, p = 0.36). In the absence of hydrops and associated hydramnios, there was no obvious relationship between umbilical venous pressure and amniotic fluid pressure.

Comment

This study of 68 pregnancies with various pathologic conditions over a wide gestational age range offers new information concerning human fetal physiology and pathophysiology. It appears to be the first systematic investigation of human amniotic fluid and fetal umbilical cord blood pressure. Each was altered in association with fetal disease.

Blood returning from the placenta courses through the large-bore ductus venosus into the inferior vena cava where it streams preferentially through the foramen ovale into the left atrium.[2] We assume that the umbilical venous pressure is an approximation of the central venous pressure and may also be sensitive to left-sided cardiac alterations. Certainly, additional animal study of factors that alter the umbilical venous pressure is desirable, but at this time our current observations support the above assumptions. Umbilical venous pressure remained within a fairly narrow range throughout gestation but rose in association with disorders likely to produce congestive heart failure or hepatomegaly. The development of hydrops, presumably as a result of high-output failure, in the fetus with a massive cervical teratoma was associated with an increase of the umbilical venous pressure into the abnormal range. The fetus with hydrops associated with

asymmetric chylothorax and a markedly displaced heart had an umbilical artery pressure of 18/4 mm Hg. We believe that this pressure is very low and consistent with some form of cardiac tamponade manifested as decreased cardiac return. Both fetuses with immune hydrops had markedly elevated umbilical venous pressure that returned to the normal range after intravascular transfusion. Although the initial elevation might partially reflect portal hypertension as a result of massive hepatomegaly,[3] the rapid return of the pressure to a normal range suggests that the high umbilical venous pressure is in part explained by myocardial dysfunction (e.g., high-output heart failure), which is quickly alleviated by intravascular transfusion. This conclusion would seem to conflict with studies of the neonate.[4] However, the neonatal studies would be subject to a wide range of variables altered by the birth process. The decline is probably not the result of a cord "spasm" since the umbilical artery systolic/diastolic ratio declined with transfusion. Furthermore, since the completion of this study we have observed the umbilical venous pressure of a fetus with a procedure-related bradycardia; it exceeded 60 mm Hg. A definitive answer will require additional study including fetal blood volume measurement.

Although the number of fetuses who did not receive pancuronium was small, there was no evidence that pancuronium altered the umbilical venous pressure. This is consistent with the known pharmacology because pancuronium causes little or no histamine release and no ganglionic blockade.[5]

The methods used for pressure measurement merit further comment. The fetal pressure transducer is traditionally placed at the level of the maternal heart during animal study. We selected the level of the fetal heart as our standard location because the fetus was the object of the study and the level of the fetal heart can be accurately identified ultrasonographically. It is possible that some fetal trunk rotation occurred from the time the transducer was set and zeroed until the time the pressures were actually measured (1 to 5 minutes). However, we were unaware of any such movement, and fetal movement during the measurement was often inhibited by neuromuscular blockade. Ideally amniotic fluid pressure measurement would occur with the transducer at the level of the needle tip. Therefore the actual amniotic fluid pressure reported in this study may be slightly elevated. However, we do not believe this possible bias is clinically relevant.

The progressive increase in amniotic fluid pressure with advancing gestation was unexpected, although in retrospect it was logical. Prior animal and human studies involving amniotic fluid pressure measurement may have occurred over too narrow a gestational age span or were focused on other areas such as uterine activity or fetal blood pressures. That women with hydramnios have elevated amniotic fluid pressure may relate to our unpublished observation that uterine decompression enhances the efficacy of tocolysis in women with hydramnios and preterm labor.

In conclusion, human amniotic fluid and fetal umbilical pressures may be measured during cordocentesis. These measurements are affected by a variety of fetal disorders. In the future, fetal umbilical pressure measurement may prove of diagnostic and therapeutic value.

REFERENCES

1. Weiner CP. Cordocentesis for diagnostic indications—two year's experience. Obstet Gynecol 1987;70:664-8.
2. Lind J, Stern L, Wgelius C. Human fetal and neonatal circulation. Springfield, Ill.: Charles C Thomas, 1964.
3. James LS. Shock in the newborn in relation to hydrops. In: Robertson JG, Dambrosio F, eds. International symposium on the management of the Rh problem. Ann Obstet Gynecol 1970;271-82.
4. Phibbs RH, Johnson P, Tooley WH. Cardio-respiratory status of erythroblastic infants. II. Blood volume, hematocrit and serum albumen concentration in relation to hydrops fetalis. Pediatrics 1974;53:13-8.
5. Drug Information 87. Chicago: American Society of Hospital Pharmacists, 1987:636.

Ovine fetal cardiorespiratory response to nicardipine

R. Harold Holbrook, Jr., MD,* Ellen M. Voss, BS, and Ronald N. Gibson, BS
Stanford, California

Nicardipine, a calcium antagonist associated with decreased uterine blood flow in near-term pregnant rabbits and fetal asphyxia after maternal administration in the rhesus monkey and sheep, was infused directly to the fetus in six chronically prepared pregnant ewes at 128 days' gestation. Changes in fetal mean arterial and diastolic blood pressure levels at 2 and 30 minutes after bolus injection of 50 μg were minimal; by 60 minutes these values had returned to preinfusion levels. No significant changes were observed after infusion of 100 μg of nicardipine. Fetal heart rate, fetal arterial blood gas values, and maternal cardiovascular variables did not change at either dose. Fetal plasma concentrations of nicardipine were 78 ± 28 ng/ml and 114 ± 48 ng/ml at 30 minutes after infusion of 50 μg and 100 μg, respectively, well within the range previously reported to be associated with fetal asphyxia. These data suggest that the previously reported fetal acidosis from maternal infusion of nicardipine may be primarily due to a decrease in maternal uterine blood flow rather than a direct fetal effect of the drug. (Am J Obstet Gynecol 1989;161:718-21.)

Key words: Premature labor, calcium channel blockers, nicardipine, fetus, sheep

Calcium antagonists are potential alternatives to 3-adrenergic agonists for preterm labor tocolysis. Nicardipine, a dihydropyridine derivative, has been found to inhibit uterine activity in the preterm pregnant rabbit,[1-3] the rhesus monkey,[4] and the postpartum rabbit.[5] Nicardipine also decreases contractility in isolated human myometrial strips[6] and delays delivery in pregnant rabbits.[5] Nifedipine, a similar dihydropyridine calcium antagonist, has been used as a tocolytic agent in human subjects, but maternal and fetal side effects have not been well studied. Maternally administered nicardipine, however, has been associated with fetal asphyxia in pregnant animals, and placental transfer has been documented. It is not clear whether fetal asphyxia is mediated through alteration in uteroplacental perfusion or by a direct action on the fetus (e.g., alterations in umbilical or ductal blood flow). To determine the effect of nicardipine on the fetus independent of changes in uterine blood flow, we administered the drug directly to the fetus and measured the fetal cardiorespiratory response.

Material and methods

Animal preparations and studies were performed in the Stanford Research Animal Facility. The study protocol was approved by the Stanford University Administrative Panel on Laboratory Animal Care. Six crossbred Western pregnant ewes at 128 days' gestation with single fetuses were fasted 24 hours before surgery and regional anesthesia was induced in the lower body with an epidural injection of 40 mg of tetracaine hydrochloride. Intravenous ketamine hydrochloride (8 mg/min) was administered to maintain sedation, and supplemental diazepam was given as needed up to a total dose of 2 mg/kg. Polyvinyl catheters (0.05 inch inner diameter, 0.09 inch outer diameter) were advanced into the ewe's descending aorta and inferior vena cava through the saphenous artery and vein. A midline abdominal incision was made to expose the uterus. Lidocaine 0.5% was used to induce local anesthesia in the fetus and polyvinyl catheters (0.03 inch inner diameter, 0.05 inch outer diameter) were placed in the fetal descending aorta and inferior vena cava through the saphenous artery and vein. A catheter was placed in the amniotic cavity for measurement of intrauterine pressure. All catheters were filled with sodium heparin, sealed with copper plugs, and tunneled to a pouch attached to the ewe's flank. The ewe received 40,000 U/kg of penicillin postoperatively and was allowed to recover for 48 hours.

With the ewe standing quietly in a study cage with free access to food and water, baseline measurements were made over a 10-minute period. Fetal arterial pressures and heart rate, maternal arterial pressures and heart rate, and amniotic pressure levels were measured on a Gould 2800S 8-channel recorder. Fetal descending aortic oxygen saturation and hemoglobin levels were measured with a Radiometer OSM3 hemoximeter, and blood gases were measured with a Corning 170 blood gas analyzer at 39° C. After baseline measurements

From the Section of Maternal-Fetal Medicine, Department of Gynecology and Obstetrics, Stanford University School of Medicine.
**Supported by National Institutes of Health Grants HD-19190 and HD-22727, Biomedical Research Support Grant S07 RR-05353, and the Mellon Foundation.*
Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.
Reprint requests: R. Harold Holbrook, Jr., MD, Room A-342, Department of Gynecology and Obstetrics, Stanford University School of Medicine, Stanford, CA 94305.
6/6/13717

Table I. Fetal and maternal cardiorespiratory responses to fetal infusion of 50 μg of nicardipine

	Preinfusion	*2 Minutes*	*30 Minutes*	*60 Minutes*
Fetal (n = 6)				
Heart rate	159 ± 13	176 ± 28	166 ± 26	171 ± 34
Mean arterial pressure (mm Hg)	48 ± 5	44 ± 3*	46 ± 3*	47 ± 3
Systolic pressure (mm Hg)	64 ± 7	60 ± 4	64 ± 3	65 ± 5
Diastolic pressure (mm Hg)	39 ± 4	37 ± 3*	37 ± 4*	39 ± 3
Hemoglobin (gm %)	11.0 ± 1.4	10.8 ± 1.9	10.7 ± 1.3	10.7 ± 2.0
Hemoglobin saturation (%)	47.7 ± 13.4	49.5 ± 11.7	50.0 ± 13.0	50.9 ± 12.6
Distal aortic pH	7.37 ± 0.03	7.37 ± 0.03	7.37 ± 0.03	7.36 ± 0.04
Distal aortic Po_2 (mm Hg)	18.2 ± 2.5	18.9 ± 2.4	18.7 ± 3.0	19.1 ± 2.9
Distal aortic Pco_2 (mm Hg)	48.2 ± 3.5	47.4 ± 3.7	46.5 ± 3.1	49.0 ± 3.1
Maternal (n = 5)				
Heart rate	107 ± 13	108 ± 19	106 ± 15	106 ± 13
Mean arterial pressure (mm Hg)	102 ± 10	105 ± 12	102 ± 11	103 ± 10
Systolic pressure (mm Hg)	141 ± 15	144 ± 17	140 ± 14	144 ± 15
Diastolic pressure (mm Hg)	83 ± 8	85 ± 10	83 ± 9	82 ± 9

All values represent the mean ± SD.
*p <0.05 versus preinfusion value.

were obtained, a 50 μg bolus of nicardipine hydrochloride (Syntex Pharmaceuticals, Palo Alto, Calif.), prepared as a fresh sterile solution of 2 mg/cc with 6 mg/cc sodium chloride added, was infused into the fetal inferior vena cava. The above measurements were then repeated at 2, 30, and 60 minutes. The animal was allowed to recover for at least 4 hours before baseline measurements were repeated. The study was repeated as above with 100 μg bolus of nicardipine. At 30 minutes after each bolus injection of nicardipine, fetal blood was drawn from the inferior vena cava and centrifuged. The serum was stored frozen at −80° C for later analysis by high-performance liquid chromatography. Nicardipine dosages were chosen on the basis of estimated fetoplacental blood volume to achieve a fetal plasma concentration of approximately 50 ng/ml at 60 minutes. This concentration is similar to that reported in studies in which fetal asphyxia developed after maternal administration.

The ewe was killed by injection of pentobarbital and saturated potassium chloride before bilateral thoracotomy. The fetus was removed from the uterus and weighed. Fetal arterial pressure was corrected by subtraction of the amniotic fluid pressure. Fetal and maternal blood pressure levels and heart rates, and fetal descending aortic blood gas and pH levels were compared before and at each time period after each nicardipine infusion by one-way analysis of variance for repeated measures. When the repeated measures analysis of variance revealed differences between time periods, Fisher's nonparametric paired least significant difference test was used to determine between which measurements the differences were significant.

Results

Fetal arterial pressure level and heart rate, descending aortic hemoglobin concentration, hemoglobin saturation, and blood gas and pH levels were measured in six animals after infusion of 50 μg of nicardipine. In five of these animals, maternal arterial pressure levels and heart rates were also obtained. Values of these variables at preinfusion and at 2, 30, and 60 minutes are listed in Table I. A small (2 to 4 mm Hg) but significant decline in fetal mean and diastolic pressure was noted at 2 and 30 minutes after infusion, but these values returned to baseline by 60 minutes. Changes in all other maternal and fetal variables were not significantly different from preinfusion values at any of the time periods.

Similar results were obtained after infusion of 100 μg of nicardipine. Maternal and fetal variables were measured in five animals and are listed in Table II. No differences in any of the variables were noted at 2, 30, or 60 minutes after infusion.

Fetal plasma concentrations of nicardipine after bolus administration of 50 μg were 527 ± 345 ng/ml, 78 ± 28 ng/ml, and 41 ± 25 ng/ml at 2, 30, and 60 minutes, respectively. With bolus administration of 100 μg of nicardipine, the fetal plasma concentrations were 414 ± 17 ng/ml, 114 ± 48 ng/ml, and 61 ± 29 ng/ml at 2, 30, and 60 minutes, respectively. The calculated half-life for nicardipine in the fetal circulation was 25.6 ± 5.2 minutes and 22.3 ± 5.9 minutes for bolus administration of 50 μg and 100 μg of nicardipine, respectively.

Comment

This study suggests that the previously reported fetal acidosis that occurs after maternal infusion of nicardipine[4] may be primarily due to a decrease in maternal uterine blood flow rather than a direct fetal effect of the drug. The fall in uterine blood flow observed in near-term pregnant animals after maternal infusion of calcium antagonists has been considered to be mainly due to peripheral vasodilation, which results in mater-

Table II. Fetal and maternal cardiorespiratory responses to fetal infusion of 100 μg of nicardipine

	Preinfusion	*2 Minutes*	*30 Minutes*	*60 Minutes*
Fetal (n = 5)				
Heart rate	161 ± 34	172 ± 21	166 ± 19	173 ± 16
Mean arterial pressure (mm Hg)	44 ± 10	42 ± 9	42 ± 8	42 ± 7
Systolic pressure (mm Hg)	60 ± 13	56 ± 11	58 ± 10	60 ± 8
Diastolic pressure (mm Hg)	36 ± 9	35 ± 8	34 ± 7	36 ± 6
Hemoglobin (gm %)	11.1 ± 2.9	10.5 ± 2.6	10.4 ± 2.0	10.8 ± 2.3
Hemoglobin saturation (%)	43.2 ± 20.0	41.3 ± 21.4	45.4 ± 19.6	42.6 ± 21.1
Distal aortic pH	7.32 ± 0.10	7.32 ± 0.10	7.31 ± 0.11	7.31 ± 0.12
Distal aortic P_{O_2} (mm Hg)	17.7 ± 3.0	17.1 ± 3.7	18.1 ± 2.7	17.3 ± 2.9
Distal aortic P_{CO_2} (mm Hg)	55.2 ± 8.2	53.6 ± 7.0	52.6 ± 7.3	55.4 ± 8.0
Maternal (n = 5)				
Heart rate	98 ± 12	101 ± 11	120 ± 38	114 ± 24
Mean arterial pressure (mm Hg)	105 ± 10	104 ± 7	104 ± 11	104 ± 9
Systolic pressure (mm Hg)	149 ± 13	146 ± 9	146 ± 12	145 ± 11
Diastolic pressure (mm Hg)	84 ± 9	85 ± 8	83 ± 12	84 ± 9

All values represent the mean ± SD.

nal hypotension and a fall in perfusion pressure. The uterine vasculature is considered to be maximally dilated near term and a diversion of flow to other regions should be expected to occur.

In the pregnant ewe, Golichowski et al.[7] and Harake et al.[8] observed that continuous maternal nifedipine infusion caused a significant fall in maternal arterial pressure levels and a (presumably reflex) rise in maternal heart rate. In Harake's study, a significant fall in uterine blood flow was observed at an infusion rate of 10 μg/kg/min, and fetal pH, arterial oxygen content, and hemoglobin saturation declined significantly. Uterine vascular resistance did not change, however, and therefore the fall in uterine blood flow could be attributed to decreased maternal arterial pressure levels. There was no change in fetal heart rate or arterial blood pressure level. There was, however, an increase in total and regional fetal cerebral blood flow consistent with fetal adaptation to asphyxia.

In the near-term pregnant rabbit,[1-3] continuous maternal nicardipine infusion at dosages that caused significant reductions in prematurely induced uterine activity were found to cause a rise in maternal heart rate and fall in maternal arterial pressure level. A fall in total uterine and placental blood flow also occurred despite a significant increase in maternal cardiac output.[2]

Ducsay et al.[4] administered a bolus of 500 μg of nicardipine to the pregnant rhesus monkey and then continuously infused 6 μg/kg/min. A significant decline in maternal arterial pressure level and increase in maternal heart rate occurred. Despite fetal plasma concentrations of nicardipine, which averaged 6% of maternal serum levels, no changes occurred in fetal heart rate or arterial blood pressure level. There was however, a significant fall in fetal arterial pH and P_{O_2}, and an increase in fetal arterial P_{CO_2}. Uterine blood flow was not measured, but the lack of change in fetal blood pressure level suggests that the fetal asphyxia was more likely a result of a decrease in uteroplacental perfusion.

In our study, bolus administration of nicardipine to the fetus resulted in fetal serum concentrations of 414 ± 17 ng/ml to 527 ± 345 ng/ml after 2 minutes, and 41 ± 25 ng/ml to 61 ± 29 ng/ml at 60 minutes after infusion. The observation that 2-minute serum levels were higher after injection of 50 μg than they were after 100 μg may be explained by an increased variability of serum levels measured soon after injection, before equilibrium is complete. The levels at 30 and 60 minutes, however, reflect the dosage injected, higher levels being associated with the injection of 100 μg. In the rhesus monkey, Ducsay et al.[4] observed fetal asphyxia at peak fetal plasma nicardipine concentrations of 7 to 35 ng/ml. Harake et al.[8] described significant asphyxial changes at fetal plasma nifedipine levels of 91 ± 8 ng/ml. Thus, it would appear that maternal administration of these agents, which results in maternal hypotension and decreased uterine blood flow, is required for these adverse fetal effects to occur.

Because calcium antagonists apparently cross the primate placenta and are vasodilators, the chronically asphyxiated fetus may be adversely affected by these agents. Redistribution of blood flow to vital organs such as the brain, heart, and adrenals through a selective peripheral vasoconstriction may be reversed by vasodilators and result in worsening of fetal asphyxia. Whereas our study did not demonstrate a significant change in fetal peripheral resistance after nicardipine infusion in the normoxic fetus, the effect of this and similar agents on the compensated asphyxiated fetus is not known.

When comparison is made of the side effects of ni-

cardipine and nifedipine, it should be recognized that different calcium antagonists display different degrees of action on uterine, vascular, and cardiac tissue.[9] In addition, calcium antagonists affect fetal and adult cardiovascular systems differently and therefore the typical adult response should not necessarily be expected in the fetus.[10]

Whereas our study confirms the tolerance of the normoxic ovine fetus to nicardipine, the reported adverse effects on uterine blood flow and limited experience with these agents in the treatment of human preterm labor limits enthusiasm for their unrestricted use. Ulmsten et al.[11] have reported that when nifedipine was used clinically to treat premature labor, maternal arterial pressure was not affected, but a transient increase in heart rate occurred. No change in fetal heart rate was observed, and there were no neonatal complications noted. However, Read and Wellby[12] found that although neither maternal heart rate nor blood pressure levels were altered by maternal administration of nifedipine, fetal heart rate was significantly increased. We (Ferguson JE II, Holbrook RH Jr., Unpublished observations) noted decreased diastolic and mean blood pressure and reflex tachycardia in 12 preterm labor patients treated with sublingual and oral nifedipine. Whereas no adverse fetal effects have been reported in human beings, maternal hypotension does occur, and there exists the potential for adverse fetal effects at higher dosages or when fetal growth retardation or chronic asphyxia are present. Further studies of the response to the hypoxic fetus to calcium antagonists and changes in uterine blood flow in human beings are warranted.

REFERENCES

1. Lirette M, Holbrook RH Jr., Katz M. Effect of nicardipine HCl on prematurely induced uterine activity in the pregnant rabbit. Obstet Gynecol 1985;65:31-5.
2. Lirette M, Holbrook RH Jr., Katz M. Cardiovascular and uterine blood flow changes during nicardipine HCl tocolysis in the rabbit. Obstet Gynecol 1987;69:79-82.
3. Holbrook RH Jr., Lirette M, Katz M. Cardiovascular and tocolytic effects of nicardipine HCl in the pregnant rabbit: comparison with ritodrine HCl. Obstet Gynecol 1987; 69:83-7.
4. Ducsay CA, Thompson JS, Wu AT, Novy MJ. Effects of calcium entry blocker (nicardipine) tocolysis in rhesus macaques: fetal plasma concentrations and cardiorespiratory changes. Am J Obstet Gynecol 1987;157:1482-6.
5. Csapo AI, Puri CP, Tarro S, Henzl MR. Deactivation of the uterus during normal and premature labor by the calcium antagonist nicardipine. Am J Obstet Gynecol 1982;142:483-91.
6. Maigaard S, Forman A, Andersson K, Ulmsten U. Comparison of the effects of nicardipine and nifedipine on isolated human myometrium. Gynecol Obstet Invest 1983;16:354-66.
7. Golichowski AM, Hathaway DR, Fineberg N, Peleg D. Tocolytic and hemodynamic effects of nifedipine in the ewe. Am J Obstet Gynecol 1985;151:1134-40.
8. Harake B, Gilbert R, Ashwal S, Power GG. Nifedipine: effects on fetal and maternal hemodynamics in pregnant sheep. Am J Obstet Gynecol 1987;157:1003-8.
9. Granger SE, Hollingsworth M, Weston AH. A comparison of several calcium antagonists on uterine, vascular and cardiac muscles from the rat. Br J Pharmacol 1985; 85:255-62.
10. Boucek RJ Jr., Shelton M, Artman M, Mushlin PS, Starnes VA, Olson RD. Comparative effects of verapamil, nifedipine, and diltiazem on contractile function in the isolated immature and adult rabbit. Ped Res 1984;18:948-52.
11. Ulmsten U, Andersson K, Wingerup L. Treatment of premature labor with the calcium antagonist nifedipine. Arch Gynecol 1980;229:1-5.
12. Read MD, Wellby DE. The use of a calcium antagonist (nifedipine) to suppress preterm labour. Br J Obstet Gynaecol 1986;93:933-7.

Effect of prolonged hypoxemia on fetal heart rate accelerations and decelerations in sheep

Alan D. Bocking, MD,* Susan White, BSc, Robert Gagnon, MD, and Howard Hansford
London, Ontario, Canada

Experiments were conducted in 10 chronically catheterized fetal sheep to determine the effect of 24 hours of hypoxemia in the absence of progressive acidemia on fetal heart rate accelerations and decelerations. Fetal hypoxemia was produced by mechanically restricting uterine blood flow with a vascular clamp placed around the maternal common internal iliac artery. Fetal arterial oxygen tension decreased from 22.3 ± 1.8 to 17.8 ± 1.5 mm Hg at 2 hours and remained low for the 24-hour experimental period. Fetal pH decreased from 7.34 ± 0.01 to 7.20 ± 0.05 at 2 hours and returned to normal values by 12 hours. No significant change was observed in the number or characteristics of fetal heart rate accelerations or decelerations during the 24-hour control period. There was a significant increase in the number of accelerations from 48 ± 4 to 63 ± 4 per hour at 8 hours of hypoxemia followed by a return to control values by 12 hours. There was no significant change in the mean amplitude or duration of accelerations. There was a significant increase in the number of decelerations per hour with an associated increase in the mean amplitude but a decrease in the mean duration of decelerations during the first 16 hours of hypoxemia. We conclude that prolonged hypoxemia in fetal sheep leads to an initial increase in the number of both accelerations and decelerations in fetal heart rate followed by a return to normal patterns indistinguishable from those of the normoexemic fetus. (Am J Obstet Gynecol 1989;161:722-7.)

Key words: Hypoxemia, fetal heart rate, fetal sheep

Assessment of the presence or absence of fetal heart rate (FHR) accelerations in the antepartum period is widely used clinically to determine fetal health.[1] In the near-term human fetus, FHR accelerations occur episodically[2] and generally in association with gross fetal body movements.[3-5] We have shown that in fetal sheep, during the latter third of pregnancy, the majority of FHR accelerations also occur in association with fetal skeletal muscle activity measured by means of electromyogram recordings.[6] It is likely therefore that the central mechanisms controlling FHR accelerations in sheep are similar to that in the human fetus.

Little is known, however, about the mechanisms that regulate accelerations and decelerations in FHR under conditions of prolonged hypoxemia that may occur in human pregnancies, during labor, or in the antepartum period. We have previously demonstrated that there is an increase in both the number and the amplitude of FHR decelerations but no change in the number or characteristics of accelerations in fetal sheep subjected to a 2-hour episode of hypoxemia that is secondary to the restriction of uterine blood flow.[7] We have now extended the period of restricted uterine blood flow to 24 hours to determine if more prolonged hypoxemia in the absence of progressive acidemia will have a different effect on FHR accelerations and decelerations in the fetal sheep. This unique experimental situation would most closely resemble that occurring in human pregnancy with prolonged uteroplacental insufficiency such as may occur with abruptio placentae during the antepartum or intrapartum period.

From the Departments of Obstetrics/Gynaecology and Physiology, University of Western Ontario, and Lawson Research Institute, St. Joseph's Health Centre.
This work was supported by a grant from the Medical Research Council of Canada.
Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.
Reprint requests: Dr. Alan D. Bocking, Department of Obstetrics and Gynecology, St. Joseph's Health Centre, 268 Grosvenor St., London, Ontario, Canada, N6A 4V2.
**Medical Research Council of Canada Scholar.*
6/6/13896

Material and methods

Surgical procedure. Surgery was performed on 10 pregnant sheep of known mating dates between 120 and 125 days' gestation, with a mean (±SEM) gestational age at the time of surgery of 122 ± 1 days. Anesthesia was induced with intravenous thiopental (Abbott Laboratories, Montreal) and maintained with a combination of 1% halothane and 50% oxygen–50% nitrous oxide. With a sterile technique, a midline incision was made in the ewe's abdomen and a polytetrafluoroethylene vascular clamp was placed around the maternal common internal iliac artery. The ovarian arteries were ligated bilaterally. Polyvinyl catheters (V4; Bolab, Lake Havasu City, Ariz.) were placed in the fetal descending aorta (n = 2) and in the brachiocephalic artery (n = 8) for arterial blood sampling. Polyvinyl

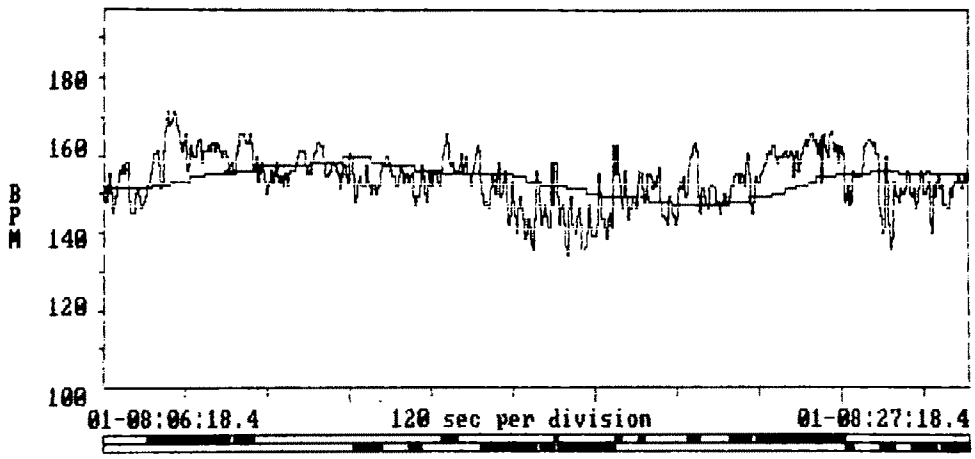


Fig. 1. Typical example of mean heart rate of a sheep fetus at 130 days' gestation with the baseline for identification of accelerations and decelerations superimposed.

Table I. Fetal arterial blood gas tensions, percent oxygen saturation, and mean FHR, before (−1) and during the period of reduced uterine blood flow

	Time				
	−1 hr	*+1 hr*	*+2 hr*	*+12 hr*	*+24 hr*
Oxygen saturation (%)	54.6 ± 3.1	31.9 ± 2.8*	31.8 ± 3.9*	27.5 ± 2.7*	29.1 ± 2.1*
P_{O_2} (mmHg)	22.3 ± 1.8	17.5 ± 0.7*	17.8 ± 1.5*	15.9 ± 1.4*	15.6 ± 1.0*
P_{CO_2} (mmHg)	49.4 ± 0.9	55.2 ± 2.0	55.0 ± 2.7	51.9 ± 1.2	52.1 ± 0.9
Base excess (mmol/L)	0.3 ± 0.6	−3.9 ± 1.5	−6.7 ± 2.0†	−2.7 ± 1.0	−1.9 ± 1.4
pH	7.34 ± 0.01	7.25 ± 0.03	7.20 ± 0.05†	7.28 ± 0.02	7.29 ± 0.02
FHR (beats/min)	177 ± 4	185 ± 12	192 ± 8†	196 ± 7†	189 ± 7†

Results are mean values ± SEM (n = 10).
*$p < 0.001$, compared with control (−1 hour).
†$p < 0.05$, compared with control (−1 hour).

catheters (VII; Bolab) were also placed in the maternal femoral vein and the amniotic cavity. Fine stainless steel wires (Cooner Sales Co., Chatsworth, Calif.), were placed over the fetal sternum for recording of the electrocardiogram (ECG). The uterine incision was repaired and the control cable for the vascular clamp, as well as all catheters and electrodes, were exteriorized to the ewe's flank.

Sodium penicillin G (1,000,000 units) was infused into the fetus and the amniotic cavity at the time of surgery and daily for the next 3 days. Sodium penicillin G (800,000 units) and streptomycin (250 mg) were injected intramuscularly to the ewe at the same time intervals. The ewes were housed in individual cages, with free access to food and water, and were allowed at least 5 days to recover from surgery before any experiments commenced. The use of animals for these studies was approved by the University of Western Ontario Council on Animal Care in keeping with guidelines provided by the Canadian Council on Animal Care.

Experimental protocol. All experiments began at 10 AM and consisted of a 24-hour control period during which continuous recordings of FHR were made. The vascular clamp was then manipulated to reduce the fetal arterial oxygen saturation by approximately 40%. Fetal arterial blood samples were drawn initially at 5, 10, and 15 minutes to ensure a stable reduction in fetal arterial oxygen saturation. Fetal arterial blood samples were subsequently obtained at intervals of 2 to 4 hours for a further 24 hours for measurement of blood gas tensions, hemoglobin, and fetal arterial oxygen saturation.

Data analysis. The fetal ECG was recorded with an AC electroencephalogram preamplifier (model 7P511, Grass Instruments, Quincy, Mass.) with successive R-R intervals recognized by a microprocessor rate meter (JRAK Biosignals, Malvern, Australia). An error rejection algorithm excluded any intervals that were <80% or >120% of the previous valid interval. FHR was then sampled by a microprocessor (Hartronix, Toronto) every 200 msec and stored for subsequent off-line analysis. A baseline FHR was determined by the micropro-

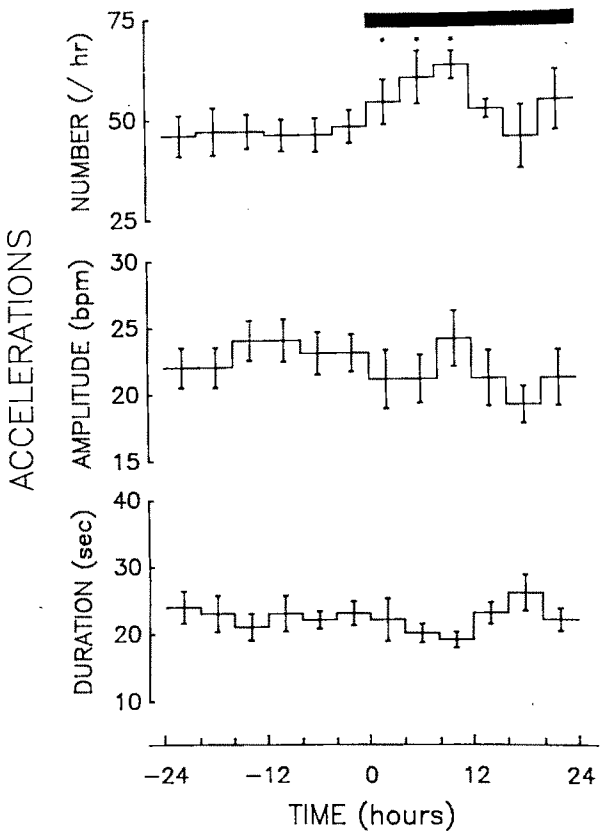


Fig. 2. Mean number per hour, amplitude, and duration of FHR accelerations in 4-hour epochs for the 24 hours before and 24 hours during hypoxemia indicated by the *solid bar*. $^*p < 0.05$.

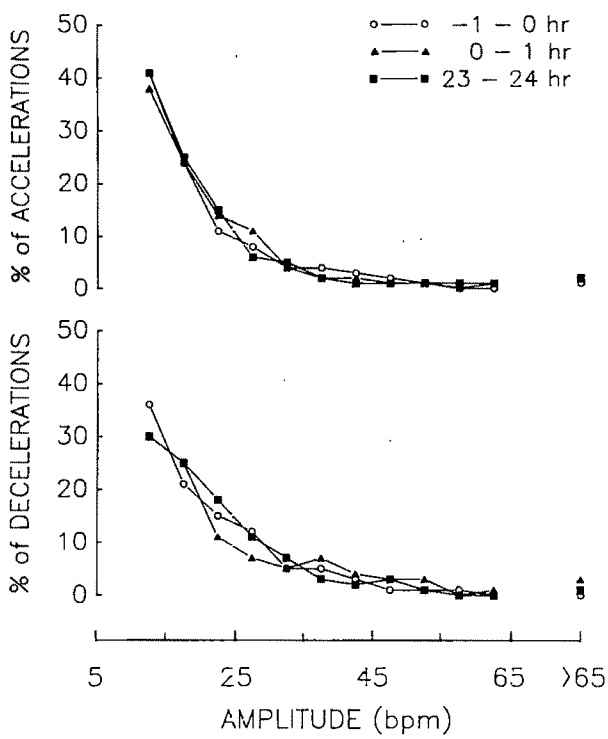


Fig. 3. Frequency distribution of the amplitude of accelerations and decelerations in intervals of 5 beats/min before and at 1 and 24 hours of hypoxemia.

cessor as the mean FHR over the previous 5 minutes, and accelerations and decelerations were identified as transient increases or decreases in the FHR of >10 beats/min lasting for ≥5 seconds. Fig. 1 demonstrates a typical example of the mean FHR of a sheep fetus at 130 days' gestation with the baseline superimposed.

Arterial oxygen tension, carbon dioxide tension, and pH were measured with an ABL glood gas analyzer (Radiometer, Copenhagen) at 37° C and then corrected for a fetal temperature of 39° C. Arterial oxygen saturation and hemoglobin were measured with an OSM2 Hemoximeter instrument (Radiometer). All results are presented as mean values ± SEM. Statistical significance was determined with either a one-way or two-way analysis of variance with repeated measures where appropriate.[8]

Results

Blood gas tensions and fetal arterial oxygen saturation. There was a significant decrease ($p < 0.001$) in both fetal P_{O_2} and fetal arterial oxygen saturation from control values of 22.3 ± 1.8 mm Hg and 54.6% ± 3.1%, respectively, which was maintained for the duration of the 24 hours (Table I). There was a small but significant decrease ($p < 0.05$) in fetal arterial pH from 7.34 ± 0.01 during the control period to 7.20 ± 0.05 at 2 hours of hypoxemia although pH subsequently returned to near-normal values This decrease in pH was largely due to the decrease ($p < 0.05$) in base excess from 0.3 ± 0.6 before the onset of hypoxemia to −6.7 ± 2.0 at 2 hours (Table I). There was a small, nonsignificant increase in fetal arterial P_{CO_2} from 49.4 ± 0.9 mm Hg during the control period to a maximum value of 55.2 ± 2.0 mm Hg at 1 hour of hypoxemia. There was no significant change in fetal hemoglobin concentrations from control values of 9.1 ± 0.5 gm/dl during the experimental period.

Mean FHR. There was an initial decrease in the mean FHR after manipulation of the vascular clamp, as reported previously.[9] This decrease was followed by a tachycardia with the maximum increase in mean FHR seen at 12 hours (Table I). There was no significant change in the fail time of FHR analyzed from 3.4% ± 0.9% during the 24-hour control period to 4.5% ± 1.9% during the 24 hours of hypoxemia.

Accelerations. There was no significant change in the mean number per hour, amplitude, or duration of accelerations during the 24 hours before the onset of hypoxemia (Fig. 2).

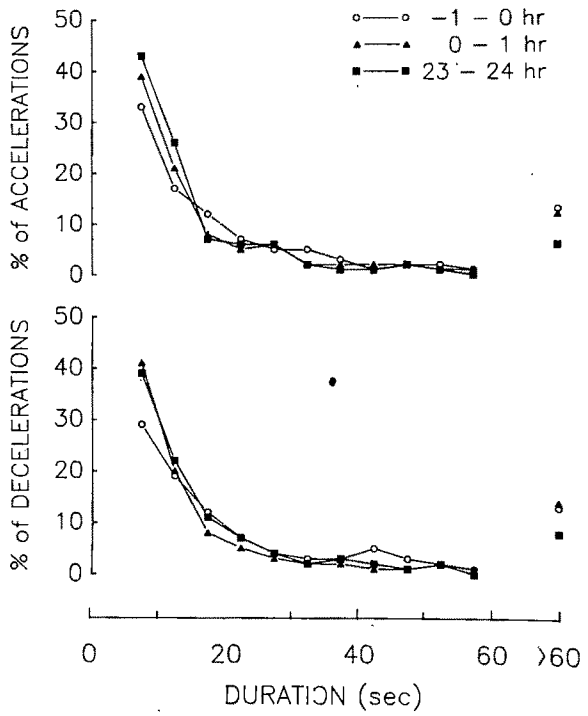


Fig. 4. Frequency distribution of the duration of accelerations and decelerations in 5-second intervals before and at 1 and 24 hours of hypoxemia.

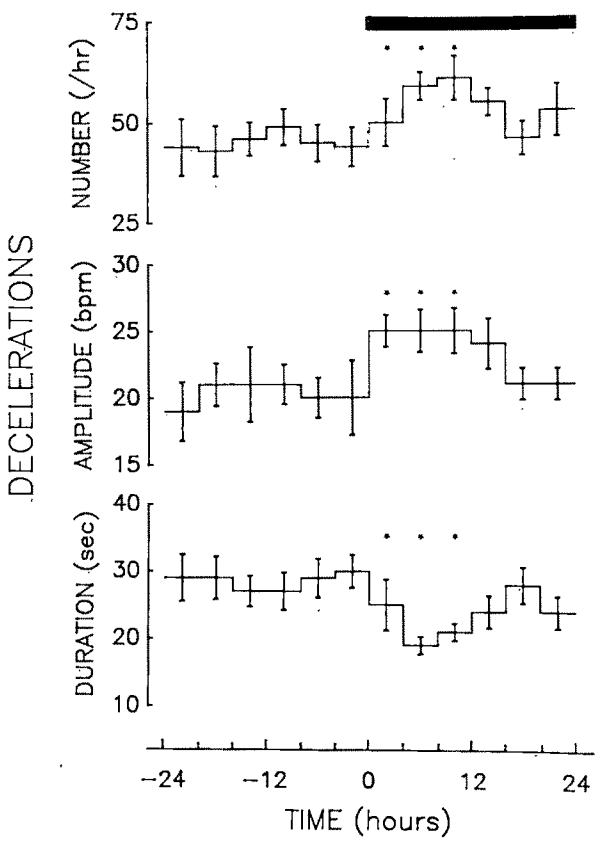


Fig. 5. Mean number per hour, amplitude, and duration of FHR decelerations in 4-hour epochs for the 24 hours before and 24 hours during hypoxemia, indicated by the *solid bar*. $*p < 0.05$.

There was a significant increase ($p < 0.01$) in the number of accelerations from 48 ± 4 per hour during the 4 hours immediately before the onset of the restriction in uterine blood flow to a maximum of 63 ± 4 per hour at 8 hours of hypoxemia. This was followed by a return to control values for the number of accelerations per hour by 12 hours of hypoxemia (Fig. 2). There was no significant change in the mean amplitude or duration of accelerations (Fig. 2).

There were no significant changes in the frequency distribution of the amplitude of accelerations at 1 hour or at 24 hours of hypoxemia when compared with the control period (Fig. 3).There was also no significant change in the frequency distribution of the duration of accelerations at 1 and 24 hours of hypoxemia (Fig. 4).

Decelerations. There was no significant change in the mean number per hour, amplitude, or duration of decelerations during the 24 hours before the onset of hypoxemia (Fig. 5).

There was a significant increase ($p < 0.01$) in the number of decelerations from 44 ± 5 per hour during the 4 hours before the onset of the restriction in uterine blood flow to a maximum of 61 ± 6 at 8 to 12 hours of hypoxemia (Fig. 5). The number of decelerations per hour then returned to control values.There was a significant increase ($p < 0.05$) in the mean amplitude of decelerations from 20 ± 1.2 beats/min during the control period to 25 ± 1.6 beats/min during the first 16 hours of hypoxemia followed by a return to control values (Fig. 5). There was also a significant decrease in the mean duration of decelerations from 29.9 ± 2.5 seconds during the control period to 19 ± 1.3 seconds at 4 to 8 hours of hypoxemia. This was followed by a return to control values (Fig. 5).

There was no significant change in the frequency distribution of amplitude (Fig. 3) or duration (Fig. 4) of decelerations at 1 and 24 hours of hypoxemia when compared with the control period.

Comment

In this study we have shown that prolonged hypoxemia in fetal sheep, which is secondary to the restriction of uterine blood flow, leads to an initial increase in the number of FHR accelerations and decelerations. This extends the observations of our previous study in which we found that 2 hours of hypoxemia of similar severity in fetal sheep leads to an increase in the number of decelerations.[7] These results are consistent with the findings of Dalton et al.[10] and Parer et al.,[11] that short-term hypoxemia induced by having the ewe inspire a

reduced oxygen gas mixture leads to an increase in both beat-to-beat variability and the 2-minute standard deviation of fetal heart periods. Huch et al.[12] also demonstrated that FHR variability in the human fetus during labor may be either increased or unchanged with hypoxemia as indicated with a transcutaneous Po_2 electrode.

In our study the increase in accelerations and decelerations was maintained for 12 to 16 hours, during which time the fetal arterial oxygen saturation remained significantly depressed at approximately 60% of control values. In fetal sheep FHR variability does not decrease with acutely induced maternal hypoxemia until severe metabolic acidemia has developed.[10] In our study acidemia, which was both metabolic and respiratory, occurred initially but pH returned to near-normal values by 12 hours. The effect of metabolic acidemia alone on FHR accelerations and decelerations is not known although it may contribute to the changes observed in this study.

It is of particular interest that the pattern of FHR accelerations and declerations returned to that of the normoexemic fetus by 16 hours, despite a persistent reduction in both fetal arterial Po_2 and fetal arterial oxygen saturation. This same time period of adaptation by the sheep fetus to prolonged hypoxemia that is secondary to the restriction of uterine blood flow occurs for behavioral activity.[13] The presence of FHR accelerations is closely linked to skeletal muscle activity in both the sheep and the human fetus.[3-6] Therefore the return to FHR patterns similar to that of the normoexemic fetus observed in this study may be a further indication of the behavioral adaptation to prolonged hypoxemia by the sheep fetus.

In these experiments, as in our previous studies, we observed an initial reflexively mediated bradycardia followed by a prolonged tachycardia. By 24 hours of hypoxemia, mean FHR returned to near-normal values although the redistribution of blood flow within the fetus, to the brain, heart, and adrenal glands, persists.[9] Since oxygen delivery to the brain is maintained by this increase in blood flow, despite a constant reduction in both fetal arterial oxygen saturation and Po_2, the alterations in FHR regulation observed under these experimental conditions do not appear to be mediated by either arterial oxygen tension or oxygen content alone. The increase in the number of FHR accelerations and decelerations and in mean FHR during the first 16 hours of hypoxemia may, however, be related to a prolonged increase in fetal plasma catecholamine concentrations, which has recently been observed under similar conditions of prolonged hypoxemia secondary to the restriction of uterine blood flow (Hooper S, Coulter C, and Harding R. Monash University, Melbourne, Australia, personal communication). Although short-term infusion of epinephrine to fetal sheep leads to an increase in FHR variability,[10] the effect of a prolonged increase in circulatory plasma catecholamine concentrations on FHR accelerations and decelerations in sheep is unknown.

Acutely induced hypoxemia in fetal rhesus monkeys also results in an increase in FHR variability, although the effects are greater on short-term than on long-term variability.[14] The appearance of late decelerations in FHR has been reported to occur before the loss of accelerations in spontaneous onset hypoxemia with progressive acidemia in rhesus monkeys.[15] There are no reports in the literature regarding the effects of more prolonged hypoxemia in the absence of acidemia on FHR variability in nonhuman primates.

It is of note that these findings of an initial increase in the number of accelerations and decelerations in fetal sheep with prolonged hypoxemia differ from that thought to occur in the human fetus. The prolonged absence of accelerations in the FHR (>80 minutes) is associated with a very high incidence of fetal hypoxemia and acidemia and with an increase in both perinatal morbidity and mortality.[16, 17] Smith et al.[18] have recently reported a decrease in the number of accelerations, as well as in the mean minute range, in preterm human fetuses delivered by cesarean section. These fetuses were subsequently found to have lower umbilical artery Po_2 values than fetuses with normal FHR characteristics. It is possible that if we had maintained the reduction in oxygen delivery to the fetus for a longer period of time we would eventually have observed a decrease in the number of FHR accelerations, as is seen in the human fetus. In addition, it is important to note that the sheep fetuses studied in this experimental protocol were all previously healthy and appropriately grown for gestational age. The effects of reduced uterine blood flow on FHR accelerations and decelerations in the growth-restricted sheep fetus are unknown, although they may more closely resemble those seen in the human fetus.

We thank Drs. B. S. Richardson, J. Patrick, and J. R. G. Challis for their interest in this work, Mrs. V. Ramsay for her excellent technical assistance, and Mrs. H. Cheung for statistical advice.

REFERENCES

1. Rochard F, Schifrin BS, Goupil F, Legrand H, Blottiere J, Sureau C. Nonstressed fetal heart rate monitoring in the antepartum period. AM J OBSTET GYNECOL 1976; 126:699-706.
2. Patrick J, Campbell K, Carmichael L, Natale R, Richardson B. Patterns of gross fetal body movements over 24-hour observation intervals during the last 10 weeks of pregnancy. AM J OBSTET GYNECOL 1982;142:363-71.
3. Timor-Tritsch IE, Dierker LJ, Zador I, Hertz RH, Rosen MG. Fetal movements associated with fetal heart rate ac-

celerations and decelerations. AM J OBSTET GYNECOL 1978;131:276-80.
4. Patrick J, Campbell K, Carmichael L, Probert C. Influence of maternal heart rate and gross fetal body movements on the daily pattern of fetal heart rate near term. AM J OBSTET GYNECOL 1982;144:533-8.
5. Rabinowitz R, Persitz E, Sadovsky E. The relation between fetal heart rate accelerations and fetal movements. Obstet Gynecol 1983;61:16-8.
6. Bocking AD, Harding R, Wickham PJD. Relationship between accelerations and decelerations in heart rate and skeletal muscle activity in fetal sheep. J Dev Physiol 1985;7:47-54.
7. Bocking AD, Harding R, Wickham PJD. Effects of reduced uterine blood flow on accelerations and decelerations in heart rate of fetal sheep. AM J OBSTET GYNECOL 1986;154:329-35.
8. Winer BJ. Statistical principles in experimental designs. 2nd ed. New York: McGraw Hill, 1971.
9. Bocking AD, Gagnon R, White SE, Homan J, Milne KM, Richardson BS. Circulatory responses to prolonged hypoxemia in fetal sheep. AM J OBSTET GYNECOL 1988; 159:1418-24.
10. Dalton KJ, Dawes GS, Patrick JE. Diurnal, respiratory, and other rhythms of fetal heart rate in lambs. AM J OBSTET GYNECOL 1977;127:414-24.
11. Parer JT, Dijkstra HR, Vredebregt PPM, Harris JL, Krueger TR, Reuse ML. Increased fetal heat rate variability with acute hypoxia in chronically instrumented sheep. Eur J Obstet Gynecol Reprod Biol 1980;10:393-9.
12. Huch A, Huch R, Schneider H, Rooth G. Continuous transcutaneous monitoring of fetal oxygen tension during labour. Br J Obstet Gynaecol 1977;84(suppl):1-39.
13. Bocking AD, Gagnon R, Milne KM, White SE. Behavioural activity during prolonged hypoxemia in fetal sheep. J Appl Physiol 1988;65:2420-6.
14. Ikenoue T, Martin CB, Murata Y, Ettinger BB, Lu PS. Effect of acute hypoxemia and respiratory acidosis on the fetal heart rate in monkeys. AM J OBSTET GYNECOL 1981;141:797-806.
15. Murata Y, Martin CB, Ikenoue T, et al. Fetal heart rate accelerations and late decelerations during the course of intrauterine death in chronically catheterized rhesus monkeys. AM J OBSTET GYNECOL 1982;144:218-23.
16. Brown R, Patrick J. The nonstress test: how long is enough? AM J OBSTET GYNECOL 1981;141:646-51.
17. Leveno KJ, Williams ML, DePalma RT, Whalley PJ. Perinatal outcome in the absence of antepartum fetal heart rate acceleration. Obstet Gynecol 1983;61:347-55.
18. Smith JH,Anand KJS, Cotes PM, et al. Antenatal fetal heart rate variation in relation to the respiratory and metabolic status of the compromised human fetus. Br J Obstet Gynaecol 1988;95:980-9.

Make researching easier with the Ten-Year Cumulative Index (1978-1987) to the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY

The Ten-Year Cumulative Index is a complete guide to more than 30,000 pages of original articles, case reports, letters, and editorials published in the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY from 1978 through 1987 (volumes 130 to 157). Numbering more than 800 pages, the Cumulative Index includes both a subject and an author index. This hard-cover volume is $64.50 ($69.00 international). Prices include shipping. *Payment must accompany all orders and must be in U.S. funds, drawn on a U.S. bank.*

Contact The C.V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318 USA; phone (314) 872-8370, ext. 394. In the U.S., call toll-free: 1-800-325-4177, ext. 394. Please allow six weeks for delivery.

Prolonged hyperinsulinemia reduces plasma fatty acid levels in the major lipid groups in fetal sheep

Paul L. Ogburn, Jr., MD, Marshall Goldstein, MD, Jane Walker, BS, and Barbara S. Stonestreet, MD

Providence, Rhode Island

The offspring of women with poorly controlled diabetes have increased total body fat. Experimental fetal hyperinsulinemia has previously been associated with increased fat accumulation in rhesus monkey fetuses and increased lipid content and lipogenesis in rat fetuses. We hypothesized that prolonged fetal hyperinsulinemia would alter plasma total fatty acids and the individual fatty acids in each major lipid group. Fatty acids determined by gas-liquid chromatography were measured in the arterial blood of 12 chronically catheterized fetal sheep after 10 to 12 days of insulin ($n = 7$) or placebo ($n = 5$) infusion. Plasma concentrations of insulin were 412 ± 63 μU/ml and 9 ± 3 μU/ml (mean ± SE) in the insulin- and placebo-treated fetal sheep, respectively. Prolonged fetal hyperinsulinemia was associated with lower circulating total fatty acid levels in each lipid class except cholesterol esters. Although the precursor of arachidonic acid, 18:2 (linoleic acid), was not decreased in any lipid class, insulin infusion was associated with reduced arachidonic acid in each lipid class. We conclude that prolonged fetal hyperinsulinemia decreases circulating total fatty acid and the individual fatty acids in each of the major lipid classes, except for the cholesterol esters. We speculate that these reductions in circulating fatty acids represent an insulin-mediated increase in tissue deposition. (Am J Obstet Gynecol 1989;161:728-32.)

Key words: Insulin, fetus, fatty acids, arachidonic acid, infant of diabetic mother

In pregnancies complicated by diabetes mellitus, maternal hyperglycemia (and consequently fetal hyperinsulinemia) has been suggested to be the cause of perinatal morbidities in the offspring.[1] Large-for-gestational-age infants of mothers with diabetes have been reported to have increased fat accumulation.[2] Fetal hyperinsulinemia in the absence of hyperglycemia accelerates fetal growth in the rhesus monkey fetus[3] and enhances fatty acid synthesis in overgrown rat fetuses.[4] Because insulin increases fetal growth, including the deposition of fat, one might anticipate that fetal hyperinsulinemia would be associated with altered plasma fatty acids in each of the major lipid components. Therefore, in conjunction with other studies of fetal hyperinsulinemia,[5] we evaluated the effect of hyperinsulinemia on total fatty acid levels and individual fatty acids in each major lipid components by comparing plasma values in insulin- and placebo-treated fetal sheep to test the hypothesis that the plasma fatty acid patterns are selectively altered by insulin treatment.

From the Brown University Program in Medicine, Departments of Obstetrics and Gynecology and Pediatrics, and Women and Infants' Hospital of Rhode Island.

Supported by the National Institute of Health and Human Development, Diabetes Center Grant No. P-50 HD11343, and the Mayo Foundation.

Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.

Reprint requests: Barbara S. Stonestreet, MD, Department of Pediatrics, Women and Infants' Hospital, 101 Dudley St., Providence, RI 02905-2401.

6/6/14714

Material and methods

The preparation of these animals and the experimental protocol have been previously reported in detail.[5] Surgery was performed on 12 pregnant mixed breed Eastern ewes with known breeding dates. Polyvinyl catheters were placed in the fetal descending aorta and inferior vena cava through pedal arteries and veins. The ewes were housed in the animal care facility postoperatively and for the duration of the experimental protocol and were allowed free access to food and water.

At 4 to 6 days after surgery, and at 123 to 126 days' gestation (term gestation is 150 days), the chronically catheterized fetal sheep were assigned to prolonged insulin- ($n = 7$) or placebo-infused ($n = 5$) groups. Insulin or placebo was then infused into a fetal vein continuously for 9 to 12 days. In the insulin-treated fetal sheep, 5 U/ml of purified porcine insulin (Iletin II, Eli Lilly and Co., Indianapolis, Ind.) was administered in a solution of 0.9% saline, 1.6% glycerol, 0.25% normal sheep serum, 1000 U/ml penicillin G, and 100 U/ml heparin at a rate of 3 ml/day of insulin. In the control group, a placebo infusion was administered at the same rate. After a mean of 11 days of prolonged infusion in each group, fetal arterial plasma samples were obtained to determine plasma insulin and circulating fatty acid levels in the major lipid groups.

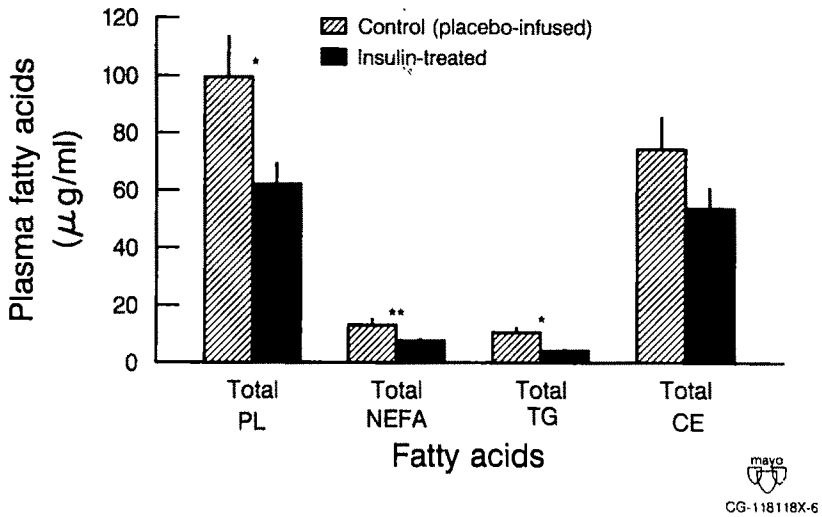


Fig. 1. Plasma concentrations of total fatty acids were significantly decreased in insulin-infused fetal sheep in phospholipids *(PL)*, nonesterified fatty acids *(NEFA)*, and triglycerides *(TG)*, but not in cholesterol esters *(CE)*. (Mean ± SE, $*p \leq 0.01$, $**p \leq 0.02$.)

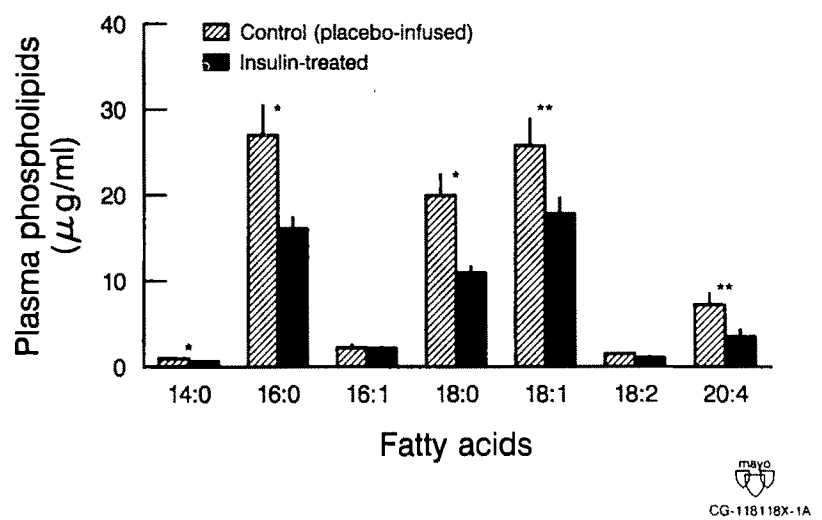


Fig. 2. Plasma concentrations of major fatty acids were decreased in phospholipids in insulin-infused fetal sheep. Major individual fatty acids measured included myristic (14:0), palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), linoleic (18:2), and arachidonic (20:4). (Mean ± SE, $*p \leq 0.01$, $**p \leq 0.02$.)

Analytic methods. Plasma insulin concentrations were determined by a double antibody radioimmunoassay.[6] Fetal arterial samples obtained to assess fatty acids in the major lipid groups were immediately centrifuged and the plasma was frozen at −20° C until analysis. For each analysis, 1 ml of plasma was extracted with chloroform/methanol (1:1) with a modified Bligh and Dyer technique.[7] Substituted forms of heptadecanoic acid (17:0) were added as internal standards to quantitate the phospholipids, triglycerides, nonesterified fatty acids, and cholesterol esters. Details of extraction and production of the methyl esters have been previously published.[5] The fatty acid methyl esters were extracted with petroleum ether and stored under nitrogen at −4° C until quantitation with capillary column gas-liquid chromatography (United Technologies/Packard Model 439, Downers Grove, Ill.). Fused silica capillary columns were used with a liquid phase of 007F/FAP, 50 m × 0.25 mm (Quadrex, New Haven, Conn.). Fatty acids were quantitated on a computing integrator (Spectra Physics model 4200, San Jose, Calif.) by comparison with the heptadecanoic acid internal standards. Retention times were determined by known standards. Other fatty acids were seen in much

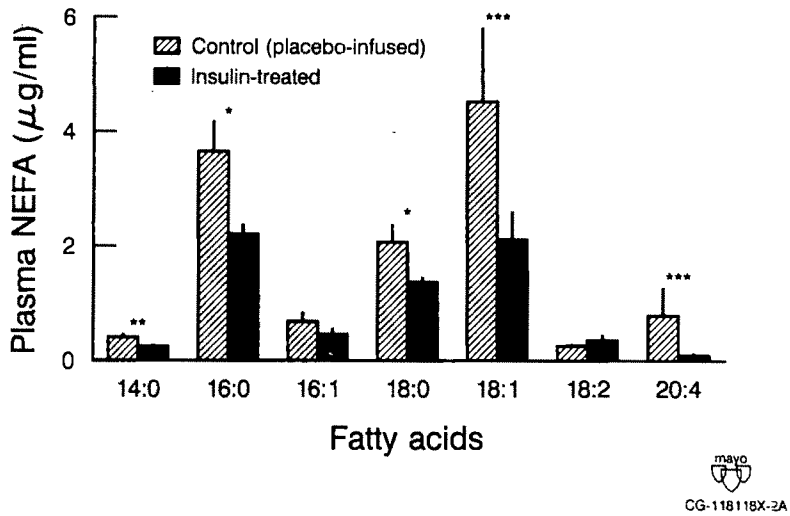


Fig. 3. Plasma concentrations of major fatty acids were decreased in nonesterified fatty acids in insulin-infused fetal sheep. Major individual fatty acids measured included myristic (14:0), palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), linoleic (18:2), and arachidonic (20:4). (Mean ± SE, $*p \leq 0.01$, $**p \leq 0.02$, $***p \leq 0.05$).

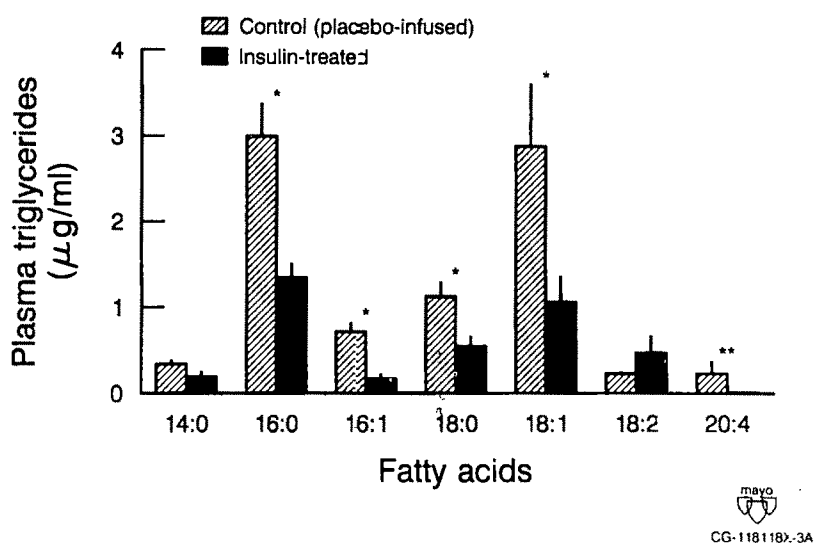


Fig. 4. Plasma concentrations of major fatty acids were decreased in triglycerides in insulin-infused fetal sheep. Major individual fatty acids measured included myristic (14:0), palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), (18:2), and arachidonic (20:4). (Mean ± SE, $*p \leq 0.01$, $**p \leq 0.02$.)

smaller quantities and are not part of the calculations.

Statistical analysis. The two-tailed unpaired Student *t* test was used to evaluate differences between means of the insulin- and placebo-treated fetal sheep for all fatty acid values reported. Results were reported as mean ± SEM; $p < 0.05$ was considered statistically significant.

Results

After 11 days of infusion, the insulin concentration was 412 ± 63 μU/ml and 9 ± 3 μU/ml (mean ± SE) in the insulin- and placebo-treated fetal sheep, respectively. The total plasma fatty acids of phospholipids, nonesterified fatty acids, and triglycerides each were significantly decreased in the insulin-infused fetal sheep (Fig. 1). Circulating fatty acid quantities and patterns for phospholipids, nonesterified fatty acids, and triglycerides are shown in Figs. 2, 3, and 4. Most of the major individual fatty acids, including arachidonic acid, were decreased in the insulin-treated sheep. Linoleic acid did not follow this pattern in any lipid groups. Cholesterol esters, unlike the other lipid groups, did

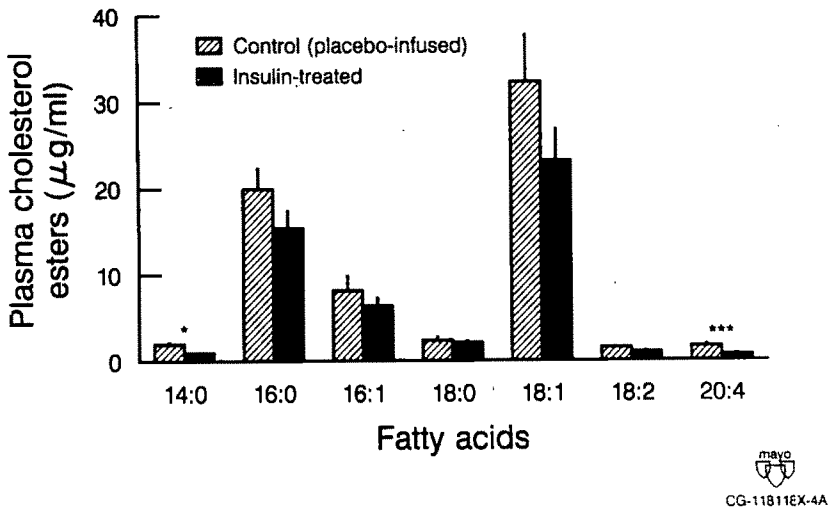


Fig. 5. Plasma concentrations were not consistently altered in cholesterol esters in insulin-infused fetal sheep. Major individual fatty acids measured included myristic (14:0), palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), linoleic (18:2), and arachidonic (20:4). (Mean ± SE, $*p \leq 0.01$,$***p \leq 0.05$.)

not show significant decreases in the circulating fatty acids in the insulin-treated fetal sheep (Fig. 5).

Comment

Insulin treatment for 11 days selectively reduced circulating phospholipid, triglyceride, and nonesterified fatty acids in the fetus. Because insulin promotes the movement of glucose and nonesterified fatty acids into cells including adipocytes for triglyceride production,[8] these reduced plasma levels may reflect increased tissue deposition for fat accumulation in the hyperinsulinemic fetus.[3,4] This concept is supported by insulin enhanced fatty acid synthesis in hyperinsulinemic rat fetuses.[4] Moreover, sustained hyperinsulinemia may diminish lipolysis, contributing to the lower circulating nonesterified fatty acid levels for production of liver phospholipids and triglycerides.[9] Therefore we speculate that the reduced total fatty acid plasma levels in our hyperinsulinemic fetal sheep may reflect increased fat synthesis and reduced lipolysis for the production of lipid-rich tissues in the fetus.

Linoleic acid is the precursor of arachidonic acid and, therefore, an essential fatty acid. Unlike arachidonic acid, however, it did not show significant decrease in response to insulin infusion. This may be, in part, because of relatively low levels of linoleic acid in the circulation of fetal sheep. It is possible that most of the linoleic acid available to the ovine fetus is converted to arachidonic acid before significant accumulation occurs in the fetal compartment. Another possibility might be that elongation of linoleic acid to arachidonic acid is reduced by insulin treatment of the fetus. On the other hand, preferential uptake of linoleic acid, and thus its removal from circulation by fetal tissues of diabetic rats, has been reported.[10]

Alterations in cholesterol esters were not as great as those of the other lipid components, which suggests that production in the fetus is less insulin-dependent. There is evidence that cholesterol esters are transported to the fetus from maternal circulation, and very little may be made directly by the fetus.[11]

It is well established that increased insulin availability decreases nonesterified fatty acids and triglycerides in the plasma of adults, fetuses, and neonates in some animal models.[8-11] There is also evidence that, at least in human beings and sheep, fetal plasma lipid levels are much lower than those of the maternal compartment.[9-13]

Prior work has suggested that hyperinsulinemia in the fetus leads to increased deposition of fatty acids into fat and other tissue stores.[1,2,4,8-11] The present study suggests that the immediate source of these fatty acids is in part the circulating lipids (triglycerides, nonesterified fatty acids, and phospholipids) whose levels are decreased by the action of insulin.

We acknowledge Dennis Berard for his excellent technical assistance and the continued support of William Oh, MD.

REFERENCES

1. Pederson J. Hyperglycemia-hyperinsulinism theory and birth weight. In: The pregnant diabetic and her newborn. 2nd ed. Williams and Wilkins, 1977.
2. Whitelaw A. Subcutaneous fat in newborn infants of diabetic mothers: an indication of quality of diabetic control. Lancet 1977;1:15-8.
3. Susa JB, McCormick KL, Widness JA, et al. Chronic hyperinsulinemia in the fetal rhesus monkey. Diabetes 1979;28:1058-63.
4. Vileisis RA, Oh W. Enhanced fatty acid synthesis in hyperinsulinemic rat fetuses. J Nutr 1983;113:246-52.

5. Stonestreet BS, Ogburn PL Jr, Goldstein M, Oh W, Wadness JA. Effects of chronic fetal hyperinsulinemia on plasma arachidonic acid and prostaglandin concentrations. AM J OBSTET GYNECOL [In press].
6. Hales CN, Randle PH. Immunoassay of insulin with insulin antibody precipitates. Biochem J 1963;88:137-46.
7. Bligh EG, Dyer WJ. A rapid method for total lipid extraction and purification. Can J Biochem Physical 1959; 37:911-7.
8. Avruch J, Carter JR, Martin DB. The effect of insulin on the metabolism of adipose tissue. In: Freinkel N, Steiner D, eds. Handbook of physiology: the endocrine pancreas. Washington, D.C.: American Physiological Society, 1972: 545-62.
9. Alexander DP, Britton HG, Cohen NM, Nixon DA. Plasma concentrations of insulin, glucose, free fatty acids, and ketone bodies in the fetal and newborn sheep and the response to glucose load before and after birth. Biol Neonat 1969;14:178-93.
10. Goldstein R, Levy E, Shafrir E. Increased maternal-fetal transport of fat in diabetes assessed by polyunsaturated fatty acid content in fetal lipids. Biol Neonat 1985;47: 343-9.
11. Roux JF, Yoshioka T. Lipid metabolism in the fetus during development. Clin Obstet Gynecol 1970;13:595-620.
12. Ogburn PL Jr, Williams PP, Johnson SB, Holman RT. Serum arachidonic acid levels in normal and preeclamptic pregnancies. AM J OBSTET GYNECOL 1984;148:5-9.
13. Ogburn PL Jr, Turner SI, Williams PP, Johnson SB, Holman RT. Essential fatty acid patterns in preeclampsia. Zentralbl Gynakol 1986;983-9.

Pharmacokinetics of azidothymidine during late pregnancy in Long-Evans rats

B. B. Little, MA, PhD, R. E. Bawdon, PhD, J. T. Christmas, MD, S. Sobhi, BS, and L. C. Gilstrap III, MD

Dallas, Texas

Azidothymidine (Zidovudine) is used to treat symptomatic human immunodeficiency virus infection. It has the ability to reverse seropositivity as well as decrease serum viral titers, and perhaps decrease potential for transmission of the virus. An animal model (pregnant Long-Evans rats) was used to investigate the potential for placental transmission of azidothymidine. The drug crosses the placenta to reach concentrations in the placenta and fetus that are comparable to 75% and 58%, respectively, of those in the maternal serum by 2 hours after administration. By 4 to 6 hours after administration azidothymidine concentrations in the placenta and fetal liver significantly exceed maternal concentrations. (AM J OBSTET GYNECOL 1989;161:732-4.)

Key words: Azidothymidine, pharmacokinetics, pregnancy, human immunodeficiency virus, placental transfer

Azidothymidine (AZT) (Zidovudine, Burroughs Wellcome, Research Triangle Park, N.C.) is used in the treatment of symptomatic human immunodeficiency virus (HIV) infection. AZT has recently been reported to transiently reverse HIV seropositivity and reduce serum viral titers.[1] Furthermore, the drug may reduce the infectious state of an HIV-positive person.[2] The risk of perinatal HIV transmission from mother to fetus is unknown but appears high with 25% to 60% of infants born to HIV-positive mothers becoming seropositive in early infancy.[3-5]

It is conceivable that the drug may be used to treat women of reproductive age, and such women may become pregnant. AZT's ability to cross the placenta is of fundamental importance to evaluate this situation. There are no published data available with regard to placental transfer of this antiviral agent.

The purpose of this study was to evaluate the placental transfer of AZT in a rat animal model.

Material and methods

Long-Evans rats were chosen as the animal model for analysis of placental transfer of AZT because (1) this species has been used in similar studies, (2) the size is convenient for handling and dissection, and (3) the rats have a known heritage for many generations and

From the Department of Obstetrics and Gynecology, The University of Texas Southwestern Medical Center.
Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.
Reprint requests: Dr. Bert Little, Department of Obstetrics and Gynecology, The University of Texas Southwestern Medical Center, Dallas, TX 75235-9032.
6/6/13889

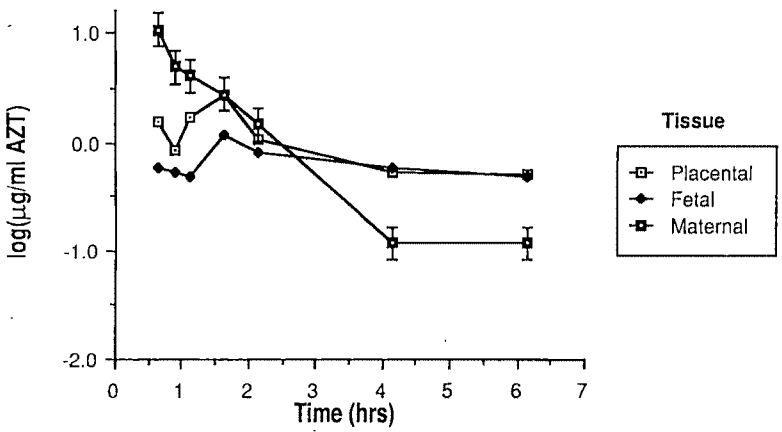


Fig. 1. AZT concentrations in maternal, placental, and fetal compartments (maternal values ± 1 SEM).

Table I. Concentrations of AZT (micrograms per deciliter) in maternal, placental, and fetal tissues

Time (hours)	*Maternal*		*Placental*		*Fetal*	
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*
0.5	8.9	2.3	1.3	0.4	0.5	0.0
0.75	4.1	4.0	0.7	0.1	0.4	0.1
1.0	3.4	1.8	1.4	0.6	0.4	0.1
1.5	2.3	2.0	2.3	1.7	1.0	0.8
2.0	1.2	1.0	0.9	0.2	0.7	0.4
4.0	0.1	0.1	0.4	0.1	0.5	0.0
6.0	0.1	0.1	0.4	0.2	0.4	0.1

are genetically homogeneous. A total of 16 timed pregnant Long-Evans rats were obtained from a commerical vendor and maintained on regular rat chow and water as desired in our Animal Resources Center until the eighteenth day of pregnancy. This study was approved by the Institutional Review Board for Animal Research. On the eighteenth day of pregnancy rats were orally given 30 mg/kg of AZT, and were killed by decapitation at the following time intervals: 0.5, 0.75, 1.0, 1.5, 2.0, 4.0, and 6.0 hours. Two rats were assigned to each treatment group, and two rats were not pregnant. The reason two rats per time period were used is because (1) this is the minimum number of animals possible to compute SD, error, and Student *t* test statistics, and (2) a paired Student *t* test (degrees of freedom = 2) can detect differences in concentration of the magnitude of a half-life as statistically significant with this sample size ($\alpha = 0.05$, $\beta = 0.90$). Maternal blood was collected at the time of decapitation, and fetuses and placentas were immediately removed by cesarean section. These samples were frozen at −4° C after collection and thawed to room temperature at the time of analysis.

Maternal serum, placental tissue extracts, and fetal liver homogenates were analyzed by high-performance liquid chromatography for AZT concentrations. All assays were performed with a guard column between the injection pump and the high-performance liquid chromatography column. Samples for assay were prepared for analysis by direct injection of the centrifuged tissue homogenate. Serum specimens were assayed by precipitation with an equal volume of acetonitrile. The specimens were vortexed for 15 seconds and centrifuged at 3000 *g* for 15 minutes. Assays were accomplished with a Waters 481 detector (wavelength = 254 mm), a C_{18} Micro-band-a-pak column and a Beckman 110 pump. The mobile phase was 16% acetonitrile and 84% 0.1 mol/L phosphate buffer with a pH of 2.5. The flow rate was 2.0 ml/min.[6] Between-batch and within-batch reproducibility studies were performed, and differences were <5%, well within acceptable limits.

Results

AZT concentrations in maternal serum dropped quickly, decreasing by half in the first hour (Table I), which correlates with the reported half-life of the drug in human patients (60 minutes).[7] By 30 minutes after administration, placental tissues attained concentrations of about one seventh, and fetal liver contained

Table II. Paired Student *t* test of difference in AZT concentrations in maternal, placental, and fetal compartments

	Maternal versus placental		*Placental versus fetal*		*Maternal versus fetal*	
Time	*t**	*<p*	*t*	*<p*	*t*	*<p*
0.5	6.2	0.03	20.0	0.01	21.0	0.01
0.75	1.7	0.2	0.2	0.8	1.8	0.3
1.0	2.1	0.2	2.8	0.1	3.7	0.08
2.0	0.0	0.9	0.9	0.4	0.9	0.4
4.0	15.0	0.01	1.9	0.2	7.9	0.02
6.0	5.9	0.05	0.0	0.9	4.2	0.05

*Degrees of freedom = 2.

concentrations of about one eighteenth of those in maternal serum, significantly different by paired Student *t* test ($p < 0.01$) (Table II). At 2 hours after dosing, concentrations in all three tissues were similar. Maternal serum and placental tissue achieved concentrations slightly higher than the fetus (Table I).

There was rapid clearance of AZT from maternal serum, with nearly undetectable levels by 4 to 6 hours after administration. However, at 4 to 6 hours after administration, both fetal and placental AZT levels remained four to five times higher than maternal levels ($p < 0.05$; Fig. 1), near the lower range for the serum of human adults who receive therapeutic doses.

Comment

Results of the present study indicate that, in the rat, AZT rapidly crosses the placenta. Whereas the drug rapidly cleared from the maternal compartment, it appears to be concentrated in the fetoplacental compartment. There are at least two mechanisms by which the findings of this study could be explained. Approximately 40% of AZT becomes glucuronoside in the first pass through the liver in adults. This AZT glucuronoside is inactive as an antiviral agent and ultimately is excreted by the kidneys.[8] Decreased hepatic metabolism in the immature fetal liver may, therefore, be responsible for persistence of significant levels in the fetal compartment.

A second possible mechanism for the findings of fetal and placental concentration of the agent involves the method of placental transfer. Preliminary studies at our institution with explanted human placental cotyledons suggested that AZT is actively transported across the human placenta into the fetal compartment.[6] Active transport could easily explain the results of this study with persistent levels in both fetal liver and placental tissue. Safety of AZT during pregnancy is, therefore, an issue because if maternal exposures occur, embryofetal exposure also seems probable. the teratogenic potential of the drug appears low in rats born to mothers who were given several times the usual human dose during early or late embryogenesis (unpublished observations). However, rodent animal models are typically poor predictors of human teratogenic potential.[9]

Further studies of AZT in human beings or nonhuman primates are needed confirm a low teratogenic potential, and to verify active placental transport of this agent. Caution must be used when extrapolating findings of animal studies of both placental transfer and teratogenicity to the condition in human beings because the composition of the rat placenta and development of the rat conceptus are different from the human placenta and conceptus. Although both are hemochorial placentas, in the rat there is a layer of cellular trophoblast interposed between the chorionic villus and maternal blood. This may cause species differences in the drug's kinetics across the placenta. However, despite differences in placental morphology, the results of this study concur with our findings in explanted human placental cotyledons,[6] and suggest that substantial amounts of AZT cross the placenta and reach the developing conceptus.

REFERENCES

1. Jackson GG, Paul DA, Falk LA, et al. Human immunodeficiency virus (HIV) antigenemia (p24) in the acquired immunodeficiency syndrome (AIDS) and the effect of treatment with zidovudine (AZT). Ann Intern Med 1988; 108:175-80.
2. Fischl MA, Richman DD, Grieco MH, et al. The efficacy of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex: a double-blinded, placebo-controlled trial. N Engl J Med 1987;317:185-91.
3. Minkoff H, Nanda D, Menez R, Fikrig S. Pregnancies resulting in infants with acquired immunodeficiency syndrome: follow-up of mothers, children, and subsequently born siblings. Obstet Gynecol 1987;69:288-91.
4. Arico M, Azzini M, Caselli D, Maccario R, Marseglia G, Michelone G. AIDS: outcome of children bortn to HTLV-III/LAV infected mothers. Helv Paediat Acta 1986;41: 477-86.
5. Borkowsky W, Paul D, Bebenroth D, Krasinski K, Moore T, Chandwani S. Human-immunodeficiency-virus infections in infants negative for anti-HIV by enzyme-linked immunoassay. Lancet 1987;1:1168-71.
6. Fortunato SJ, Bawdon RE, Swan KF, Sobhi S. Transfer of azidothymidine across the in vitro perfused human placenta. In: Proceedings of the Society for Gynecologic Investigation, 1989;36:82.
7. Yarchoan R, Klecker RW, Weinhold KJ, et al. Administration of 3'-azido-3'-deoxythymidine, an inhibitor of HTLV-III/LAV replication, to patients with AIDS or AIDS-related complex. Lancet 1986;1:575-80.
8. Richman DD, Fischl MA, Grieco MH, et al. The toxicity of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. N Engl J Med 1987; 317;192-7.
9. Shepard TH. Human teratogenicity. Adv Pediatr 1986; 33:225-68.

Ultrastructural aspects of preeclampsia

I. Placental bed and uterine boundary vessels

Douglas R. Shanklin, MD, and Baha M. Sibai, MD

Memphis, Tennessee

Biopsy specimens were obtained under direct vision at the time of cesarean section from 42 patients (33 preeclamptic and nine normotensive patients) and from three hysterectomies (all in normotensive patients). Mean gestational age was 32.8 ± 0.9 weeks (range, 26 to 40 weeks) in women with preeclampsia and 36.1 ± 1.1 weeks in normotensive women (range, 32 to 41 weeks). Tissues were obtained from the central placental bed and from nonplacental sites. Specimens were examined histologically and by electron microscopy. Ultrastructural changes in small vessels, primarily venules, were compared with preeclamptic blood pressure. Extensive ultrastructural endothelial injury was found consistently in both site and nonsite areas in all of the specimens from women with preeclampsia but not in normotensive women ($p < 0.0001$). There was no apparent correlation between the type or degree of endothelial damage and maternal hypertension. The same types and relative severity of specific vascular injury were present in both placental and nonplacental sites. Endothelial and derivative vascular injury occurs more or less uniformly in the uterus in preeclampsia, especially along the boundary zone between maternal and fetal tissues. (Am J Obstet Gynecol 1989;161:735-41.)

Key words: Placental site biopsy. preeclampsia, vascular injury, ultrastructural changes, uterine boundary zone

The continuing search for both the cause and pathogenesis of preeclamptic toxemia (gestosis humanis) over the past 20 years has turned to the integrity of uterine vessels and changes in coagulation, which may relate to endothelial injury as shown by histologic and electron microscopic methods.[1-18] Histologic,[1-3, 9, 10, 13] transmission ultrastructural[5-7, 12, 13, 14, 18, 19] and scanning electron microscopic[17] studies have considered uterine arteries at several levels of the arborization, as shown especially clearly by Sheppard and Bonnar.[18, 19] Interest has been directed primarily at spiral arteries* and more general features of the placental bed.[1, 3, 5-7, 12-14, 17-19] These largely European studies have identified a number of lesions: acute atherosis,[1, 7, 10, 13] hyperplastic arteriosclerosis,[13] and alterations[1, 9, 13, 18, 19] in the expected physiologic restructuring of the spiral arteries during pregnancy.[3, 5, 6, 13, 14, 17-19] The cited articles account for at least 426 placental bed biopsies and samples of placental site tissue from uteri studied after cesarean section hysterectomy or pregnancy termination by earlier hysterectomy. Two large series were identified only as "over 100"[13] and "several hundred"[3] biopsies, comprising a possible total of perhaps 750 individual cases studied to date. This material was derived from clinical backgrounds of normal pregnancy,† preeclampsia or other hypertensive states,[2, 7, 9, 10, 13, 18, 19] and intrauterine growth retardation.[1, 10, 18, 19] Some studies included multiple categories of clinical condition of either mother or fetus.[1, 9, 10, 18, 19] Several studies also examined placentas,[2, 7, 10, 13, 18, 19] including two in which the placenta remained in situ after hysterectomy.[2, 13] The basic techniques for obtaining placental bed biopsies and an overview of the information obtained up to 1986 were summarized in a critical review by Robertson et al.[14] The observation that the placental bed is a heterogeneous tissue field, making sampling difficult, has been emphasized in a number of reports.[3, 13, 14] Less often, but no less emphatically, several comparative studies have indicated that no distinction can be made between normotensive and hypertensive cases by histologic or ultrastructural examination of spiral arteries,[18, 19] despite the finding by Brosens et al.[1] that acute atherosis was found principally in hypertension combined with fetal growth retardation and not in normotensive pregnancies, a finding later disputed presumptively by study of 39 placentas but not placental beds.[10]

In light of these studies and the ongoing need to elucidate primary vascular events in early preeclampsia, we began in mid-1987 to collect central placental bed biopsy material at time of cesarean section delivery with

From the Departments of Pathology and Obstetrics and Gynecology, University of Tennessee, Memphis.

Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.

Reprint requests: Douglas R. Shanklin, MD, Department of Pathology, University of Tennessee, Memphis, 800 Madison Ave., Memphis, TN 38163.

**References 1, 3, 5-7, 9, 12-14, 17-19.*

†References 1, 3, 5, 6, 9, 12-14, 17-19.

6/6/13891

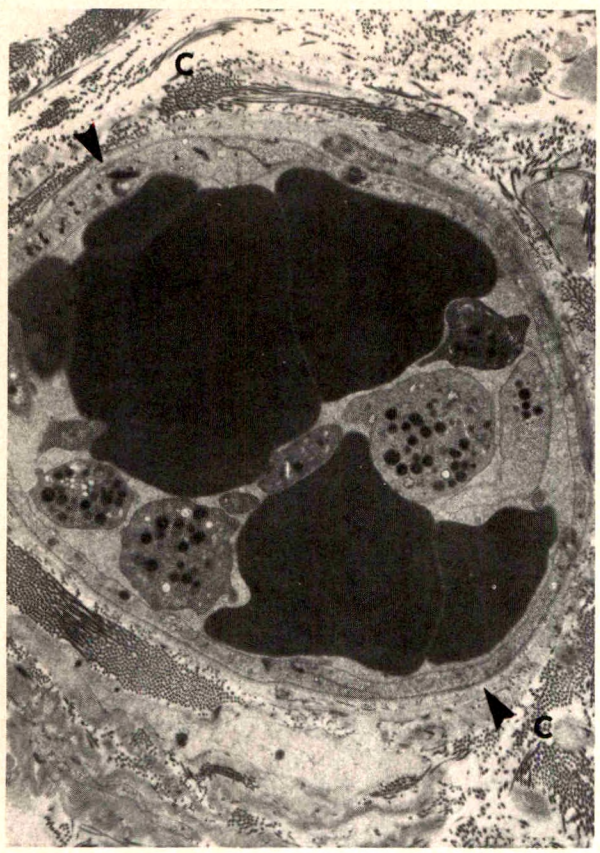


Fig. 1. Normal small venule in placental bed region of the uterine boundary zone, preeclampsia. Platelets and erythrocytes indicate a diameter of the vessel at 15 to 20 μm. Note the excellent preservation of platelets. Bundles of collagen *(C)* are outside the vascular wall *(arrows)*, which is composed of flat endothelium with some overlapping of cells and tight junctions. Occasional normal venules are found in the boundary zone of women with preeclampsia. Figs. 1 to 3 show the range of change from normal to severe endothelial injury. (Original electron microscopic magnification ×3150.)



Fig. 2. Segmentally injured large venule in placental bed region of the uterine boundary zone, preeclampsia. Vessel diameter is approximately 20 to 30 μm. Enlarged endothelial nuclei *(N)* and segmentally swollen cytoplasm *(arrows)*. Note also cavitated endothelial mitochondria *(M)*. Similar change in smaller venules or somewhat greater change in vessels of this size would show reduction in lumen. (Original electron microscopic magnification ×1600.)

simultaneous biopsy of nonplacental site uterine boundary tissue, with emphasis on smaller blood vessels. Histologic and transmission electron microscopic studies to date are the subject of this report. Extensive ultrastructural endothelial injury was found consistently in both placental site and uterine nonplacental site boundary myometrium in all of the specimens from the preeclamptic patients but not in specimens from normotensive women ($p < 0.0001$).

Material and methods

Informed consent for placental bed and uterine boundary biopsies was obtained from various patients in the prenatal clinics of the E. H. Crump Hospital who were scheduled for cesarean section for obstetric or perinatal indications. A total of 42 patients, 33 with preeclampsia (systolic blood presure >140 mm Hg, or diastolic blood pressure >90 torr or both, with proteinuria >300 mg/24 hours), and nine normotensive women had biopsies. Three cesarean section hysterectomies from normotensive patients were also studied; thus a total of 45 patients (12 normotensive and 33 hypertensive) was studied. At the time of cesarean section, biopsy specimens approximately 0.8 × 0.6 × 1.0 cm in size were excised by sharp dissection and were immediately placed in the appropriate fixative. Placental site samples were taken from the center of the attachment site just after placental removal, and comparable nonsite samples were taken from the opposite uterine wall. On receipt in the pathology laboratory, appropriate accession numbers were assigned and the samples were trimmed into pieces for paraffin or plastic embedding according to ordinary techniques, beginning with 10% neutral buffered aqueous formalin for histology and net 1.0% glutaraldehyde buffered in Sörenson's solution for ultrastructural study.

Paraffin sections were cut at 5 μm and were stained by hematoxylin and eosin for light microscopy. Histologic preparations were available for all 45 patients.

Plastic sections for electron microscopy were pre-

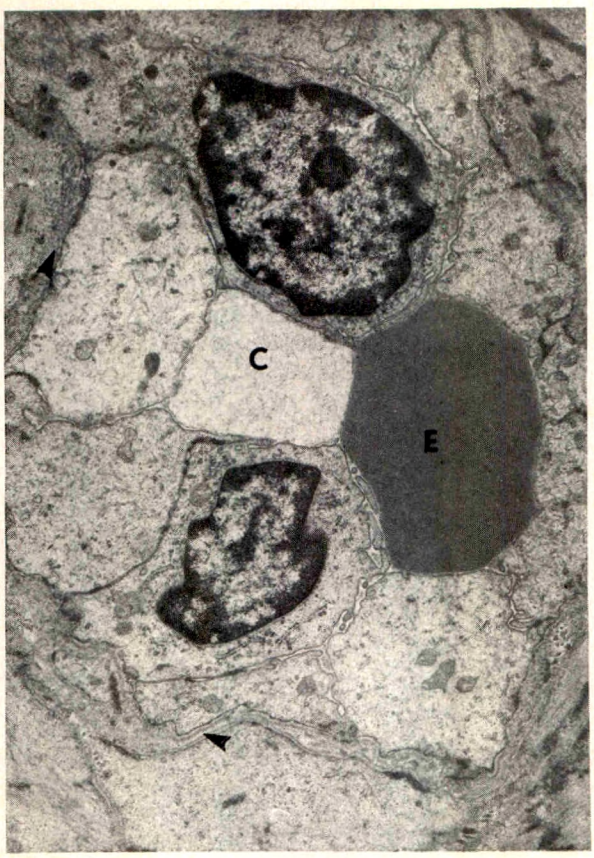


Fig. 3. Marked reduction of lumen of large venule, placental bed region of uterine boundary zone, preeclampsia. One intact erythrocyte *(E)* is pressed by greatly swollen endothelial cells including herniated endothelial cytoplasm from a different plane *(C)*. Venular basement membrane is irregular but intact *(arrows)*. (Original electron microscopic magnification ×8000.)



Fig. 4. Partial surface erosion, swollen endothelium, large venule, nonplacental site region of uterine boundary zone, preeclampsia. Considerable swelling is marked by zonal and linear erosion *(arrows)*. Closely proximate adventitial collagen lies next to hypertrophic uterine muscle *(M)*. (Original electron microscopic magnification ×4000.)

pared in the usual manner with lead acetate staining. Electron micrographs were taken at ×1600 to ×2500 original magnification in a Zeiss EM-10C and were printed at an average further enlargement of ×2.7. Electron micrographs were available for 27 of 33 preeclamptic women and for 9 of 12 normotensive women.

Results

Vascular and ultrastructural changes were compared between the two groups and with preeclamptic maternal blood pressure.

Clinical aspects. The average gestational age in preeclamptic pregnancies was 32.8 ± 0.9 weeks (range, 26 to 40 weeks) and 36.1 ± 1.1 weeks in normotensive pregnancies (range, 32 to 41 weeks; the difference was not significant, $t = 0.50$, $p > 0.5$). Maximum recorded blood pressures fulfilled criteria used in the primary diagnosis of preeclampsia (systolic blood pressure >140 mm Hg, diastolic blood pressure >90 torr, or both, with proteinuria >300 mg/24 hr). The variability of blood pressure in each patient was noted, but since no correlation with average or maximum values and tissue findings could be demonstrated, further correlation with the variability was not undertaken. There were no maternal deaths in this series. Perinatal mortality was low; there were no stillbirths and only one neonatal death in the hypertensive group, which yielded a group rate of 3.0% and 2.2% overall. None of these patients had eclamptic seizures.

The differences in blood pressures were strongly significant (systolic: $t = 10.5$, $p < 0.001$; diastolic: $t = 10.8$, $p < 0.001$). The more than 3-week difference in mean gestational age suggested some significance by Student's t test ($t = 2.32$, $0.05 > p > 0.02$). The F value for variation of population, however, was 5.38 (not significant), which devalued the t test result. We anticipate that enlargement of the normotensive portion of the series will further reduce this limited difference without impairing the distinction with regard to vascular injury.

Pathologic aspects. Histological findings were helpful in assessing the ultrastructural changes and will be described first.

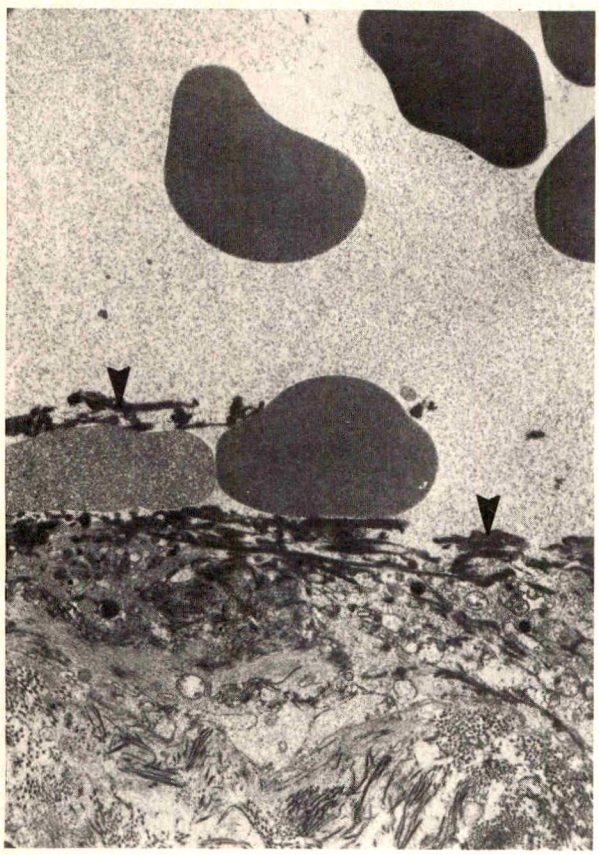


Fig. 5. Linear fibrin deposition *(arrows)* on eroded endothelium, large venule, nonplacental site region, uterine boundary zone, preeclampsia. Note intermingling of cellular debris and strands of fibrin. (Original electron microscopic magnification ×4000.)

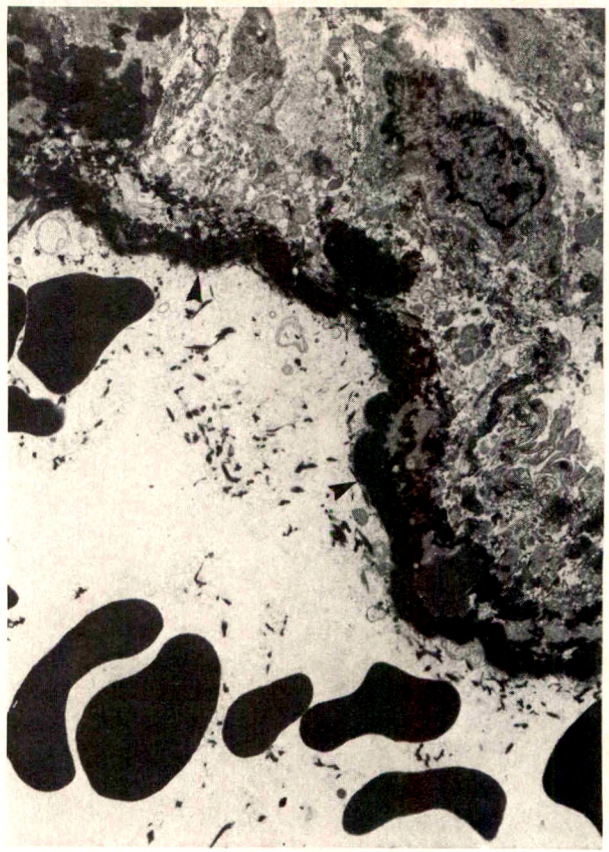


Fig. 6. Compacted heavy fibrin deposition *(arrows)* and loose luminal fibrin replacing extensively eroded endothelium, nonplacental site region, uterine boundary zone, preeclampsia. (Original electron microscopic magnification ×2500.)

Histologic changes. Histologic changes that were readily observed included some apparent medial hypertrophy of smaller arteries and arterioles, primarily but not exclusively in the placental site, dilated veins and venules with and without thrombosis, occasional placental site trophoblastic giant cells, focal hemorrhage, some interstitial edema, and uterine smooth muscle reflecting the expected gestational hypertrophy. The adaptive changes in spiral arteries were seen in most specimens, but no distinction with regard to degree or character of the change was observed between preeclamptic and normal subjects. Decidua and regressed or atrophic endometrial glands were observed less often. Occasional lymphocytes were found in the junctional tissues and occasional circulating blood elements, such as polymorphonuclear leukocytes, macrophages, and platelets were found in addition to many erythrocytes. Whereas overt thrombosis was uncommon, small foci of fibrin deposit were found more often, especially in relation to tears and erosions of the endothelium, and in overt tears into the perivascular space and myometrium. None of the changes were present such that unequivocal, consistent identification of placental site as opposed to nonplacental site sampling was possible. Placental site trophoblast, when present, would identify the site, but only one third of these biopsies contained trophoblast. The possibility that trophoblastic site giant cells may be found beyond the placental bed was enhanced by one case in which both biopsies contained them.

More importantly, an overall view of the boundary between the uterus and the placental sac is possible from a composite of these observations. Vascular injury and tears into uterine soft tissues, with hemorrhage, are commonplace; essentially these processes occur both in the placental site and elsewhere throughout the boundary zone either equally randomly or with comparable frequency. In fact, such observations make it possible to define the boundary zone as the combination of the atrophic or regressive endometrium, which is displaced and compressed by the pregnancy sac, plus the interdigitating connective tissues of the inner myometrium including blood vessels.

The ultimate maternal vascularization of the placental site is therefore a modification of this region, with

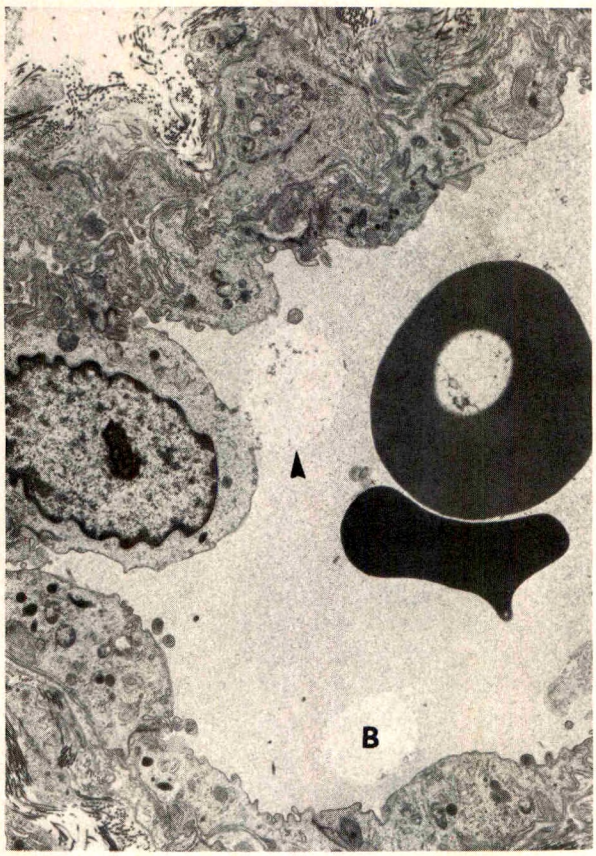


Fig. 7. Rupturing bleb *(arrow)* of endothelium, large venule, nonplacental site region of uterine boundary zone, preeclampsia. This is one possible precursor to overt paraluminal erosion of endothelium. Note a similar structure *(B)* near lower edge of photograph, protruding from a different level in the block. (Original electron microscopic magnification ×3150.)

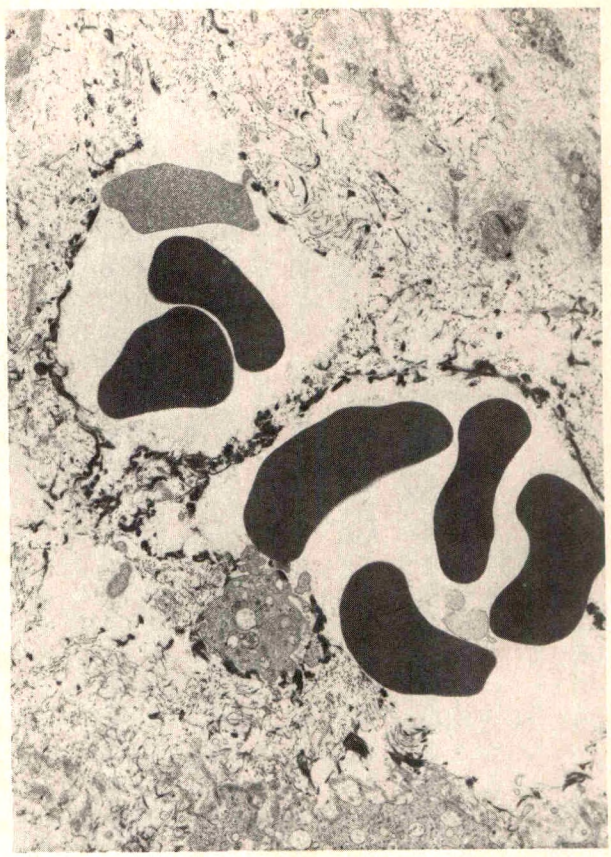


Fig. 8. Microhemorrhage into connective tissue of nonplacental site region of uterine boundary zone, preeclampsia. Note fibrin deposition onto surfaces of the dissection planes of the hemorrhage and the absence of venular media or basement membrane. (Original electron microscopic magnification ×2500.)

the added change of hypertrophy and dilation in the spiral arteries with additional changes in the deeper arterial arcades of the myometrium. Since the pathologic changes seemed to us to be more or less uniform throughout the boundary zone, the larger vessels beneath the placental site itself would appear to be less significant. Our subsequent studies of the ultrastructural details of these regions have confirmed the unitary and integral nature of the boundary zone.

Ultrastructural changes. There was no apparent correlation between the type or degree of endothelial damage and the level of maternal hypertension. Endothelial injury was pervasive in the preeclamptic women, but the same types and relative severity of specific changes were present in samples from both placental and nonplacental boundary sites. We concentrated on smaller vessels, particularly the venules, as the most likely site for injury (Figs. 1 and 2). The restricted size of electron microscopic samples signified that all kinds and degrees of change were not present in all cases. Nevertheless, a coherent spectrum of change was obvious. The endothelium was both swollen (Fig. 3) and eroded (Fig. 4). Even mild swelling was associated with enlargement of endothelial nuclei (Fig. 2); the result was apparent reduction of lumen (which was sometimes very extreme), with marked cytoplasmic swelling of endothelium and a reduced number of nuclei per cross-sectional field (Fig. 3). Luminal fibrin polymerization (Fig. 5) was observed in the case of both swollen and eroded endothelium, but was more obvious and more common with erosion (Fig. 6). In some cases erosion was incomplete with a light deposit of fibrin, but in others complete erosion was related to a heavy deposit (Fig. 6). Endothelial cytoplasmic tears short of erosion of the whole thickness were found, and as the sample became larger, at least one source for these focal tears became apparent: the endothelium occasionally showed vacuoles that enlarged into blebs bulging from the surface (Fig. 7). These blebs would then rupture, exposing the endothelial cytosol to the vascular lumen. Subendothelial edema was seen less often. Platelets

were found but not as a regular feature of these early lesions. Instead, linear or circumferential fibrin deposit seemed to be the principal means by which the earliest repair of the injury was attempted. Hemorrhages into the deeper boundary tissues, although not common, were almost always associated with fibrin deposit at that level (Fig. 8). The vacuoles have been identified as endothelial mitochondria, which initially show swelling with loss of cristae. A general expansion of cytoplasm, probably from electrolyte shifts, was a conspicuous feature. Platelet adhesion was observed, but was not a consistent feature. Altered permeability was seen occasionally with subendothelial plasma and fluid accumulation. Atherotic changes were not seen by electron microscopy; in two cases mucinous degeneration of larger vessels was seen on light microscopy.

Comment

As has been noted, prior studies have concentrated heavily on spiral arteries* in the placental bed.[1, 3, 5-7, 12, 13, 14, 17-20] Critical comparative studies have shown no effective distinction in spiral arteries in the placental bed between hypertensive and normotensive pregnancies,[18, 19] findings not explained by procedural and technical difficulties in obtaining biopsies.[14]

On a priori grounds, we considered that the effects of changes in coagulation factors seen in preeclamptic toxemia[4, 15, 16] and other aspects of the vascular lesion might be more easily identified in smaller vessels of the placental-maternal tissue boundary. The spiral arteries are subject to such profound physiologic adaptation,[3] and pathologic changes especially in late pregnancy,[13] that subtle lesions might not be easily distinguished. Moreover, recent challenges[10, 18, 19] to the putative specificity of acute atherosis and other changes in hypertensive pregnancies[7, 13] have led researchers to consider pathogenetic effects at differing zones or segments of the arterial tree.[1, 9, 12] In fact, the scope of sampling may need to be extended in three dimensions, since significant differences in abnormal uterine waveform velocity have now been described between the fundal and the low arcuate segment vascular fields.[11] Our observations extend this work with emphasis on the proximal venous side of the uterine boundary circulation. Understanding of the phenomenon of altered coagulation has also changed, with more precise and sensitive methods now available.[16] Some changes are present very early in pregnancy, such as decreased fibrinolytic activity (present by 12 weeks), and decreased fibrinolytic capacity (seen by 20 weeks).[4] Saleh et al.[16] examined clotting factors by a variety of techniques. They found levels of serum fibronectin to be increased and α_2-antiplasmin to be decreased in preeclampsia. Both levels returned toward normal post partum, with a greater relative change in patients with severe preeclampsia.[16]

It is tempting to consider that the increased fibronectin, a substance known to be produced by both liver and endothelium,[16] originates in the injured smaller vessels we have examined. Obviously, further study is necessary to establish the relationship of these observations. In particular, correlation with early changes in the coagulation mechanism must await the availability of appropriate uterine tissue from early second trimester, if not before. Earlier tissue samples might also provide information on the vascular adaptations that result, near term, in a lack of significant differences in vascular resistance and other circulatory parameters between preeclamptic pregnancy and the nonpregnant state, whereas normal pregnancy differs from both.[20] Meanwhile we are attempting to describe the mature vascular lesions in the third trimester. The existence of a circulating factor toxic to endothelium has been suggested by recent evidence.[15] It is unclear whether this is a primary agent or a factor involved in further pathogenesis promoting extension of the lesions.

The ultrastructural changes are those of endothelial injury, probably to cytoplasmic organelles and very possibly to mitochondria. Both diffuse and organelle swelling occur, and either leads to erosion of endothelium. Our observations suggest that this erosion begins with loss of the cortical cytoplasm rather than slough of the entire endothelial cell. Fibrin is deposited during the erosive process just as would occur in most forms of endothelial injury. Occasionally fluid appears to collect behind the endothelium. This can occur either by transudation or as dissection from another point along the vessel. Of interest are the recent studies of Glawanakowa and Popowa.[8] They examined peripheral blood for circulating desquamated endothelial cells and found a progressive rise during pregnancy, with a further increase in patients with edema-proteinuria-hypertension gestosis (preeclampsia). Once erosion has begun, whole-cell desquamation can proceed from that focus. Our later-stage lesions (Figs. 7 and 8) can represent either whole-cell desquamation or multistage erosion. Confirmation of the results of Glawanakowa and Popowa will contribute to a better understanding of the vascular effects of pregnancy as modified by preeclampsia. A major source for circulating endothelial cells might be the venules we have examined, since they are numerous. Our experience has shown that small vessels, particularly venules, are injured in preeclampsia irrespective of their location in the boundary zone. It remains to be determined whether comparable vascular injury occurs elsewhere in the uterus or in

*References 1, 3, 5-7, 9, 12-14, 17-19.

other body sites. Changes in endothelial organelles such as mitochondria suggest a metabolic link that merits further exploration.

We thank Deborah Hollis for preparation of tissues for electron microscopy and Frank Moretta for the electron micrographs.

REFERENCES

1. Brosens I, Dixon HG, Robertson WB. Fetal growth retardation and the arteries of the placental bed. Br J Obstet Gynecol 1977;84:656-63.
2. Brosens I, Renaer M. On the pathogenesis of placental infarcts in pre-eclampsia. J Obstet Gynaecol Br Commonw 1972;79:794-9.
3. Brosens I, Robertson WB, Dixon HG. The physiological response of the vessels of the placental bed to normal pregnancy. J Pathol Bacteriol 1967;93:569-80.
4. Condie RG, Ogston D. Sequential studies on components of the haemostatic mechanism in pregnancy with particular reference to the development of pre-eclampsia. Br J Obstet Gynaecol 1976;83:938-42.
5. De Wolfe F, De Wolf-Peeters C, Brosens I. Ultrastructure of the spiral arteries in the human placental bed at the end of normal pregnancy. AM J OBSTET GYNECOL 1973; 117:833-48.
6. De Wolf F, De Wolf-Peeters C, Brosens I, Robertson WB. The human placental bed: electron microscopic study of trophoblastic invasion of spiral arteries. AM J OBSTET GYNECOL 1980;137:58-70.
7. De Wolf F, Robertson WB, Brosens I. The ultrastructure of acute atherosis in hypertensive pregnancy. AM J OBSTET GYNECOL 1975;123:164-74.
8. Glawanakowa W, Popowa G. Untersuchungen über die desquamation des gefässendothels bei EPH-gestosen. Cor Vasa 1988;30:140-5.
9. Khong TY, De Wolf F, Robertson WB, Brosens I. Inadequate maternal vascular response to placentation in pregnancies complicated by pre-eclampsia and by small-for-gestational age infants. Br J Obstet Gynaecol 1986; 93:1049-59.
10. Khong TY, Pearce JM, Robertson WB. Acute atherosis in preeclampsia: maternal determinants and fetal outcome in the presence of the lesion. AM J OBSTET GYNECOL 1987;157:360-3.
11. Leiberman JR, Meizner I, Mazor M, Insler V. Blood supply to the uterus in preeclampsia. Eur J Obstet Gynecol Reprod Biol 1988;28:23-7.
12. Marais WD. Human decidual spiral arteries; ultrastructural of the intima in normal vessels. J Obstet Gynaecol Br Commonw 1968;75:552-67.
13. Robertson WB, Brosens I, Dixon HG. The pathological response of the vessels of the placental bed to hypertensive pregnancy. J Pathol Bacteriol 1967;93:581-91.
14. Robertson WB, Khong TY, Brosens I, De Wolf F, Sheppard BL, Bonnar J. The placental bed biopsy: review from three European centers. AM J OBSTET GYNECOL 1986; 155:401-12.
15. Rodgers GM, Taylor RN, Roberts JM. Preeclampsia is associated with a serum factor cytotoxic to human endothelial cells. AM J OBSTET GYNECOL 1988;159:908-14.
16. Saleh AA, Bottoms SF, Welch RA, Ali AM, Mariona FG, Mammen RF. Preeclampsia, delivery, and the hemostatic system. AM J OBSTET GYNECOL 1987;157:331-6.
17. Sheppard BL, Bonnar J. Scanning electron microscopy of the human placenta and decidual spiral arteries in normal pregnancy. J Obstet Gynaecol Br Commonw 1974; 81:20-9.
18. Sheppard BL, Bonnar J. The ultrastructure of the arterial supply of the human placenta in pregnancy complicated by fetal growth retardation. Br J Obstet Gynaecol 1976; 83:948-59.
19. Sheppard BL, Bonnar J. An ultrastructural study of uteroplacental spiral arteries in hypertensive and normotensive pregnancy and fetal growth retardation. Br J Obstet Gynaecol 1981;88:695-705.
20. Sohn Ch, Fendel H. Arterielle renale und uterine Durchblutung in normalen und gestotischen Schwangerschaften. Z Geburtshilfe Perinatol 1988;192:43-8.

Birth weight prediction from remote ultrasonographic examination

Joseph A. Spinnato, MD, Robert D. Allen, and Hiram W. Mendenhall, MD
Mobile, Alabama

Multiple linear regression by the least squares method was used to remodel five existing static equations to permit birth weight estimates from remote ultrasonographic parameters. By incorporating lapse time (examination-to-delivery interval), the new equations can be used to estimate birth weight 0 to 35 days after ultrasonographic examination. For each model, R^2 approached 1.0. The generated equations were tested on 167 nonmodel cases. Among the three best equations, 65% to 67% of estimates were within 100 mg/kg birth weight. The mean absolute error ranged from 86 to 91 gm/kg. With a mean lapse time of 16.8 ± 11 days, the accuracy of birth weight estimates equaled or exceeded that reported for existing static formulas. The accuracy of the estimates was maintained across the entire range of lapse times and birth weights observed. In intrapartum situations when ultrasonography is unavailable or technically difficult, birth weight can be estimated from remote ultrasound parameters. (AM J OBSTET GYNECOL 1989;161:742-7.)

Key words: Ultrasonography, estimated fetal weight

In a previous report[1] we described the development of an equation to predict birth weight from ultrasonographic examinations. By incorporating the interval from ultrasonographic examination to delivery (lapse time), the model predicted birth weight based on ultrasonographic measurements taken as early as 35 days before delivery. The rationale for the model was based on the hypothesis that ultrasonographic measurements of fetal dimensions at a point in gestation could be coupled with elapsed time to mathematically predict a birth weight based on observed growth in a population.

We extrapolated this concept to five existing formulas by including a lapse-time modifier in the structure of these existing equations. The lapse-time modifier for each equataion was determined individually from model building that analyzed the initial static estimate and the lapse time that occurred versus actual birth weight.

Material and methods

This study had two phases. In phase I, model building generated new equations based on five static equations. In Phase II, the new equations were tested on cases other than model cases.

Phase I—Model development. The records of all patients seen in the Perinatal Unit of the University of South Alabama Medical Center between October 1984 and September 1986 for obstetric ultrasonographic evaluation were reviewed. The patient population was a racially mixed (55% black), low-income clinic population. Patients were included in the study if they met the following criteria: (1) the pregnancy was a singleton gestation; (2) the biparietal diameter, occipitofrontal diameter, femur length, transverse and anteroposterior diameters of the fetal abdomen had been measured by ultrasonography; (3) data on the outcome of the delivery were available; (4) the interval (lapse time) from ultrasonographic examination to delivery was ≤35 days; and (5) birth weight was ≤5000 grams.

All ultrasonographic examinations were performed by one of three experienced ultrasonographers using a linear array real-time scanner (ADR Model 4000) with a 3.5 MHz transducer. As in our previous study,[1] standard imaging and measurement techniques were used.

Model generation and all statistical methods were performed with the Statistical Analysis System (SAS) and the IBM-4341-II mainframe computer of the University of South Alabama. Multiple linear regression using the least squares method was used to construct the models. Using the five formulas (Table I), the fetal weight estimates determined on the day of ultrasonographic examination (in logarithmic form) and the

From the Department of Obstetrics and Gynecology, University of South Alabama Medical Center.
Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.
Reprint requests: Joseph A. Spinnato, MD, Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of Louisville, Louisville, KY 40292.
6/6/14093

Table I. Static equations

Author	*Equation*
1. Hadlock et al.[5]	Log (weight) = 1.326 − 0.00326 AC × FL + 0.0107 HC + 0.0438 AC + 0.158 FL
2. Hadlock et al.[6]	Log (weight) = 1.5662 − 0.0108 HC + 0.0468 AC + 0.171 FL + 0.00034 HC^2 − 0.003685 (AC × FL)
3. Hadlock et al.[6]	Log (weight) = 1.5115 + 0.0436 AC + 0.1517 FL − 0.00321 (AC × FL) + 0.0006923 (BPD × HC)
4. Ott et al.[8]	Log (weight) = 0.04355 HC + 0.05394 AC − 0.0008582 HC × AC + 1.2594 (FL/AC) − 2.0661
5. Shepard et al.[2]	Log (weight) = −1.7492 + 0.166 (BPD) + 0.046 (AC) − 2.646 (AC × BPD)/1000

AC, Abdominal circumference; *FL*, femur length; *HC*, head circumference in centimeters; *BPD*, biparietal diameter.

lapse time observed were analyzed versus the logarithm of actual birth weight. For each equation the constructed model generated a lapse-time modifier.

After model development, the modified equations were tested with the model cases. The mean error (estimated − actual birth weight), mean absolute error (absolute value of estimated − actual birth weight), and the standardized absolute error (absolute error/kilogram birth weight) were determined for each modified equation. The equations were examined for accuracy across the range of birth weights and lapse times observed. To allow comparisons to previous reports, the observed error was converted to percentage error by the formula ([predicted weight − actual weight]/actual weight × 100) to generate a mean ± SD of percent error. The correlation coefficient (Pearson's *r*) of estimated versus actual birth weight was determined, and the percent of estimates within 50, 100, and 150 gm/kg of actual birth weight was calculated. One- and two-way analyses of variance were used to test for differences among means. Tukey's studentized range test was used to evaluate differences among group means. The significance level was set at alpha = 0.05.

Phase II—Model validation. The modified equations were validated by use of different cases from those used to develop the models. Patients seen in the Perinatal Unit for fetal ultrasonographic evaluation from October 1986 to August 1987 were reviewed for inclusion in the validation phase of the study. The inclusion criteria and ultrasonographic method were unchanged from phase I. Modified equations developed in phase I were used to generate birth weight estimates that were compared to actual birth weight. Accuracy of the estimates was determined as outlined in phase I.

Table II. Descriptive statistics of included cases for both phases of the study*

Variable	*Model development (N = 245)*	*Validation (N = 167)*
Examination gestational age (wk)	35.4 ± 3.2 (25-42)	34.7 ± 2.9 (30-42)
Birth gestational age (wk)	37.6 ± 2.9 (28-44)	37.1 ± 2.8 (30-44)
Lapse time (days)	15.7 ± 10.7 (0-35)	16.8 ± 11.0 (0-35)
Femur length (mm)	68.9 ± 6.7 (46-82)	68.0 ± 6.1 (55-82)
Abdominal circumference (mm)	302.5 ± 32.7 (188-388)	302.5 ± 35.0 (207-396)
Head circumference (mm)	314.4 ± 21.2 (243-356)	310.7 ± 19.0 (261-364)
Birth weight (gm)	2978 ± 680 (1134-4876)	3074 ± 713 1375-4870)

*Data are presented as mean ± SD, with range in parentheses.

Table III. Modified equations

1. Log [birth weight] = 1.0009 [Log initial weight] + 0.0043 (LT)
2. Log [birth weight] = 1.0009 [Log initial weight] + 0.0043 (LT)
3. Log [birth weight] = 1.0 [Log initial weight] + 0.0043 (LT)
4. Log [birth weight] = 1.001 [Log initial weight] + 0.0034 (LT)
5. Log [birth weight] = 0.9994 [Log initial weight] + 0.0047 (LT)

Birth weight in grams. *LT*, Lapse time in days.

Phase I—Model development. From 259 ultrasonographic examinations for which delivery outcome was known, 245 examinations and paired delivery outcomes fulfilled the study criteria. Table II presents the descriptive statistics of these cases.

A model to predict birth weight from remote ultrasonographic examination was generated for each of the five equations. For each model, R^2 approached 1.0. In each, the intercept did not differ significantly from zero and was deleted from the modified equation (Table III). The coefficients for each model's parameters are described in Table IV. Both lapse time and the logarithm of the initial fetal weight estimate contributed significantly to the birth weight predictions, $p \leq 0.0001$.

The modified equations were tested on the model cases. Modified equations 1, 2, and 3 performed quite similarly. Remarkably accurate birth weight estimates were noted. For each estimate, the mean error was very small and did not differ statistically from zero. The percent mean error was <1%. The mean absolute error per kilogram actual birth weight was less than 100 gm/kg. There was no significant difference in the ac-

Table IV. Description of models

Model	*LT coefficient*	*SE*	*Zero coefficient*	*SE*	R^2
1	0.00428326	0.0003	1.00086433	0.0016	0.9998
2	0.00429440	0.0003	1.00293973	0.0016	0.9998
3	0.00434518	0.0003	1.00002378	0.0016	0.9998
4	0.00344469	0.0004	1.00112376	0.0022	0.9996
5	0.00473645	0.0004	0.99972076	0.0025	0.9995

LT coefficient, Lapse-time coefficient; *SE,* standard error of the estimate; *Zero coefficient,* coefficient of the log of the original static estimate.

Table V. Validation phase—Overall performance of the new equations in descending order of accuracy*

Equation	*Error (gm) (mean ± SD)*	*% Error (mean ± SD)*	*Absolute error (gm) (mean ± SD)*	*Maximum absolute error (gm)*	*Standardized absolute error (gm/kg) birth weight*	*r†*
1	−112 ± 314	−3.6 ± 10.7	267 ± 198	1175	90.0	0.91
2	−112 ± 315	−3.6 ± 10.8	268 ± 199	1197	90.2	0.91
3	−118 ± 319	−3.8 ± 10.8	272 ± 202	1288	91.3	0.91
4	−187 ± 426	−5.4 ± 15.2	389 ± 254	1090	132.7	0.84
5	−154 ± 468	−4.6 ± 16.1	410 ± 273	1296	138.5	0.82

*Lapse time mean = 16.8 ± 11 days, range 0 to 35 days. Error estimates expressed as negative numbers indicate underestimation of actual birth weight; positive numbers indicate overestimation.

†Pearson's correlation coefficient for estimated versus actual birth weight.

Table VI. Validation phase—Accuracy of birth weight estimates within segments of lapse time

Lapse time (days)	*Equation 1*	*Equation 2*	*Equation 3*	*Equation 4*	*Equation 5*
≤7 (n = 48)					
Absolute error*	280 ± 202	280 ± 204	288 ± 206	371 ± 257	455 ± 313
Standardized absolute error†	100.0	100.0	102.3	135.7	162.0
8-14 (n = 22)					
Absolute error*	209 ± 174	213 ± 174	226 ± 169	382 ± 201	424 ± 220
Standardized absolute error†	66.3	67.3	71.6	123.0	136.9
15-21 (n = 30)					
Absolute error*	328 ± 254	329 ± 255	337 ± 268	420 ± 230	451 ± 311
Standardized absolute error†	102.7	102.9	105.3	141.5	146.0
22-28 (n = 35)					
Absolute error*	230 ± 166	230 ± 167	229 ± 170	388 ± 242	337 ± 223
Standardized absolute error†	79.7	80.0	79.3	133.5	117.7
29-35 (n = 32)					
Absolute error*	273 ± 169	273 ± 170	267 ± 171	394 ± 318	373 ± 243
Standardized absolute error†	90.8	90.8	88.6	125.5	120.3

*In grams, mean ± SD.

†Absolute error per kilogram actual birth weight in grams per kilogram.

curacy of estimates with increasing lapse time. In contrast, although the mean error for modified equations 4 and 5 did not differ statistically from zero, the percent mean error was between 1% and 2%, and the mean absolute error per kilogram actual birth weight exceeded 100 gm/kg for both. The accuracy of estimates was not stable over the range of lapse times for modified equation 4 nor over the range of birth weights for either modified equations 4 or 5.

Phase II—Model validation. One hundred sixty-seven cases fulfilled the study criteria for the validation phase. The descriptive statistics are presented in Table II.

Table V shows the overall accuracy of the modified equations. Modified equations 1, 2, and 3 again proved superior to 4 and 5. The accuracy of individual estimates, as best expressed by absolute error and standardized absolute error, was larger for the modified

Table VII. Validation phase—Accuracy of equations within birth weight segments

Weight group (gm)	*Equation 1*	*Equation 2*	*Equation 3*	*Equation 4*	*Equation 5*
1000-2000 (n = 12)					
Absolute error*	195 ± 152	193 ± 155	191 ± 149	370 ± 185	355 ± 185
Standardized absolute error†	117.3 (3.7)	116.4 (3.8)	115.8 (4.1)	228.5 (17.6)	218.8 (11.7)
2000-3000 (n = 69)					
Absolute error*	251 ± 184	252 ± 184	250 ± 184	339 ± 222	332 ± 221
Standardized absolute error†	96.9 (−4.8)	97.1 (−4.9)	96.6 (−5.2)	127.6 (−7.4)	128.6 (−5.3)
3000-3500 (n = 34)					
Absolute error*	273 ± 194	273 ± 195	280 ± 192	504 ± 246	492 ± 238
Standardized absolute error†	85.7 (−5.3)	85.8 (−5.4)	87.9 (−6.2)	158.7 (−10.6)	154.7 (−12.7)
3500-4000 (n = 38)					
Absolute error*	314 ± 238	314 ± 243	328 ± 260	435 ± 307	526 ± 335
Standardized absolute error†	83.5 (−2.8)	83.4 (−2.7)	87.3 (−2.6)	116.3 (−6.8)	140.4 (−3.2)
4000-5000 (n = 14)					
Absolute error*	272 ± 183	281 ± 174	281 ± 157	250 ± 197	326 ± 321
Standardized absolute error†	61.5 (−1.7)	63.7 (−1.4)	64.0 (−1.0)	56.8 (0.7)	74.6 (0.6)

*In grams, mean ± SD.

†Absolute error per kilogram actual birth weight in grams per kilogram. Percent mean error in parentheses.

Table VIII. Validation phase—Comparisons of new equations to their static counterpart for cases delivering within 1 week of ultrasonographic examination*

Equation		*Error (gm) (mean ± SD)*	*% Error (mean ± SD)*	*Absolute error (gm) (mean ± SD)*	*Maximum absolute error (gm)*	*Standardized absolute error (gm/kg) birth weight*
1	Original	−252 ± 316	−7.6 ± 12	333 ± 226	957	110.4
	Modified	−140 ± 318	−4.1 ± 12	280 ± 202	883	100.0
2	Original	−297 ± 318	−9.1 ± 12	362 ± 240	990	120.0
	Modified	−141 ± 319	−4.1 ± 12	280 ± 204	886	100.0
3	Original	−238 ± 324	−7.2 ± 12	331 ± 225	940	109.7
	Modified	−144 ± 326	−4.2 ± 12	288 ± 206	878	102.3
4	Original	−163 ± 446	−2.8 ± 17	382 ± 277	948	126.6
	Modified	−61 ± 450	−0.6 ± 17	371 ± 257	882	135.7
5	Original	−246 ± 530	−6.6 ± 19	470 ± 342	1338	155.8
	Modified	−163 ± 532	−3.9 ± 20	455 ± 313	1199	162.0

*n = 48, mean birth weight 3017 gm, mean lapse time 3.1 ± 1.8 days. Error estimates expressed as negative numbers indicate underestimation of actual birth weight; positive numbers indicate overestimation.

equations 4 and 5 when compared with equations 1, 2, and 3.

The ability of the modified equations to maintain accuracy over the range of lapse times observed is presented in Table VI. For each modified equation, analysis of variance for differences among lapse time group segments failed to demonstrate differences for mean error, mean absolute error, or mean absolute error per kilogram birth weight ($p > 0.05$). In other words, increasing lapse time caused no deterioration in the accuracy of estimates.

Table VII shows the data on the accuracy of estimates across the range of birth weights encountered. Equations 1, 2, and 3 were consistently accurate across the range of birth weight groups, with no significant differences noted for mean absolute error or mean absolute error per kilogram birth weight (analysis of variance $p > 0.05$). A trend toward overestimating the weight of small infants as expressed by percent mean error was seen with equation 1 ($p = 0.09$) and equation 2 ($p = 0.07$) and was significant for equation 3 ($p = 0.02$). The estimates of equations 4 and 5 were not stable across weight groups for each measure of accuracy considered ($p < 0.005$).

In Table VIII, the estimates from the original static equations are compared with the estimates from the modified equations for patients delivering within 1 week of examination. The new equations, which adjusted for a mean lapse time of 3.1 ± 1.8 days, were associated with a notable reduction in mean error, percent mean error, mean absolute error, and maximum observed error. For each equation the modification less-

Table IX. Validation phase—Percent of birth weight estimates noted within 50, 100, and 150 gm per kilogram actual birth weight (n = 167)

Equation	Estimates (%) within		
	50 gm/kg	100 gm/kg	150 gm/kg
1	32.3	64.7	83.8
2	31.1	65.9	83.8
3	29.9	65.9	82.0
4	20.4	43.1	59.9
5	19.2	39.5	61.1

ened the overall tendency to underestimate birth weight.

The relative accuracy of each modified equation is shown in Table IX as the percent of each equation's estimates that fall within ranges of absolute error per kilogram birth weight. The performance of equations 1, 2, and 3 were virtually identical to each other; 65% to 67% of the estimates fell within 100 gm/kg actual birth weight. The estimates of equations 4 and 5 were considerably less accurate.

Comment

The remodeling of existing static equations to predict birth weight from remote ultrasonographic data was successfully accomplished. The accuracy of each equation's conversion was directly related to the accuracy of its original static estimate. The relative inaccuracy of the modified equations 4 and 5 is heralded by the less accurate predictions made by the parent static formulas (Table VIII).

The accuracy of birth weight predictions of the modified equations 1, 2, and 3 is remarkable and compares favorably to other studies of static formulas. Shepard, et al.[2] reported that 54.8% and 50.7% of the estimates from equations 1 and 2, respectively, were within 100 gm/kg birth weight. Warsof et al.[3] reported four equations with percent of estimates within 100 gm/kg that ranged from 52% to 57%. Weiner et al.[4] evaluated the use of eight formulas with preterm infants; the percent of estimates within 100 gm/kg ranged from 19% to 63%. In each of these studies the estimates were made within 48 to 72 hours of delivery. In this study 65% to 67% of the estimates were within 100 mg/kg birth weight. Warsof et al.[3] reported the mean absolute error per kilogram birth weight to range from 104 to 125 gm/kg among four equations tested. The modified equations 1, 2, and 3 had mean absolute errors that ranged from 86 to 91 gm/kg. Equations 1 and 2 (from Hadlock et al.[5]) may offer advantages over our previously reported equation[1] as a result of improved accuracy across birth weight ranges.

Hadlock et al.[5,6] reported 19 static formulas in which the standard deviation of percent mean error ranged from ±7.3% to 12.9%. Ott et al.[7] observed a range from 13% to 18% for the same statistic among eight formulas. The same authors noted a range from ±8.7% to 11.1% among six other formulas.[8] In this study, the standard deviation of percent error ranged from ±10.1% to 10.8% for the three modified formulas derived from Hadlock et al. Shephard et al.[2] reported the mean absolute error to be ±343.4 and ±349 gm for their equations 1 and 2 for all estimates, and a maximum absolute error observed of 1266 and 1099 gm, respectively, for each equation. The mean absolute error for the modified formulas 1 through 3 was <280 gm, and the maximum absolute error ranged from 962 to 1288 gm (Table V).

The results of this study validate the concept of incorporating a lapse-time factor in equations to predict birth weight from remote ultrasonographic data. Weight estimates were improved even when birth occurred within 1 week of ultrasonographic examination. The accuracy of the estimates was maintained across the full range of lapse times observed. When lapse time exceeded 35 days, unacceptable deterioration of accuracy is noted (our unpublished observations). The use of these equations when lapse time exceeds 35 days is discouraged. Although the number of modified equations is limited, this concept probably could be used to remodel virtually any static formula.

These modified equations predict growth subsequent to ultrasonographic examination on the basis of the mean growth observed in our population. Although no attempt was made to exclude growth-retarded or macrosomic infants during model development, the use of the modified equations for pregnancies at risk for excess or subnormal fetal growth may result in significant overestimation or underestimation. Furthermore, these equations have been tested only as birth weight predictors, and their accuracy remote from delivery for interval weight estimations is not suggested, implied, or tested. Rates of fetal growth remote from delivery may differ from that in the 35 days before delivery.

The clinical use of the modified equations is straightforward. The weight estimate determined by the static parent formula is coupled with the lapse time that has occurred, and a birth weight estimate is determined by the modified form of that equation.

The results achieved in this study and the parameter coefficients determined may be specific to our population. Full validation of the modified equations requires testing outside our population. Although the concept of lapse-time factoring may be confirmed by others, the coefficients for the equations' parameters may differ in other populations.

We thank A. L. Bower, RN, RDMS, P. D. Driskell, RN, P. Z. Walters, RN, and Maggie Steptoe, MS, for

technical assistance, and P. C. Wagner, BSN, RNC, for help with data collection.

REFERENCES

1. Spinnato JA, Allen RD, Mendenhall HW. Birth weight prediction from remote ultrasound examination. Obstet Gynecol 1988;71:893-8.
2. Shepard MJ, Richards VA, Berkowitz RL, Warsof SL, Hobbins JC. An evaluation of two equations for predicting fetal weight by ultrasound. AM J OBSTET GYNECOL 1982;142:47-54.
3. Warsof SL, Wolf P, Couleban J, Queenan JT. Comparison of fetal weight estimation formulas with and without head measurements. Obstet Gynecol 1986;67:569-73.
4. Weiner CP, Sabbagha RE, Viasrub N, Socol ML. Ultrasound fetal weight prediction: role of HC and FL. Obstet Gynecol 1985;65:812-7.
5. Hadlock FP, Harrist RB, Sharman RS, Deter RL, Park SK. Estimation of fetal weight with the use of head, body, and femur measurements—a prospective study. AM J OBSTET GYNECOL 1987;151:333-7.
6. Hadlock FP, Harrist RB, Carpenter RJ, Deter RL, Park SK. Sonographic estimation of fetal weight. Radiology 1984; 150:535-40.
7. Ott WJ, Doyle S, Flamm S. Accurate ultrasonic estimation of fetal weight. Am J Perinatol 1985;2:178-82.
8. Ott WJ, Doyle S, Flamm S, Wittman J. Accurate ultrasonic estimation of fetal weight. Am J Perinatol 1986;3:307-10.

Dating the early pregnancy by sequential appearance of embryonic structures

Wendy B. Warren, MD, Ilan Timor-Tritsch, MD, David B. Peisner, MD, Sashi Raju, and Mortimer G. Rosen, MD

New York, New York

A total of 97 transvaginal scans were performed from 4 to 12 weeks' gestation in normal and accurately dated gestations. The sequential appearance of six structures were examined: (1) the gestational sac only was present during week 4; (2) the yolk sac appeared in week 5; (3) the fetal pole with detectable heart motion was first seen in week 6; (4) the single unpartitioned ventricle in the brain marked week 7; (5) the falx cerebri appeared during week 9; and (6) the appearance and the disappearance of the physiologic midgut herniation were seen in week 8 and week 11, respectively. Inasmuch as the time in gestation at which these structures appear characterizes the gestational age more than any measurement at this age, we propose a practical method to determine the correct gestational age in early first-trimester pregnancy. (AM J OBSTET GYNECOL 1989;161:747-53.)

Key words: Transvaginal ultrasonography, embryologic development, pregnancy, first trimester

Pregnancy dating is an important consideration in several diagnostic and therapeutic processes in perinataology. The later in pregnancy the "dating" process is performed, the larger the confidence limits become.[1] Measurement of the crown-rump length by ultrasonography is considered to be the most accurate method to establish gestational age in the first trimester. However, crown-rump length is measurable by abdominal ultrasonography only after 6½ weeks' gestation.[2-5] The accuracy of early first-trimester crown-rump length measurements by abdominal ultrasonography has been estimated to be ±5 days at best.[6] Attempts have been made to date the very early gestation by measuring the size of the gestational sac.[1, 7] However this was not widely accepted as a useful clinical tool because the confidence limits of these measurements are ±12 days and the size of the sac varies because of variable influences of the bladder and scanning plane.[1]

In earlier publications we described the ability of a 6.5 MHz transvaginal transducer probe to image embryonic and extraembryonic structures in the first trimester.[8] In early anatomic studies of human embryos, the structures of developing embryos have been used as a method to determine their postconceptional age.[9, 10] We hypothesize that the sequential appearance

From the Department of Obstetrics and Gynecology, and the Division of Ultrasound, Columbia University College of Physicians and Surgeons.
Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.
Reprint requests: Ilan E. Timor, MD, Department of Obstetrics and Gynecology, Columbia Presbyterian Medical Center, 622 W. 168th St., Room 1212, New York, NY 10032.
6/6/14092

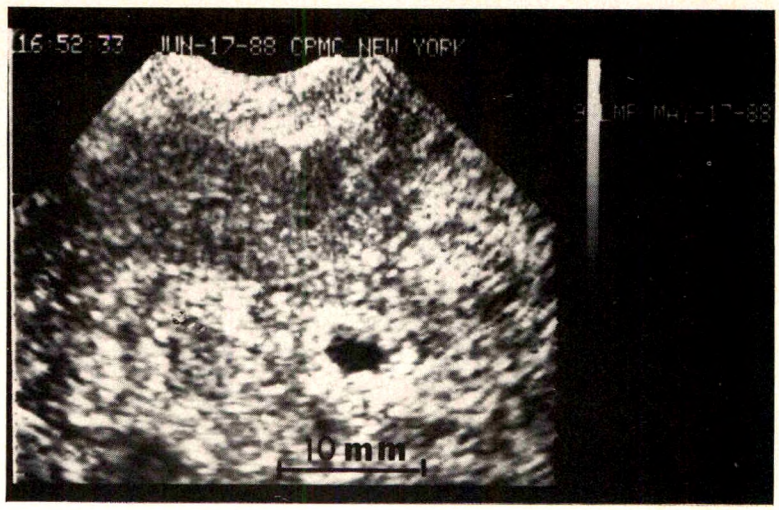


Fig. 1. Week 4. Intrauterine gestational sac of 3.5 × 2.5 mm without yolk sac.

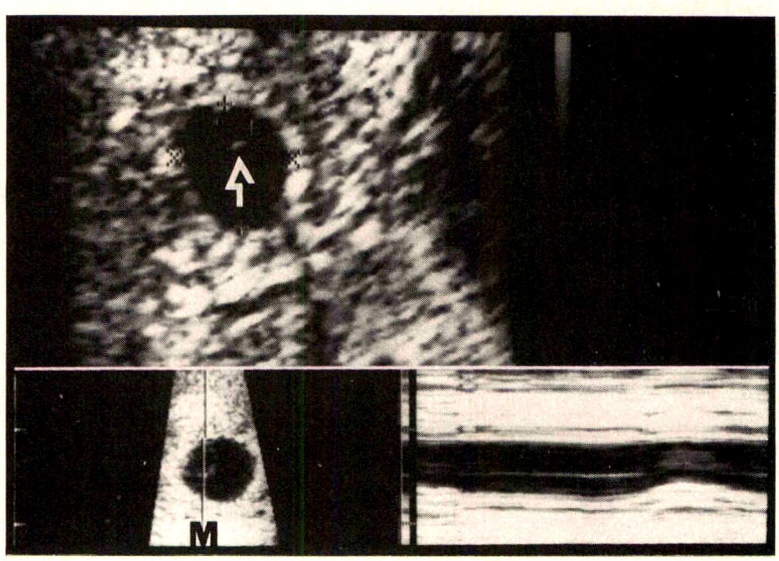


Fig. 2. Week 5. 2.2 mm Yolk sac *(open arrow)* within a 6.6 × 7.0 mm chorionic sac. Embryonic pole is not yet seen. No heart motion was detected by M-mode sweep *(M)*.

of embryonic structures by transvaginal ultrasonography can be used in the dating process of first-trimester pregnancies. The purpose of this study is to develop an ultrasonographic method of dating for practical clinic application in the first trimester.

Material and methods

We performed 104 first trimester transvaginal ultrasonography examinations. For purposes of cross-sectional analysis in this study, only one examination for each embryo-fetus was used. To ensure the enrollment of patients with most accurate dating, we included only patients who had artificial insemination, participated in our in vitro fertilization program, or had regular menstrual periods with certain dates of their last menstrual period (LMP) or conception.

Gestational ages in this study are given in weeks and days from the LMP or from the day of conception to which 14 days were added. For the purpose of analysis and potential clinical application, the studies were grouped by weeks of gestational age (e.g., 4 weeks = 4 weeks and 0 days to 4 weeks and 6 days).

The ESI-1000 scanner (Elscint, Haifa, Israel) equipped with the ED65 transvaginal probe with a 10 mm diameter crystal that has a frequency of 6.5 MHz was used for all studies. To shorten examination time,

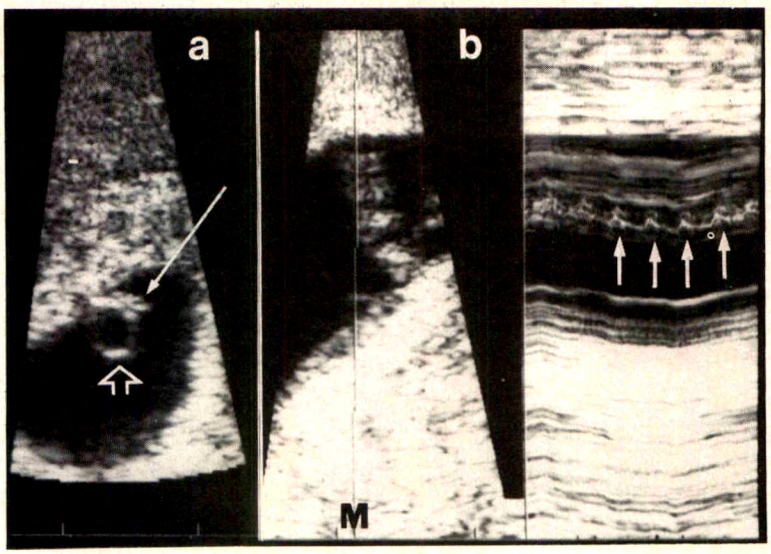


Fig. 3. Week 6. **a,** 4 mm Embryonic pole *(long arrow)* is imaged tangentially to yolk sac *(open arrow)*. **b,** M-mode cursor *(M)* clearly shows heart motion *(small arrow)*.

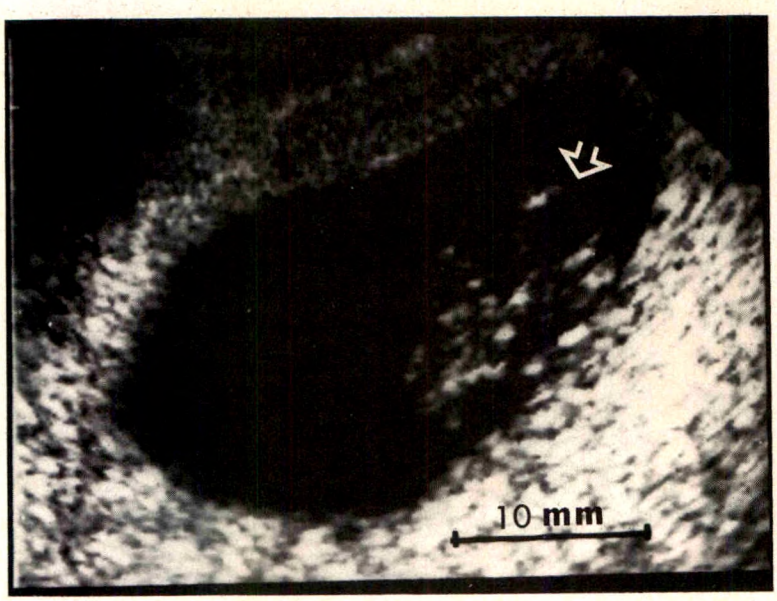


Fig. 4. Week 7. Unpartitioned ventricle in head *(open arrow)*.

video recording of the scanning session was used in gestations >9 weeks. The reason for this was that fetal movement starts to be significant at this time, interfering with a detailed, effective, and short examination.

The hard copies and the video tape recordings of each gestation were evaluated for the presence and the absence of six embryonic-anatomic structures known to occur at certain gestational ages: (1) the gestational sac (Fig. 1); (2) the yolk sac (Fig. 2); (3) fetal pole with detectable heart motion (Fig. 3); (4) the single unpartitioned ventricle in the developing brain (Fig. 4); (5) the falx that divides the ventricle (Fig. 5); and (6) the physiologic midgut herniation (Fig. 6).

Results

Table I contains the distribution of the scanned pregnancies throughout gestation.

For each gestational week the mean gestational age by crown-rump length was compared to the mean gestational age as determined clinically by date of conception. Table I shows that the clinical dating for each week >6 weeks' gestation is within 1 day of the gestational

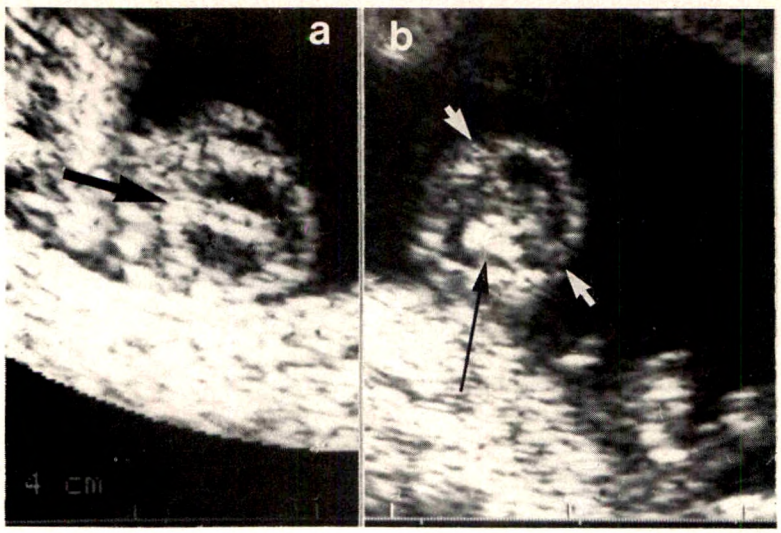


Fig. 5. Week 9. **a,** Falx *(black arrow)* divides ventricle. **b,** In addition to falx *(white arrows)*, hyperechogenic choroid plexus is visualized *(black arrow)*.

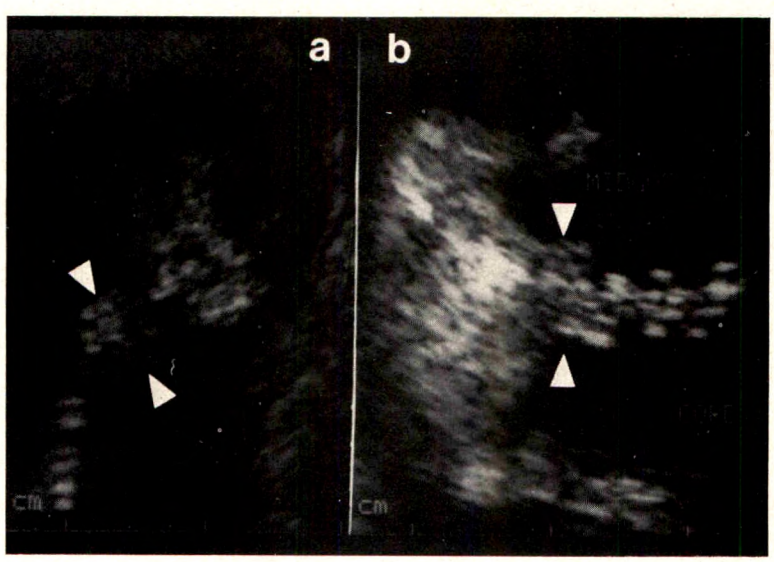


Fig. 6. Weeks 8 to 11. Physiologic midgut herniation *(between arrowheads)* is demonstrated on a horizontal **(a)** and midsagittal **(b)** section of abdominal wall at cord insertion.

age derived from the measured mean crown-rump length.[2] At 11 weeks' gestation, however, gestational age by LMP was within 2 to 3 days of the gestational age by crown-rump length.

As to the presence or the absence of embryonic and extraembryonic structures, the following observations were made.

Week 4, n = 6. The sonolucent intrauterine gestational sac without a yolk sac was seen throughout week 4. The earliest observation was in three patients at 4 weeks and 3 days. The latest this observation was made was at 5 weeks and 1 day (one patient).

Week 5, n = 11. The yolk sac is the dominant structure of week 5. It was observed as early as 5 weeks and 0 days in three patients. The latest a yolk sac was seen with no heart motion in a fetal pole was at 6 weeks and 1 day.

Week 6, n = 15. The fetal pole with fetal heart motion was first seen in week 6. In two embryos the fetal heart was seen as early as 6 weeks and 0 days.

Week 7, n = 17. The appearance of the undivided ventricle in the forebrain marks 7 weeks' gestation. Some variation was observed; in two instances the single ventricle appeared as early as 6 weeks and 6 days. In one embryo this structure with no falx was seen as late as 9 weeks and 2 days.

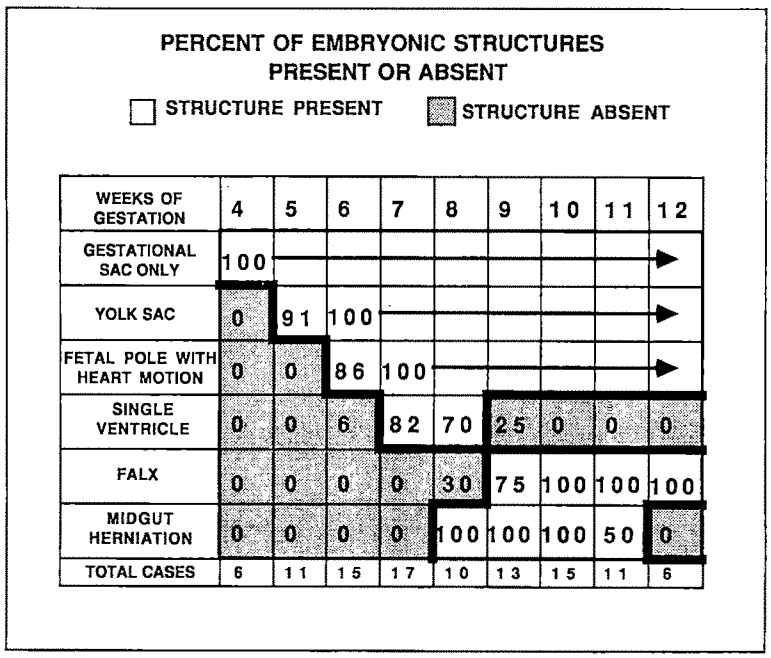


Fig. 7. Summary of detection of six embryonic structures in first trimester of pregnancy. *Solid line* differentiates weeks of gestation in which majority of embryos-fetuses displayed change in ultrasonographic appearance.

Table I. Distribution of the patient population according to weeks' gestation by clinical dating and by mean crown-rump length

Week	*No.**	*Mean gestational age by clinical dating*	*Mean crown-rump length† (mm)*	*Gestational age by mean crown-rump length† (weeks + days)*
4	6	4 + 4	—	—
5	11	5 + 2	2.0‡	—
6	15	6 + 2	6.4	6 + 3
7	17	7 + 3	11.8	7 + 4
8	10	8 + 3	17.4	8 + 3
9	13	9 + 2	26.1	9 + 3
10	15	10 + 2	34.5	10 + 2
11	11	11 + 2	48.0	11 + 5
12	6	12 + 3	53.0	12 + 3

*Total = 104.
†Crown-rump length by Robinson and Fleming.[2]
‡$n = 2$.

Week 8, n = 10. The falx appeared and divided the two lateral ventricles scanned in week 8 in three of 10 embryos. The earliest the falx was seen was at 8 weeks and 1 day. The physiologic ventral herniation of the midgut was observed in all four embryos in which the abdominal wall was imaged.

Week 9, n = 13. During week 9, 10 of 13 fetuses showed the presence of the falx partitioning the ventricle. In all 13 fetuses the physiologic midgut herniaton was present.

Week 10, n = 15. In week 10 all the fetuses showed the falx in the forebrain. The area of the cord insertion could be evaluated in 11 of 15 fetuses and the midgut herniation was seen in all 11 fetuses.

Week 11, n = 11. In week 11, the midgut herniation was present in four of eight fetuses in which the umbilical cord insertion was imaged.

Week 12, n = 6. No fetus showed a midgut herniation after 12 completed weeks' gestation.

Comment

In a study of human embryo specimens accumulated at the Carnegie Institution of Washington, Johns Hopkins University, Streeter[10] used the number of somites

present in the embryos to classify their stage of development into groups. For each group, he carefully described the development of organ systems in an attempt to determine the age. He used this structural method to determine age because, "It is in embryos of the first seven weeks that the external form and structural organization are more informative as to age than is size alone." Beyond 8 weeks from conception, according to Streeter, "the weekly increment in growth and weight is large enough to provide an adequate index of relative development."

Obstetric transvaginal ultrasonography allows the use of the high-frequency, 6.5 MHz probe with a lateral resolution of about 0.6 mm and axial resolution of 0.3 mm.[11] This type of high resolution enables the accurate measurement of extremely small structures such as 2 mm yolk sac and the early embryonic pole, and the detection of even thinner structures at fluid interfaces, such as the falx, as we have described in previous studies.[8] We used six embryonic structures detected by transvaginal ultrasonography for purposes of dating the early pregnancy with consideration to the hypothesis of Streeter that this could be a useful method to date the gestation.

After testing the accuracy of the gestational ages of our study groups by crown-rump length, we were confident to expand the evaluation to the six embryonic structures imaged throughout the group.

Despite our efforts to accurately date every pregnancy included in the study, there were several embryos that were slightly different from the overwhelming majority of the embryos of the same gestational age. The following is a list of examples:

1. In one embryo at 6 weeks and 1 day, only a yolk sac was seen. This was commonly observed during week 5.
2. In two embryos the single ventricle, usually seen during week 7, was seen at 6 weeks and 6 days.
3. In one embryo an unpartitioned ventricle with no falx was seen as late as 9 weeks and 2 days.
4. In one case the falx, which marks the week 9, was seen as early as 8 weeks and 1 day.

These slight variations seen in a small percentage of the studied embryos may be a result of unforeseen variations in the actual gestational ages despite our best effort to control this variable. Theoretically, they may also represent some minor differences in timing of the actual structural development as Streeter suspected.[10]

We therefore suggest a practical way to date the early first-trimester pregnancy, summarized in Fig. 7. For each of the six anatomic structures analyzed, we placed a dividing mark between the gestational weeks during which the anatomic structure was seen in the majority of cases and the weeks during which it was mostly absent. From these lines we have derived a steplike map for easy dating purposes.

When a gestational sac is visualized without a yolk sac, the gestation is in week 4 from the LMP. If a yolk sac is detected in the gestational sac, but no fetal heart can be discerned, the gestation is in week 5. If there is fetal heart motion, but a cerebral ventricle cannot be visualized, then the gestation is in week 6. If there is a single ventricle but no falx and no physiologic midgut herniation, then it is 7 weeks' gestation. If either a falx or the midgut herniation can be seen, it is >8 weeks' gestation. If there definitely is no midgut herniation, the normal fetus is >12 weeks' gestation (Fig. 7). The converse is also true—if a ventral hernia is imaged in a well-dated fetus >12 weeks' gestation, this should be considered abnormal or, at the least, highly suspicious.

These normative data may also be used to follow-up expected development and detect as early as possibly any deviation from the normal. In studies done in basic embryology it has been suggested that structure definition within any given embryo-fetus is at least as reliable, and probably is more reliable, than the currently employed length measurements to determine gestational age in the early pregnancy. More work is needed to prove this assumption as it applies to ultrasonographic techniques to date the early pregnancy.

In conclusion:

1. The high-frequency high-resolution vaginal ultrasound probe detects certain embryonic structures at consistent gestational ages.
2. Accurate dating of early pregnancies by this technique is feasible based on the chronologic and sequential appearance of these structures.
3. We propose a simple chart as a practical dating guide for clinical use in the first-trimester pregnancy with uncertain dates or to monitor the structural development of an embryo-fetus with known and accurate dating. A line was drawn to mark the gestational weeks during which a majority of the embryos-fetuses displayed a change in the ultrasonographic appearance. (Fig. 7)

REFERENCES

1. Sabbagha RE. Gestational age. In: Sabbagha RE, ed. Diagnostic ultrasound applied to obstetrics and gynecology. 2nd ed. Philadelphia: Lippincott, 1980.
2. Robinson HP, Fleming JEE. A critical evaluation of sonar "crown-rump length" measurements. Br J Obstet Gynaecol 1975;82:702.
3. Drumm JE, Clinch J, MacKenzie G, et al. The ultrasonic measurement of fetal crown-rump length as a method of assessing gestational age. Br J Obstet Gynaecol 1976; 83:417.
4. MacGregor SC, Tamura RK, Sabbagha RE, et al. Underestimation of gestational age by conventional crown-rump length growth curves. Obstet Gynecol (In press).
5. Green JJ, Hobbins JC. Abdominal ultrasound examina-

tion of the first trimester fetus. AM J OBSTET GYNECOL 1988;159:165-75.
6. Romero R, Jeanty P, Hobbins JC. Diagnostic ultrasound in the first trimester of pregnancy. Clin Obstet Gynecol 1984;27:286-313.
7. Hellman LM, Koboyashi M, Fillisti L, Lavenhar M. Growth and development of the human fetus prior to the twentieth week of gestation. AM J OBSTET GYNECOL 1969;103:789.
8. Timor-Tritsch IE, Farine D, Rosen MG. A close look at early embryonic development with the high-frequency transvaginal transducer. AM J OBSTET GYNECOL 1988; 159:676-81.
9. Moore KL. The developing human. Philadelphia: WB Saunders, 1988.
10. Streeter GL. Developmental horizons in the human embryos. Contrib Embryol Carnegie Inst 1942;30:211.
11. Timor-Tritsch IE, Rottem S, Thaler I. Review of transvaginal ultrasonography: a description with clinical application. Ultrasound Quarterly, 1988;6:1-34.

Macrosomia in the postdate pregnancy: Is routine ultrasonographic screening indicated?

Judith L. Chervenak, MD, Michael Y. Divon, MD, Jordan Hirsch, MD, Barbara A. Girz, DO, and Oded Langer, MD

Bronx, New York, and Newark, New Jersey

Macrosomia is a potential but often overlooked consequence of the postdate pregnancy. A total of 317 consecutive patients with well-dated pregnancies who were seen because of fetal surveillance at >41 weeks' gestation had an estimation of the fetal weight based on femur length and abdominal circumference at the initial visit. The incidence of macrosomia at 41 weeks' gestation was 25.5%. There was a higher incidence of cesarean section because of arrest and protraction disorders in the postdate pregnancies in which the infant was macrosomic (22%) versus those in which the infant was not macrosomic (10%, $p < 0.01$). In a control group of 100 consecutive women delivered between 38 and 40 weeks' gestation, the incidence of macrosomia was 4%, significantly lower than the rate in the postdate patients ($p < 0.01$). Incidence of cesarean section because of arrest and protraction disorders was significantly lower in this group (6%, $p < 0.05$). The sensitivity and specificity of an estimated fetal weight >4000 gm to predict a birth weight >4000 gm were 60.5% and 90.7%, respectively, with a positive predictive value of 70% and a negative predictive value of 87%. We conclude that routine ultrasonographic screening for macrosomia may be a valuable adjunct to current fetal surveillance protocols used in the postdate pregnancy. (AM J OBSTET GYNECOL 1989;161:753-6.)

Key words: Fetal weight, macrosomia, postdates, ultrasonography

Approximately 18.5% of all pregnancies continue beyond 41 weeks' gestation and 3.9% to 10% continue beyond 42 weeks' gestation.[1] These pregnancies have an increased risk of perinatal morbidity and mortality that can be reduced with appropriate antepartum fetal surveillance.[2]

There is no consensus with regard to the optimal form of fetal surveillance to use in the postdate pregnancy. Current protocols suggest the use of the biophysical profile, nonstress test, contraction stress test, amniotic fluid index, umbilical artery Doppler systolic/diastolic ratio, or some combination of the above.[3-5] These tests are primarily designed to detect fetal hypoxia or acidosis as a result of uteroplacental insufficiency.

Another recognized but often overlooked consequence of the prolonged pregnancy is fetal macrosomia. The postdate fetus should continue to grow, although at a reduced rate, in the absence of uteroplacental insufficiency.[6] Thus an otherwise healthy fetus may become macrosomic with continuation of the pregnancy. Macrosomia, although rarely screened for unless clinically suspected, is by itself associated with an increased risk of maternal and perinatal morbidity and mortality. The purpose of this study was to determine (1) the incidence of macrosomia in pregnancies ≥41 weeks' gestation, (2) the incidence of cesarean section because of arrest and protraction disorders in this sub-

From the Albert Einstein College of Medicine and the New Jersey Medical School.
Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.
Reprint requests: Michael Y. Divon, MD, Albert Einstein College of Medicine, Department of Obstetrics and Gynecology, Room 703, 1825 Eastchester Road, Bronx, NY 10461
6/6/14148

set of patients, and (3) the predictive value of a single ultrasonographically determined estimated fetal weight as a routine screening tool for macrosomia in the postdate pregnancy.

Material and methods

The study group consisted of all patients seen for fetal surveillance at >41 weeks' gestation from January 1987 through June 1988 at a hospital that serves a private middle-class population. Only patients with singleton uncomplicated pregnancies with intact membranes were included in the study. Gestational age was confirmed by early first examination and ultrasonogram at <20 weeks' gestation in patients with certain last menstrual periods. All patients had negative glucose screen results at 26 to 28 weeks' gestation.

After the initial visit, these patients were routinely seen twice weekly to undergo nonstress tests and ultrasonographic determination of amniotic fluid volume.

Morphometric measurements were made with the use of real-time scanning equipment (either an ATL-Ultramark IV, ATL, Bothell, Wash., or an Aloka 633, Cozometrics, Wallingford, Conn.). At that time, measurements of biparietal diameter (BPD), femur length, and abdominal circumference were made with standard techniques. Estimated fetal weights were determined with the standard equation of Hadlock, which is based on measurements of the abdominal circumference and femur length. Estimated fetal weights were not used as part of a management protocol. Actual birth weight, route of delivery, and indications for cesarean section were determined. Percentage differences between estimated and actual birth weights were determined for each patient. The sensitivity, specificity, and positive and negative predictive values of an estimated fetal weight >4000 gm to predict a birth weight >4000 gm were determined.

The postdate patients were compared with a control group of 100 consecutive patients between 38 and 40 weeks' gestation who had no antepartum complications. These patients were drawn from the same patient population. The incidence of macrosomia, which was defined as a birth weight >4000 gm, was determined for both groups. The incidence of cesarean section because of protraction and arrest disorders was determined for the study group with birth weights above and below 4000 gm, as well as for the control group.

A protracted active phase was defined as cervical dilatation of <1.2 cm/hr in nulliparous patients and <1.5 cm/hr in multiparous patients. A prolonged deceleration phase was defined as >1 hour for multiparous women and >3 hours for nulliparous women. A protracted descent was defined as <1.0 cm/hr and <2.0 cm/hr in nulliparous and multiparous patients, respectively. Arrest of dilatation was defined as no progress in dilation for >2 hours during the active phase of labor. Arrest of descent was defined as no progress in descent >1 hour for nulliparous women and >0.5 hour for multiparous women.[7]

Results

A total of 472 patients were seen for fetal surveillance of their pregnancies at >41 weeks' gestation between January 1987 and June 1988. Of these, 317 patients met criteria for the study. The mean gestational age was 42 ± 0.6 weeks' gestation and the mean birth weight was 3710 ± 452 gm. There were 236 patients (74.4%) who were delivered of infants who weighed <4000 gm. A total of 81 patients (25.5%) were delivered of infants who weighed >4000 gm; 12 of these 81 patients were delivered of infants who weighed >4500 gm and 3 were delivered of infants who weighed >5000 gm.

There were 172 patients (54.2%) who had completed 41 weeks' gestation. A total of 145 (45.7%) patients had completed 42 weeks' gestation, 19 of whom had completed 43 weeks' gestation. The mean birth weight of the infants born at 41 completed weeks' gestation was 3710 ± 452 gm and the mean birth weight of infants at 42 weeks' gestation was 3705 ± 454 gm. Of the 172 neonates (25.5%) born at 41 weeks' gestation, 44 were macrosomic; 37 of the 145 neonates (25.5%) born at >42 weeks' gestation were macrosomic. There was no perinatal mortality.

The control group had a mean gestational age of 39.8 ± 0.5 weeks and a mean birth weight of 3339 ± 360 gm. The incidence of macrosomia was 6%, significantly less than in the study population ($p < 0.05$).

Of the 317 patients in the study group, 76 were delivered by cesarean section, of which 72 were primary and 4 were repeat. The incidence of primary cesarean section was 22.7%. In those with birth weights >4000 gm, 18 of 81 (22%) had cesarean sections because of arrest or protraction disorders. In those with birth weights <4000 gm the incidence of cesarean section for the same indications was 24 of 235 (10%), which was significantly lower ($p < 0.01$). Eight patients (2.5%) had outlet forceps deliveries, of which five were performed on infants <4000 gm and three involved infants >4000 gm. This was not significantly different. There were no midforceps deliveries. The incidence of cesarean section because of arrest or protraction disorders in the control group was 4%, which again was significantly lower when compared with the incidence for the postdate group in general ($p < 0.05$).

There were 66 patients (20.8%) in whom the BPD was not obtainable because of the position of the fetal head. Abdominal circumference and femur length were obtained in all patients. There were 276 patients

(87%) who had an estimated fetal weight based on the abdominal circumference and femur length that was within 15% of the actual birth weight. Of these, 216 (68%) had estimated weights within 10% of the actual birth weight. Of the 251 patients in whom an estimation of fetal weight based on BPD and abdominal circumference could be obtained, 221 (88%) had an estimate within 15% of the actual birth weight and 176 (70%) had an estimate within 10% of the actual birth weight. Table I presents the sensitivity, specificity, and positive and negative predictive values of an estimated fetal weight >4000 gm to predict a birth weight >4000 gm. The mean interval from scan to delivery was 5.4 ± 4.4 days and the mean percentage error of an estimated fetal weight was 7.5% ± 6.4%.

Table I. Predictive value of estimated fetal weight

	Birth weight	
Estimated fetal weight	*>4000 gm*	*<4000 gm*
>4000 gm	49	22
<4000 gm	32	214

Sensitivity = 61%; specificity = 91%; positive predictive value = 70%; negative predictive value = 87%.

Comment

Results of this study indicate the incidence of macrosomia at ≥41 weeks' gestation is considerable. The incidence of birth weights >4000 gm was 25.5% (44:172) at 41 weeks' gestation and 25.5% (37:145) at 42 weeks' gestation. This was significantly increased when compared with the 6% of macrosomic infants in the control group of patients delivered between 38 and 40 weeks' gestation ($p < 0.05$). Similar results were reported by Arias[8] who also found a 23.8% incidence of macrosomia in fetuses of >41 weeks' gestation. In a review of 9394 pregnancies, Boyd et al.[9] found a 16% incidence of macrosomic infants at 41 weeks' gestation and a 21% incidence at 42 weeks' gestation, both of which were significantly increased when compared with the 8.2% incidence he reported at <41 weeks' gestation. This study concluded that infants delivered at 42 weeks' gestation were almost three times as likely to weigh >4000 gm than those delivered at <41 weeks' gestation.

This relatively high incidence of macrosomia is important to recognize because of the complications associated with this condition. These include increased maternal and fetal trauma, shoulder dystocia with resulting Erb's palsy, perinatal asphyxia, meconium aspiration, increased rate of labor disorders, and postpartum hemorrhage.[10-13] We chose to evaluate the incidence of cesarean section in response to arrest and protraction disorders because of its relatively common occurrence and because such disorders are defined by standard and generally accepted criteria.[7] In our study the incidence of cesarean section because of arrest and protraction disorders in the postdate pregnancies with macrosomic infants was 22.2% and was significantly greater than the 10.1% observed for postdate pregnancies with nonmacrosomic infants ($p < 0.01$). Others[9, 13] have also demonstrated that there is an increased incidence of cesarean section because of arrest and protraction disorders in this population. Parks and Ziel[13] have advocated primary cesarean section for fetuses with an estimated fetal weight >5000 gm to avoid the potential complications of vaginal delivery of an infant believed to be ≥4500 gm.

Present protocols with regard to the conservative management of the postdate pregnancy have emphasized the detection of fetal asphyxia but have neglected to account for increasing fetal size. The rationale that is often given for prolongation beyond term of a pregnancy with good dates is the avoidance of cesarean section thought to result from induction of labor with an unfavorable cervix. It may not be appropriate, however, to screen a postdate pregnancy solely for asphyxia while allowing the fetus to continue to grow, thereby also increasing the chances for cesarean section on the basis of fetal size. Clinical suspicion based on a symphyseal-fundal height >40 cm or maternal risk factors such as maternal age, parity, prepregnant obesity, maternal diabetes, or previous birth of a large infant has been shown to be inadequate, leaving >50% of macrosomic infants undetected.[9] Whereas routine ultrasonographic screening is advocated in the diabetic pregnancy in which the incidence of macrosomatia ranges from 10% to 28%,[14, 15] it is rarely, if at all, used in the postdate pregnancy where the absolute incidence of large infants may be higher.

The third purpose of this study was to evaluate the accuracy of an ultrasonographically determined estimated fetal weight obtained at ≥41 weeks' gestation. In our study, 87% of the estimated fetal weights were within 15% of the actual birth weights and 68% were within 10% of the actual weights. The sensitivity and specificity of an estimated fetal weight >4000 gm to predict an actual birth weight >4000 gm were 61% and 91%, respectively. The positive predictive value of 70% indicates that the ultrasonographic diagnosis of macrosomia was associated with actual macrosomia in 7 of every 10 cases. The negative predictive value of 87% indicates that when ultrasonography excluded macrosomia, it was present in only 1.3 of 10 cases. These findings occurred despite the fact that the estimates were determined by multiple operators. We chose an estimation of fetal weight based on a standard equation determined from measurements of the abdominal cir-

cumference and femur length because these measurements could be easily obtained in all patients, whereas a BPD could not be adequately measured in 66 (20.8%) of the 317 patients. Various methods for the ultrasonographic detection of the macrosomic infant have been suggested. These have included evaluation of estimates of fetal weight as determined by the equation of Shephard based on measurements of the BPD and abdominal circumference, and that of Hadlock based on measurements of the abdominal circumference and femur length. Others have used various indices such as the BPD or abdominal circumference alone, or the head circumference or femur length/abdominal circumference ratios.[16, 17] The latter two are based on the premise that the abdominal circumference should be relatively increased in the macrosomic infant. The majority of these studies have focused on the infant of the diabetic mother or on the large-for-gestational-age infant, delivered at a wide variety of gestational ages. It has been suggested that the body proportions of the postdate infant may be different from that of the diabetic infant and thus it is uncertain whether the above studies can be applied to the postdate population.[16]

A study by Guzick et al.[18] that evaluated the use of standard equations to estimate fetal weight in the postdate population concluded that the predictive value of these equations was not related to gestational age. He did not, however, determine the ability to predict macrosomia. We found the sensitivity and specificity of our ultrasonographic prediction of macrosomia in the postdate population to be similar to those reported by other studies of infants at <40 weeks' gestation or in diabetic pregnancies.[17, 19] The fact that they did not vary greatly from other studies may be a reflection of the error of the technique.

The addition of the determination of an estimated fetal weight at the first visit at ≥41 weeks' gestation can be easily accomplished and may be a valuable adjunct in the management of these pregnancies. We plan further studies that will incorporate this determination into a prospective management scheme for this population. This is needed to determine whether induction of labor would, in fact, result in a lower incidence of cesarean deliveries or birth trauma when compared with expectant management.

In conclusion, this study has demonstrated that the incidence of macrosomia in postdate pregnancies is considerable, that this condition is associated with an increased rate of cesarean section because of arrest and protraction disorders, and that real-time ultrasonography can be used with reasonable accuracy to predict this condition in the postdate pregnancy.

REFERENCES

1. Sachs BP, Friedman EA. Results of an epidemiologic study of post-date pregnancy. J Rep Med 1986;31:162-6.
2. Rutherford SE, Phelan JP, Smith CV, Jacobs N. The four quadrant assessment of amniotic fluid volume: an adjunct to antepartum fetal heart rate testing. Obstet Gynecol 1987;70:353-6.
3. Yeh S, Read J. Management of post-term pregnancy in a large obstetric population. Obstet Gynecol 1982;60: 282-7.
4. Johnson JM, Harman CR, Lange IR, et al. Biophysical profile scoring in the management of the postterm pregnancy: an analysis of 307 patients. AM J OBSTET GYNECOL 1986;154:269-73.
5. Granados JL. Survey of the management of postterm pregnancy. Obstet Gynecol 1984;63:651-3.
6. Bromwich P. Big babies. Br Med J 1986;293:1387.
7. Pritchard JA, MacDonald PC, Gant NF. Williams Obstetrics, 17th ed. Norwalk, Connecticut: Appleton-Century-Crofts 1985:643.
8. Arias F. Predictability of complications associated with prolongation of pregnancy. Obstet Gynecol 1987;70: 101-6.
9. Boyd ME, Usher RH, McLean FH. Fetal macrosomia: prediction, risks and proposed management. Obstet Gynecol 1983;61:715-21.
10. Modanlou HC, Dorchester WL, Thorosian A, Freeman RK. Macrosomia. Maternal fetal and neonatal implications. Obstet Gynecol 1980;55:420-4.
11. Stevenson DK, Hopper AO, Cohen RS, et al. Macrosomia: causes and consequences. J Peds 1982;100:515-9.
12. Spellacy WN, Miller MS, Winegan A. Macrosomia—maternal characteristics and infant complications. Obstet Gynecol 1985;66:158-61.
13. Parks DG, Ziel HK. Macrosomia. A proposed indication for cesarean section. Obstet Gynecol 1978;52:407-9.
14. Coustan DR, Imarah J. Prophylactic insulin treatment of gestational diabetes reduces the incidence of macrosomia, operative delivery and birth trauma. AM J OBSTET GYNECOL 1984;150:836-42.
15. Bochner CJ, Medearis AL, Williams J, et al. Early third trimester ultrasound screening in gestational diabetes to determine the risk of macrosomia and labor dystocia at term. AM J OBSTET GYNECOL 1987;157:703-8.
16. Hadlock FP, Harrist RB, Fearneyhough TC, et al. Use of femur length/abdominal circumference ratio in detecting the macrosomic fetus. Radiology 1985;154:503-5.
17. Mintz MG, Landon MB. Sonographic diagnosis of fetal growth disorders. Clin Obstet Gynecol 1988;31:44-52.
18. Guzick DS, Lowe TW, Santos-Ramos R. Ultrasound prediction of fetal weight in prolonged pregnancy. AM J OBSTET GYNECOL 1985;151:783-6.
19. Deter RL, Hadlock F. Use of ultrasound in the detection of macrosomia: a review. J Clin Ultrasound 1985;13:519-24.

Discordant twins—A prospective study of the diagnostic value of real-time ultrasonography combined with umbilical artery velocimetry

Michael Y. Divon, MD, Barbara A. Girz, DO, Avi Sklar, MD, Denise A. Guidetti, MD, and Oded Langer, MD

Bronx, New York

This study was undertaken to evaluate the role of Doppler velocimetry combined with intertwin differences in ultrasonographically derived estimated fetal weight, biparietal diameter, abdominal circumference, and femur length as a comprehensive test for the prediction of discordancy. The following cutoff values were used to indicate abnormal test results: Δ biparietal diameter > 6 mm, Δ abdominal circumference > 20 mm, Δ femur length > 5 mm, Δ estimated fetal weight > 15%, and Δ systolic/diastolic ratio > 15%. Discordancy was identified when the birth weight difference exceeded 15%. The study population consisted of 58 consecutively evaluated third-trimester twin gestations. Eighteen sets of twins were discordant. None of these tests was uniformally successful in identifying twin discordancy; in three instances, all test results were normal. The diagnostic accuracy provided by ultrasonography was not significantly different from that provided by Doppler velocimetry. Overall the best predictor appeared to be the presence of either Δ systolic/diastolic ratio > 15% or Δ estimated fetal weights > 15%, which correctly identified 14 of the 18 discordant twins and misclassified only five of the 40 concordant pairs. This combination also had the highest positive and negative predictive values (73% and 90%, respectively). (Am J Obstet Gynecol 1989;161:757-60.)

Key words: Twin gestation, discordancy, ultrasonography, Doppler

Discordant growth in twin gestation can result from placental crowding, twin-to-twin transfusion, a poor placental implantation site, or chromosomal anomalies.[1] This high-risk condition is associated with a significant increase in perinatal morbidity and mortality.[1,2] Use of discordancy as an outcome variable has its pitfalls (i.e., discordant twins who are both normal or a growth-retarded pair, which may not be discordant).

The clinical diagnosis of discordant growth in twin gestation is almost impossible.[1] With the use of real-time ultrasonography, various indices have been suggested for identification of discordant growth. These include intrapair differences in biparietal diameters, abdominal circumferences, and femur lengths.[3-6] Since the definition of growth discordancy is based on birth weight differences, ultrasonographic estimates of fetal weight have also been useful in making this diagnosis.

Recent studies have suggested that twin discordancy is associated with unequal fetoplacental blood flows. Thus umbilical artery flow velocimetry has been advocated for prediction of this condition.[7,8] These studies, however, did not compare the diagnostic value of umbilical artery flow velocimetry in relation to other established ultrasonographic criteria for diagnosis of discordant growth in twin gestation.

The purpose of this study was to examine the diagnostic value of various ultrasonographic indices combined with Doppler assessment of umbilical artery velocity waveforms as a comprehensive test for detection of twin discordancy.

Material and methods

The study population consisted of patients evaluated at the Maternal-Fetal Assessment Center at the Albert Einstein College of Medicine. The following were criteria for inclusion in the study: (1) third-trimester twin gestation with both fetuses alive at the time of the examination, (2) well-documented dates (by reliable menstrual history in agreement with an ultrasonographic examination before 22 weeks' gestation), (3) intact membranes and mother not in labor, (4) delivery occurred within 2 weeks of ultrasonographic-Doppler evaluation. Morphometric measurements were made with real-time scanning (either ATL-Ultramark IV or an Aloka 633) equipment, and included intrapair dif-

From the Department of Obstetrics and Gynecology, The Albert Einstein College of Medicine of Yeshiva University.

Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.

Reprint requests: Michael Y. Divon, MD, Albert Einstein College of Medicine, Department of Obstetrics and Gynecology, Room 703, 1825 Eastchester Rd., Bronx, NY 10461.

6/6/13672

Table I. Coefficients of correlation between percent difference in birth weight and percent difference in ultrasonographic-Doppler indices

	R	*p*
% ΔBW vs. % ΔBPD	0.33	NS
% ΔBW vs. % ΔAC	0.49	NS
% ΔBW vs. % ΔFL	0.55	NS
% ΔBW vs. % ΔS/D	0.57	NS
% ΔBW vs. % ΔEFW	0.70	<0.05

ferences in (1) biparietal diameters—ΔBPD, (2) abdominal circumferences—ΔAC, (3) femur length—ΔFL. These measurements were performed by means of standard techniques and also allowed the calculation of the estimated fetal weights (EFW) (based on AC-FL[9]) and the intrapair difference in EFWs (ΔEFW).

Umbilical artery velocity waveforms were analyzed with a continuous-wave Doppler device (Fetal-Flo, Medasonics, Inc., Mountain View, Calif.). The locations of the umbilical cords were determined by real-time ultrasonography, and the Doppler transducer was then aimed in the direction of each umbilical cord. Thus umbilical cords were identified by their location and by the differing fetal heart rates.[10] We accepted recordings for analysis only after obtaining a steady state of at least five consecutive pulsatile arterial waveforms simultaneously displaying the nonpulsatile signal generated by umbilical venous flow (flowing in the opposite direction). No waveforms were obtained during periods of fetal breathing movements, body movements, or cardiac arrhythmias. The umbilical artery flow velocity waveform was characterized by the ratio of peak systolic/end-diastolic frequencies (S/D ratio). Three separately obtained S/D ratios were derived per fetus, and the results were averaged. The intrapair S/D ratio difference was expressed as a percentage of the larger twin's S/D ratio (ΔS/D).

The following cutoff levels were used prospectively to indicate abnormal test results: (1) ΔBPD > 6 mm, (2) ΔAC > 20 mm, (3) ΔFL > 5 mm, (4) ΔS/D > 15%, (5) ΔEFW > 15%.[4, 5, 11] Bed rest at home was recommended when ΔEFW > 15%.

The intrapair birth weight (BW) difference was expressed as a percentage of the larger twin's birth weight (ΔBW). Discordancy was defined as ΔBW > 15%.[1, 12] The coefficients of correlation (R) between %ΔBW and the percent difference in each of the ultrasonographic-Doppler variables were calculated. To evalute the usefulness of the various indices for predicting twin discordancy, we calculated the specificity, sensitivity, and positive and negative predictive values for single and combined test results.

Results

Fifty-eight sets of twins fulfilled the study criteria and formed the population for this study. Of the 58 women, 40 (69%) were delivered of concordant twins and 18 (31%) were delivered of discordant twins. There was a statistically significant difference in the mean birth weight between the concordant twins (2627 ± 526 gm; range, 1680 to 3700) and the discordant twins (2192 ± 315 gm; range, 1170 to 3050; $p < 0.01$). The mean gestational age at delivery was 37.4 ± 1.2 weeks (range, 30 to 42); there was no difference between the concordant and the discordant pairs.

Table I presents the coefficients of correlation between the percent difference in birth weight and the percent difference in each of the ultrasonographic-Doppler indices used in this study. Only the %ΔEFW was significantly correlated with the %ΔBW ($R = 0.7$, $p < 0.05$).

Table II presents the sensitivity, specificity, and positive and negative predictive values of single and combined test results. None of these five tests was uniformally successful in identification of growth discordancy in twins. Overall, the best predictor appeared to be the presence of either ΔEFW > 15% or ΔS/D > 15%, which correctly identified 14 of the 18 growth-discordant twins (sensitivity of 78% and specificity 87%). This combination of tests also had the highest positive and negative predictive values (73% and 90%, respectively). In three instances with mild birth weight discordancy, results of all tests had been normal (Table III). The diagnostic accuracy provided by Doppler velocimetry was not significantly different from that provided by real-time ultrasonography.

Comment

This study assessed the diagnostic value of real-time ultrasonography combined with umbilical artery velocimetry as a comprehensive test for prediction of twin discordancy. The results indicate that none of the tests evaluated in this study was consistently successful in identifying growth discordancy. Overall, the best predictor seemed to be the presence of either ΔEFW > 15% or ΔS/D > 15%. This combination correctly identified 14 of the 18 discordant sets of twins.

The most common definitions of growth discordancy are based on intertwin birth weight difference expressed as a percentage of the larger twin's weight.[1] A wide range of cutoff values for %ΔBW has been discussed in the literature. These values range from ΔBW > 15% to ΔBW > 40%.[1, 13] Although most clinical problems occur when the ΔBW exceeds 25%, the lower end of this spectrum was chosen for this study because we wanted to screen and include all possible pairs of discordant growth despite the obvious possibility of an increased incidence of false-positive test results. The incidence of growth discordancy in our study population was 31%. Other studies have reported a somewhat lower incidence of 19% to 23% with the same definition for discordancy.[1, 13] This difference may be explained

Table II. Sensitivity, specificity, and positive and negative predictive values for single and combinations of test results

	Sensitivity (%)	*Specificity (%)*	*Positive predictive value (%)*	*Negative predictive value (%)*
ΔS/D > 15%	66	64	55	75
ΔEFW > 15%	47	81	56	74
ΔBPD > 6 mm	30	88	50	75
ΔAC > 20 mm	53	77	53	77
ΔFL > 5 mm	28	92	63	72
Either ΔS/D > 15% or ΔEFW > 15%	78	87	73	90

Table III. Ultrasonographic and Doppler characteristics of three patients with discordant twins and normal testing

Case No.	*ΔBW (%)*	*ΔS/D (%)*	*ΔEFW (%)*	*ΔBPD (%)*	*ΔAC (%)*	*ΔFL (%)*
1	17	13	10	3	9	4
2	21	5	12	7	13	7
3	22	11	14	10	12	8

by differences in sample size and by the fact that our study population was comprised of high-risk referrals.

Real-time ultrasonography is essential for the diagnosis of discordancy in twins. Initially, discordancy was diagnosed by evaluation of the BPD. Crane et al.[3] compared the growth of the BPD between concordant twins and appropriate-for-gestational-age singleton gestations and detected no significant differences. They also showed that the mean difference in BPD among concordant twins was 1.8 mm (range, 0 to 5 mm). However, the mean difference in BPD among discordant twins was 12.3 mm (range, 5 to 31 mm). A study by Leveno et al.[11] suggested that a cutoff value of ΔBPD ≥ 6 mm is associated with discordancy and poor fetal outcome. In our study, the sensitivity and specificity of ΔBPD > 6 mm for predicting discordancy were 30% and 88%, respectively. These results imply that most discordant twins do not demonstrate significant intrapair differences in BPD and are in agreement with other studies that reported disappointing results with this variable.[2, 3, 5]

Grumbach et al.[14] evaluated the abdominal circumference in twin and singleton gestations. Their results indicated a significant decrease in the growth rate of the abdominal circumference in twins starting at approximately 32 weeks of gestation. Large intertwin differences in abdominal circumferences were reported to be associated with neonatal birth weight discordancy by Barnea et al.[4] In the present study the positive and negative predictive values for ΔAC > 20 mm were 53% and 77%, respectively. These results indicate that a substantial difference between the abdominal circumferences will correctly predict approximately one half of the discordant pairs. Several investigators evaluated this index and reported its sensitivity to range from 66% to 80% and its specificity to range from 74% to 85%.[13] The slightly lower sensitivity obtained in our study may be related to the prospective nature of the study and to the fact that we used measurements obtained by multiple perinatologists.

During the third trimester, growth of the fetal femur in twins is not significantly different from that in singleton gestations.[14] Storlazzi et al.[5] evaluated several ultrasonographic indices as predictors of discordant fetal growth and showed that ΔFL ≥ 5 mm had a positive predictive value of 75%. A slightly lower positive predictive value of 63% was calculated in our patient population.

The neonatal diagnosis of discordant growth in twin gestation is based on birth weight criteria. Therefore it is reasonable to evaluate the value of intrapair differences in EFW for making this diagnosis. In our study, %ΔEFW was the only ultrasonographic-Doppler variable that had a statistically significant correlation with %ΔBW ($R = 0.7, p < 0.05$). The sensitivity of 47% and specificity of 77% for ΔEFW > 15% are not markedly different from those obtained for ΔAC > 20 mm. This is not surprising since the various formulas available for calculating EFW emphasize the abdominal circumference much more than any other variable. As the ΔEFW increases, the likelihood of one fetus being small for gestational age is also increased. This fact was noted by O'Brien et al.,[12] who reported that ΔEFW ≥ 30% is associated with a small-for-gestational-age newborn in 66% of cases.

Abnormal umbilical artery velocity waveforms have been detected in approximately 50% of growth-retarded singleton pregnancies.[15] Such waveforms probably reflect increased placental impedance to blood flow and are consistent with histologic evidence of a

placental vascular lesion.[16] Several investigators have reported the use of umbilical artery velocimetry for surveillance of twin pregnancies.[7, 8, 10, 17, 18] Farmakides et al.[7] used an absolute cutoff value of $\Delta S/D > 0.4$ and found this test useful for identification of discordant growth. Their sensitivity and specificity values were the same as those detected by us with a relative cutoff level of $\Delta S/D > 15\%$ (i.e., 73% and 82%, respectively). However, no comparison was made between umbilical artery velocimetry and real-time ultrasonography. Saldana et al.,[10] who compared $\Delta S/D$ to ultrasonographically derived ΔEFW, concluded that Doppler velocimetry was a better predictor of discordancy. The difference between their results and ours could partially be a result of different definitions of discordancy. Whereas Saldana et al. used an absolute value of $\Delta BW > 350$ gm, we chose a relative birth weight difference of 15%. Nimrod et al.[17] evaluated a combination of ΔAC, ΔBPD, and $\Delta S/D$ in either the fetal aorta or the umbilical artery and found that 9 of their 11 discordant pairs had at least two abnormal variables. However, these combinations were associated with a high false-positive rate. Although discordant S/D ratios may reflect unequal fetoplacental circulations, the neonatal definition of discordancy is not related to umbilical arterial flow. A significant birth weight discordancy was identified in one of our patients who demonstrated concordant S/D ratios associated with twin-to-twin transfusion.

In conclusion, the results of our study indicate that Doppler velocimetry complements real-time ultrasonography for the diagnosis of discordant fetal growth in twin pregnancies. The presence of either $\Delta S/D > 15\%$ or $\Delta EFW > 15\%$ correctly identified 14 of the 18 discordant twins and misclassified only 5 of the 40 concordant pairs. Ideally, an accurate ultrasonographic estimate of fetal weights should suffice for making this diagnosis. However, ultrasonographic estimates of fetal weight have inherent inaccuracies[19] that are amplified in this situation by head compression, unfavorable fetal lie, and the fact that an overlying fetus may cover parts of the other fetus. Abnormal umbilical artery velocity waveforms are not as directly related to the definition of discordancy as is an increased ΔEFW, but they do reflect unequal fetal-placental circulations, which is probably a major contributor to growth discordancy in twin pregnancies.

REFERENCES

1. Blickstein I, Lancet M. The growth discordant twin. Obstet Gynecol Surv 1988;43:509.
2. Erkkola R, Ala-Melleo S, Piiroinen O, et al. Growth discordancy in twin pregnancies: a risk not detected by measurements of biparietal diameter. Obstet Gynecol 1985; 66:203.
3. Crane JP, Tomich PG, Kopta M. Ultrasonic growth patterns in normal and discordant twins. Obstet Gynecol 1980;55:678.
4. Barnea ER, Romero R, Scott D, et al. The value of biparietal diameter and abdominal perimeter in the diagnosis of growth retardation in twin gestation. Am J Perinatol 1985;2:221.
5. Storlazzi E, Vintzileos AM, Campbell WA, et al. Ultrasonic diagnosis of discordant fetal growth in twin gestations. Obstet Gynecol 1987;69:363.
6. Secher NJ, Kaern J, Hansen PK. Intrauterine growth in twin pregnancies: prediction of fetal growth retardation. Obstet Gynecol 1985;66:63.
7. Farmakides G, Schulman H, Saldana LR, et al. Surveillance of twin pregnancy with umbilical arterial velocimetry. AM J OBSTET GYNECOL 1985;153:789.
8. Giles WB, Trudinger BJ, Cook CM. Fetal umbilical artery flow velocity-time waveforms in twin pregnancy. Br J Obstet Gynaecol 1985;92:490.
9. Russell DL, Harrist RB, Birnholz JC, Hadlock FP, eds. Quantitative obstetrical sonography. New York: Wiley, 1985.
10. Saldana RL, Eads MC, Schaefer TR. Umbilical blood waveforms in fetal surveillance of twins. AM J OBSTET GYNECOL 1987;157:718.
11. Leveno KJ, Santos-Ramos R, Duenhoelter JH, et al. Sonar cephalometry in twin pregnancy: discordancy of the biparietal diameter after 28 weeks' gestation. AM J OBSTET GYNECOL 1980;138:615.
12. O'Brien WF, Knuppel RA, Scerbo JC, et al. Birth weight in twins: an analysis of discordancy and growth retardation. Obstet Gynecol 1986;67:483.
13. Vintzileos AM, Rodis JF. Growth discordancy in twins. In: Divon MY, ed. Abnormal fetal growth: IUGR and macrosomia. New York, New York: Elsevier (In press).
14. Grumbach K, Coleman BG, Arger PH, et al. Twin and singleton growth patterns compared using ultrasound. Radiology 1986;158:237.
15. Divon MY, Guidetti DA, Braverman JJ, et al. Intrauterine growth retardation - A prospective study of the diagnostic value of real-time sonography combined with umbilical artery flow velocimetry. Obstet Gynecol 1988;72:611.
16. Giles WB, Trudinger BJ, Baird PJ. Fetal umbilical artery flow velocity waveforms and placental resistance: pathological correlations. Br J Obstet Gynaecol 1985;92:31.
17. Nimrod C, Davies D, Harder J, et al. Doppler ultrasound prediction of fetal outcome in twin pregnancies. AM J OBSTET GYNECOL 1987;156:402.
18. Giles WB, Trudinger BJ, Cook CM, Connelly A. Umbilical artery flow velocity waveforms and twin pregnancy outcome. Obstet Gynecol 1988;72:894.
19. Benanceraf BR, Gelman R, Frigoletto FD. Sonographically estimated fetal weights: accuracy and limitations. AM J OBSTET GYNECOL 1988;159:1118.

Doppler umbilical artery waveform indices—Normal values from fourteen to forty-two weeks

Susan K. Hendricks, MD, Tanya K. Sorensen, MD, Keith Y. Wang, MD, PhD, Jeanette M. Bushnell, MSN, Elaine M. Seguin, RNC, and Rosalee W. Zingheim, BSN
Seattle, Washington

Normal values for Doppler waveform indices of the umbilical artery have been reported for gestational ages of 20 to 40 weeks in small numbers of normal patients. We evaluated 590 patient studies performed at 2-week intervals from 14 to 42 weeks' gestation on patients without medical or pregnancy complications. Readings were obtained during fetal quiet times (no fetal breathing or movements). Values for A (systolic) and B (diastolic) pressures were plotted as Pourcelot (A − B/A) and A/B ratios. Mean, SD, and 95% confidence limits were derived, and the skewness, kurtosis, and regression correlations were calculated. No diastolic flow was found in any pregnancy >15 weeks' gestation (n = 25) or in 50% of the gestations between 15 to 17 weeks (n = 25). When diastolic pressure equals zero, the Pourcelot ratio value equals one and the A/B ratio approaches infinity and loses meaning. Recent work by Thompson et al. suggests that the Pourcelot ratio fits a normal distribution from 20 to 40 weeks' gestation and that the A/B ratio (which does not fit a normal curve) may be transformed to a normal distribution by conversion of the A/B ratio to 1/1 − Pourcelot ratio. Our data supports the normality of both indices from 18 to 42 weeks' gestation, but these assumptions are not applicable as the Pourcelot ratio approaches one or as the A/B ratio approaches infinity. Knowledge of normal umbilical flow ratios at gestational ages from 18 weeks may allow early detection and directed management of high-risk pregnancies. (Am J Obstet Gynecol 1989;161:761-5.)

Key words: Doppler velocimetry, normality, Pourcelot ratio, A/B ratio

The utility of fetal velocimetry in antepartum assessment continues to be controversial. Although a plethora of research has attempted to support and clarify the use of Doppler ultrasonography for high-risk assessment, the majority of clinical practitioners remain uncertain about the position of Doppler ultrasonography in the schemata of antenatal testing. The classic methods of assessment (nonstress tests, contraction stress tests, and biophysical profile tests) are similarly inconsistent at gestational ages <28 weeks. The physiologic variability of system development in the fetus is responsible for the lack of reliability and predictability in the second trimester. The genesis and development of uterine and umbilical blood flow is phylogenically earlier than that of respiratory rhythmicity and fetal heart rate variability, and thus provokes the hypotheses that Doppler ultrasonography might be an appropriate modality for the selection and management of high-risk pregnancies at younger gestational ages than current techniques allow. To date, normal curve development has been limited by the small numbers of patients tested between 20 and 40 weeks' gestation.[1-3] Furthermore, most Doppler ultrasonographic studies have used parametric statistics to evaluate the A/B ratio. This ratio, which approaches infinity as B values approach zero, has been shown by statistical analysis to be non-normal. Our study calculates the distributions of the Pourcelot and A/B ratios (after mathematical transformation to a normal distribution by the conversion of A/B to 1/1 − Pourcelot ratio) in a large low-risk population and offers a suggestion for the future use and direction of fetal velocimetry.

From the Departments of Obstetrics and Gynecology and Radiology, University of Washington.
Supported in part by the National Institutes of Health Biomedical Research Support Grant 62-3100 and the Pacific Coast Obstetrical and Gynecologic Society Young Investigators Grant, with technical support from MedaSonics.
Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.
Reprint requests: Susan K. Hendricks, MD, Division of Maternal-Fetal Medicine, University of Washington, RH-20, Seattle, WA 98195.
6/6/13894

Material and methods

Fetal velocimetry of the umbilical arteries was performed on 590 patients with low-risk pregnancies. Patients with medical complications during or preceding pregnancy were excluded, as were all tests on patients with complications of pregnancy. Fetal anomalies were excluded by ultrasonography.

Tests were performed at 2-week intervals from 14 to 42 weeks' gestation. Readings of the umbilical artery were obtained by continuous-wave Doppler ultra-

Table I. Pourcelot ratio

Gestational age (wk)	*n*	*Mean*	*+1.96 SD*	*−1.96 SD*	*Skewness*	*Kurtosis*
14	25	1.000	1.000	1.000	—	—
16	25	0.856	1.09904	0.61296	1.148	0.481
18	26	0.807	0.98144	0.63256	−2.258	5.03
20	29	0.743	0.9488	0.5372	0.032	0.412
22	25	0.747	0.84304	0.65096	−0.431	−0.555
24	25	0.714	0.87668	0.55132	−0.871	1.211
26	30	0.681	0.85936	0.50264	0.463	−0.824
28	43	0.696	0.82536	0.56664	−0.686	0.513
30	42	0.686	0.8624	0.5096	−0.064	−0.501
32	51	0.651	0.80388	0.49812	−2.15	10.058
34	75	0.618	0.81008	0.42592	0.249	0.213
36	61	0.6	0.76268	0.43732	0.249	0.213
38	71	0.591	0.80464	0.37736	−0.114	−0.025
40	34	0.54	0.73992	0.34008	1.28	1.852
42	25	0.531	0.68976	0.37224	0.52	−0.276

Table II. A/B ratio

Gestational age (wk)	*n*	*Mean*	*−1.96 SD*	*−1.96 SD*	*Skewness*	*Kurtosis*
14	25	*	*	*	*	*
16	25	*	*	*	*	*
18	26	4.807	7.30404	2.30996	0.959	0.723
20	29	4.31	6.65612	1.96388	−0.101	0.723
22	25	4.116	5.88588	2.34612	1.152	0.854
24	25	3.781	5.90956	1.65244	0.327	−1.225
26	30	3.386	5.35384	1.41816	1.297	2.906
28	43	3.483	5.2666	1.6994	1.038	−0.223
30	42	3.449	5.41684	1.48116	0.697	0.058
32	51	3.023	4.50084	1.54516	0.989	0.611
34	75	2.763	4.03308	1.49292	0.887	2.018
36	61	2.63	4.00984	1.25016	2.364	8.776
38	71	2.656	4.4004	0.9116	2.04	6.379
40	34	2.371	4.30748	0.43452	3.127	9.53
42	25	2.203	3.085	1.321	1.33	1.366

*B = 0 (14 weeks' gestation, all 25 cases; 16 weeks' gestation, 10:25).

sonography (4 MHz) (MedaSonics Vasculab D-10, Mountain View, Calif.) in the manner originally described by Trudinger et al.[1] Patients were placed in the left lateral recumbent position during times of examination. Measurements were taken during periods of fetal inactivity and apnea, which have been shown to affect the accuracy of measurement. Real-time spectral analysis was used to identify the maximum amplitude of the umbilical arterial waveform concomitantly visualized with the umbilical venous signal. The signal was recorded for a minimum of 10 waveforms and measurements were performed by hand. An average was calculated for a minimum of five signals to obtain each separate value for A and B. Only low-risk singleton gestations were evaluated. Patients were not initially included in the study if there were antecedent maternal or fetal abnormalities or complications (hypertension, diabetes, endocrine disorders, fetal anomalies) and were removed from the study if a pregnancy-related complication arose (intrauterine growth retardation, pregnancy-induced hypertension, oligohydramnios).

Values for A (systolic) and B (diastolic) pressures were obtained and plotted as Pourcelot ratio. To decrease the potential effect of extremely high A/B ratios, which may approach infinity as values of B approach zero, values for A/B were calculated from its relationship to the Pourcelot ratio (A/B = 1/1 − Pourcelot ratio) as previously described by Thompson et al.[4] This relationship has been shown to translate the A/B curve into a normal distribution. Mean, SD, and 95% confidence intervals were calculated and fitted to regression curves. Distribution curves were developed for Pourcelot and A/B ratios at each gestational age and for the total distribution, and the skewness and kurtosis (graphic measures of symmetry and distribution) of these curves were analyzed.

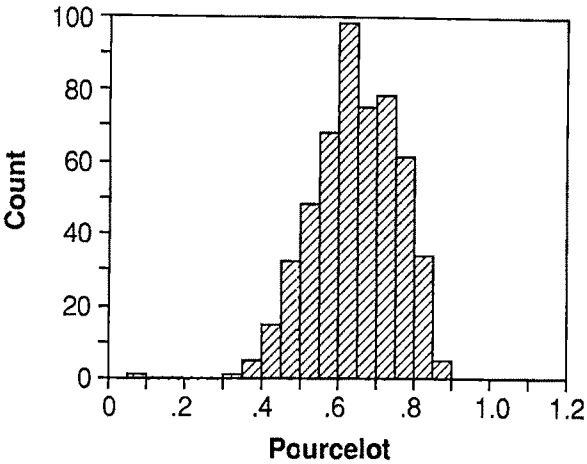


Fig. 1. Frequency distribution, Pourcelot ratio.

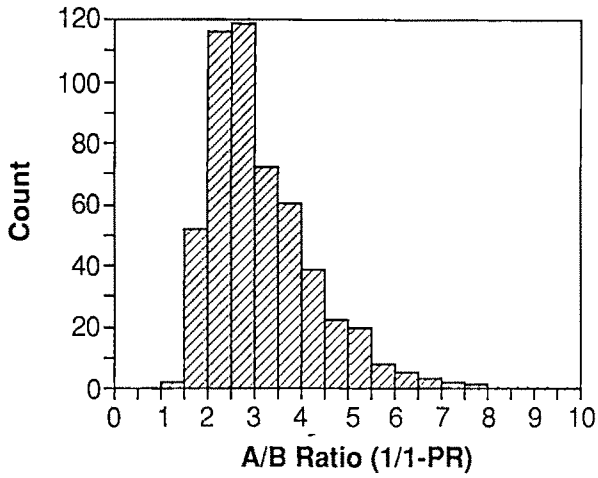


Fig. 2. Frequency of distribution, A/B ratio.

Results

The n for each gestational age and the calculated values of mean, SD, and 95% confidence intervals for Pourcelot and A/B ratios are listed in Tables I and II. Pourcelot ratio has been shown to be normal at all gestational ages in previous studies by Thompson et al.[5] This assumption of normality holds for the A/B ratio only when converted directly from the Pourcelot ratio for each gestational age group, which has little significance at gestational ages <18 weeks (when B = O and Pourcelot ratio = 1). For each gestational age ranking, skewness and kurtosis were calculated to graphically estimate the normality of the distribution for each ratio. Skewness and kurtosis were measured for the entire population set, excluding 14 and 16 weeks' gestation (Tables I and II). All values of B at 14 weeks' gestation were zero (n = 25), and 13 of 25 values at 16 weeks' were zero, which makes statistical manipulation of these subsets meaningless (Pourcelot ratio = 1 and A/B = infinity). The skew of these data was immense, which rendered the use of parametric statistics invalid. Skewness, a measure of symmetry, was calculated at 0.258 for Pourcelot ratio and at 0.439 for the A/B ratio for the data from 18 to 42 weeks' gestation, which strengthened the argument of normality (showing a nonsignificant positive, or right-handed, skew). Kurtosis, a graphic measure of distribution shape, was calculated at 1.043 for Pourcelot ratio and at 1.002 for the A/B ratio (demonstrating a nonsignificant leptokurtosis). The distribution curves for the total data set of Pourcelot and A/B (1/1 − Pourcelot ratio) ratios are shown in Figs. 1 and 2.

The gestational progression of Pourcelot ratio is represented in Fig. 3. The mean and 95% confidence intervals are plotted. Regression lines were fitted to the curve with a correlation coefficient of r = 0.95. The mean and 95% confidence intervals of the A/B ratio are plotted in Fig. 4 with the fitted regression curves (r = 0.89). These calculations were obtained by a direct conversion of A/B from the Pourcelot ratio by the formula (1/1 − Pourcelot ratio) as previously described.

Comment

Doppler waveform indices have been studied by multiple investigators as a possible measure of fetal well-being since original reports by Fitzgerald and Drumm (1977)[6] and McCallum et al. (1978)[7] reported the technical ability to measure the blood flow velocity waveforms in the umbilical artery of the fetus. Normal studies have been reported by some investigators but in relatively small numbers and generally encompassing the gestational ages between 28 and 40 weeks.[1-3] Thompson et al. have reported normal values for 35 patients between 20 and 40 weeks' gestation with the n at each gestational age ranging from 15 studies at 20 weeks to 34 studies at 36 weeks.[4] In this study we report a series of 590 normal low-risk patient studies from 14 to 42 weeks' gestation. The assumption of normality for Pourcelot ratio as proved by Thompson et al.[4] is reconfirmed by our data for gestations of 18 to 42 weeks. Similarly, calculation of the mean and 95% confidence intervals of the A/B ratio by direct conversion from the normal distribution of the Pourcelot ratio (A/B = 1/1 − Pourcelot ratio) transforms the A/B ratio into a normal distribution and allows the valid use of parametric statistical methods. This statistical treatment does not appear valid for gestations <18 weeks because of the high percentage of fetuses without diastolic flow.

Early studies of fetal well-being as predicted by fetal velocimetry were encouraging. Various investigators have found a good correlation between an increased

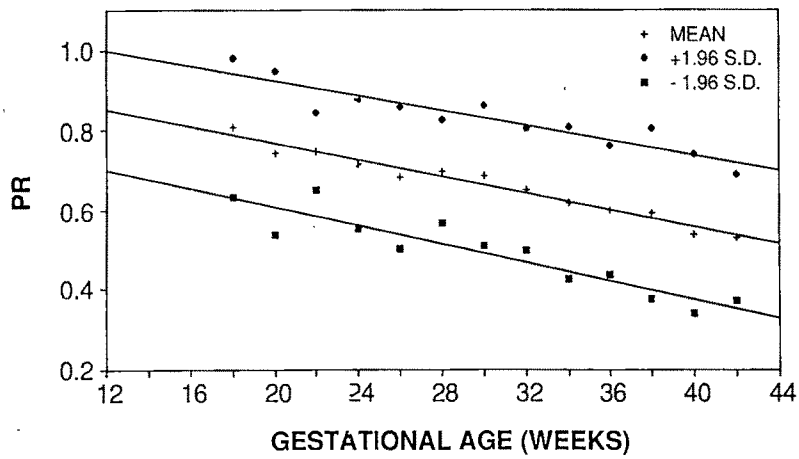


Fig. 3. Normal values for Pourcelot ratio.

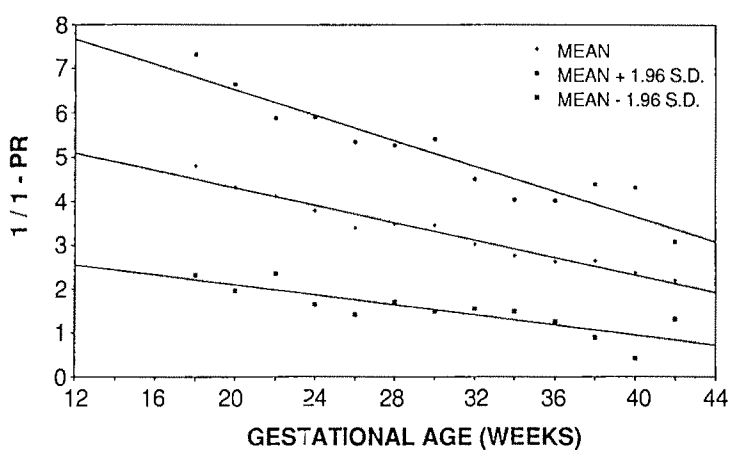


Fig. 4. Normal values for A/B ratio (1/1 − Pourcelot ratio).

A/B ratio and the presence of intrauterine growth retardation, which is currently the only Food and Drug Administration-sanctioned use of fetal Doppler ultrasonography in the United States.[8-10] Trudinger et al.[11] noted that the sensitivity of fetal Doppler ultrasonography was greater than that of traditional means of antepartum tests (the nonstress test) while maintaining equivalent specificity and predictive values. However, the place of fetal velocimetry in the spectrum of routine antepartum tests for the majority of high-risk pregnancy conditions remains unclear. If a uniqueness exists in the comparison of Doppler ultrasonography with other fetal testing methods, it may be that this modality offers the ability to screen high-risk pregnancies early in gestation with a reproducible testing mechanism. Knowledge of normal values for the Pourcelot and A/B ratios at early gestational ages may permit identification and assessment of high-risk pregnancies at earlier gestational ages than that allowed by the limitations of current antepartum test modalities, which creates the possibility of earlier intervention and therapy.

We thank Kathleen Nelson for her invaluable contributions to this work.

REFERENCES

1. Trudinger BJ, Giles WB, Cook CM, Bombardier J, Collins L. Fetal umbilical artery flow velocity waveforms and placental resistance: clinical significance. Br J Obstet Gynaecol 1985;92:23-30.
2. Schulman H, Fleischer A, Stern W, Farmakides G, Jagani N, Blattner P. Umbilical velocity wave ratios in human pregnancy. Am J Obstet Gynecol 1984;148:985-90.
3. Stuart B, Drumm J, FitzGerald DE, Duigan NM. Fetal blood velocity waveforms in normal pregnancies. Br J Obstet Gynaecol 1980;87:780-5.
4. Thompson RC, Trudinger BJ, Cook CM, Giles WB. Normal values for umbilical artery waveform indices. Br J Obstet Gynaecol 1988;95:589-91.
5. Thompson RC, Trudinger BJ, Cook CM. Doppler ultra-

sound waveform indices: A/B ratio, pulsatility index, and Pourcelot ratio. Br J Obstet Gynaecol 1988;95:581-8.
6. Fitzgerald DE, Drumm JE. Non-invasive measurement of the fetal circulation using ultrasound: a new method. Br Med J 1977;2:1450-8.
7. McCallum WD, Williams CS, Napel S, Daigle RE. Fetal blood velocity waveforms. Am J Obstet Gynecol 1978; 132:425-9.
8. Erskine RL, Ritchie JWK. Umbilical artery blood flow characteristics in normal and growth retarded fetuses. Br J Obstet Gynaecol 1985;92:605-10.
9. McCowan LM, Erskine MB, Ritchie K. Umbilical artery Doppler blood flow studies in the preterm, small for gestational age fetus. Am J Obstet Gynecol 1987;156: 655-9.
10. Fleischer A, Schulman H, Farmakides G, Bracero L, Blattner P, Randolph G. Umbilical artery velocity waveforms and intrauterine growth retardation. Am J Obstet Gynecol 1985;151:502-5.
11. Trudinger BJ, Cook CM, Jones L, Giles WB. A comparison of fetal heart rate monitoring and umbilical artery waveforms in the recognition of fetal compromise. Br J Obstet Gynaecol 1986;93:171-5.

Screening with Doppler velocimetry in labor

Leonard Feinkind, MD, Ovadia Abulafia, MD, Isaac Delke, MD, Joseph Feldman, MD, and Howard Minkoff, MD

Brooklyn, New York

Doppler flow velocimetry was performed on 273 nonselected patients in labor. All patients were at least 2 cm dilated and 80% effaced (58% > 4 cm) and were delivered of infants within 24 hours. Fetal heart rate tracing performed at the same time was read by another observer and compared with Doppler flow velocity measurements (systolic/diastolic ratio, pulsatility index). A significant correlation was noted ($r = 0.234$, $p < 0.001$) between the two modalities. Both fetal heart rate tracings and umbilical flow velocity measurements were correlated with fetal outcome. Significant associations were noted between the umbilical systolic/diastolic ratio and fetal distress, 5-minute Apgar scores, the need for intubation independent of gestational age, and umbilical arterial cord pH and base excess. It appears that the information obtained from flow velocimetry had additional benefits compared with that of fetal heart rate tracings alone. (Am J Obstet Gynecol 1989;161:765-70.)

Key words: Doppler ultrasonography, flow velocimetry, labor

Traditionally, the nonstress test (NST) has been used in the antepartum period to monitor fetal well-being.[1,2] More recently in the intrapartum period it has become routine in many institutions to obtain an initial "triage" NST when the patient is first seen. This initial NST is said to have predictive value for the occurrence of fetal distress.[2,3] Other modalities have been used in conjunction with the screening NST to increase its predictive value. We report on the use of Doppler flow velocimetry to identify patients at increased risk for poor perinatal outcome. Doppler velocimetry was chosen for this study because it has previously been used to identify the fetus in jeopardy.[4] The fetus subjected to a chronic decrease in uterine blood flow or umbilical blood flow may be diagnosed with Doppler flow velocimetry and is more susceptible to episodes of decreased Po_2 levels that normally occur during labor. Neonatal asphyxia may occur more rapidly in these patients. The purpose of this study is to examine in labor the relationship between the NST, umbilical artery flow velocity, and perinatal outcome.

From the Health Science Center at Brooklyn, State University of New York.
Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.
Reprint requests: Leonard Feinkind, MD, SUNY Health Science Center at Brooklyn, Box 24, 450 Clarkson Ave., Brooklyn, NY 11203-2098.
6/6/13673

Methods

A total of 273 random patients in labor underwent a 20-minute Doppler flow velocimetry examination during the period from April 1987 to November 1987. All patients were ≥2 cm dilated and 80% effaced (58% > 4 cm) and were delivered of infants within 24 hours. The systolic/diastolic (S/D) ratio and pulsatility index (PI) (maximum velocity − minimum velocity/mean velocity) were recorded. Continuous-wave Doppler ultrasonography with a 4 mHz transducer and

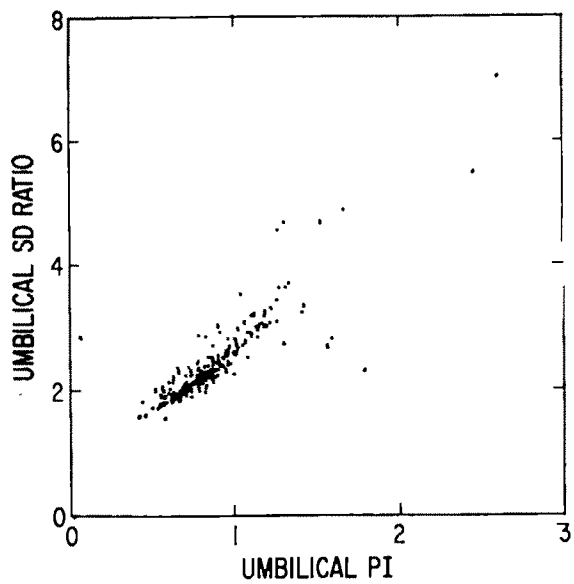


Fig. 1. Relationship between umbilical S/D ratio and umbilical PI.

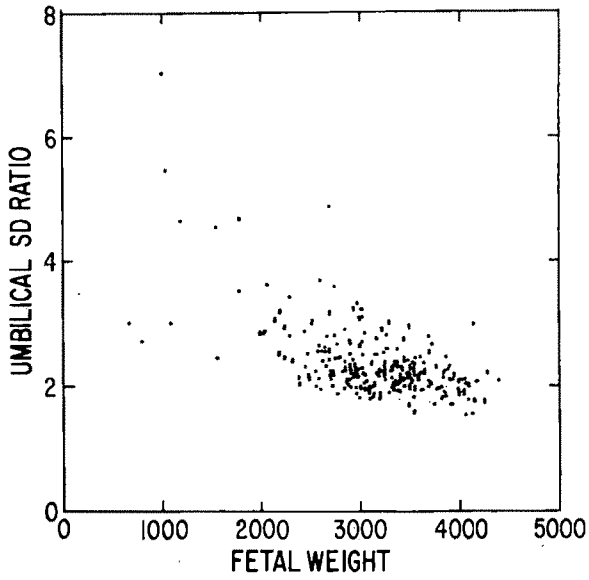


Fig. 2. Relationship between umbilical S/D ratio and fetal weight.

a power density of 6.5 mw/cm^2 (Multigon Industries, North Yonkers, N.Y.) was used to obtain readings on the umbilical and uterine arteries. The S/D ratio and PI of these vessels were computed. The uterine artery signals were obtained transabdominally in the right and left lower quadrants. While the patient was in the left lateral position, the hand-held transducer was adjusted until the best signal was obtained. Two recordings from the right uterine artery and two recordings from the left uterine artery were averaged. The values of three umbilical recordings were also averaged. Care was taken to note the occurrence of umbilical vein fluctuations and irregular arterial waveforms. All measurements were taken in the absence of fetal breathing with at least 10 consistent waveforms present. All patients were simultaneously monitored by internal or external means with a Corometrics (Wallingford, Conn.) model 111 or 115 fetal monitor. During the Doppler examination the corresponding fetal tracing was analyzed for the same 20-minute period by another investigator. In addition, the last 30 minutes of fetal tracing immediately before delivery was also analyzed.

The Lyons scoring system was used to evaluate the fetal tracings on a score of 1 to 10.[5] This was done by a separate observer who was unaware of the results of the Doppler velocimetry and the fetal outcome. The initial tracing (tracing 1) when the Doppler flow velocimetry was performed and the last 30-minute tracing before delivery (tracing 2) were scored. Intraobserver variation was also analyzed by repeat readings taken on 50 random patients. The information gathered from the Doppler flow velocimetry was not used in the clinical management of the patient. Attending staff not involved in the study made all clinical decisions without knowledge of the Doppler flow velocity measurements. Furthermore, all cases of fetal distress were reviewed separately by an independent observer. The gestational age was calculated by last menstrual period or ultrasonogram, with confirmation of gestational age by the Dubowitz[6] examination performed by a pediatrician.

Various dependent variables that were analyzed included fetal heart rate tracing, meconium-stained amniotic fluid, oxytocin administration, gestational age, sex, and fetal weight at the time of delivery, 1- and 5-minute Apgar scores, delivery mode, fetal distress (persistent late decelerations, fetal bradycardia to 60 beats/min, or a scalp pH <7.2 that required operative delivery), and the need for neonatal resuscitation (intubation or oxygen by Ambu bag).

Another of the outcome variables analyzed was arterial umbilical cord blood gases. Because of the practical constraints of running a busy delivery unit only 198 samples were obtained. Blood gas samples that were not analyzed by the laboratory within 15 minutes were discarded. Blood samples that had not arrived in the laboratory by this time were noted to have prolonged delays in transport and were considered unreliable. This left 135 arterial blood gas samples available for analysis. No bias could be detected in those cases in which blood was sent for analysis.

Statistics. Multiple regression analysis was used to account for the possible interrelations of several variables. However, the results indicate little improvement in predictive value when several variables were considered simultaneously or individually. Thus the results are presented in terms of Pearson product moment

Table I. Pearson product moment correlation coefficients between Doppler flow velocimetry, fetal heart rate tracings, and various selected outcome variables ($n = 273$)

	Umbilical PI	*Umbilical SD ratio*	*Fetal tracing 1*
Fetal tracing 1	−0.23*	−0.24*	
Gestational age	−0.31†	−0.37†	0.06
Fetal weight	−0.46†	−0.60†	0.16
5-minute Apgar score	−0.26†	−0.29†	0.28†
Fetal tracing 2	−0.20‡	−0.22‡	0.27*
Intubation	0.20‡	0.21*	−0.22†
Meconium	−0.04	0.01	−0.16

*$p < 0.001$.
†$p < 0.0001$.
‡$p < 0.01$.

correlation coefficients between pairs of variables. In the use of these correlation coefficients it is helpful to think in terms of r^2. This product would then express the proportion of the variation in the outcome variable that can be explained by its relationship with the independent variables.

The Student t test, computed with both pooled and separate variances, was used to compare means. In the evaluation of fetal distress, the Student t test and the Kolmogorov-Smirnov two-sample test were used. The latter test was chosen because it evaluates the discrepancy in the entire distribution of two populations rather than focusing solely on the means. Calculations were performed on an IBM XT computer with a statistical program (Systat, Evanston, Illinois); $p < 0.05$ was considered significant.

Results

The population at Kings County Hospital primarily comprises low socioeconomic black and Hispanic patients. The average maternal age was 25.9 years (SD = 6.3, minimum = 15, maximum = 45). A total of 16% (44:273) of the patients acknowledged smoking tobacco and 5% acknowledged taking "street" drugs (12 took crack, one patient took methadone, one took methadone and crack, and one took alcohol and crack). The average gestational age was 39 weeks (6.5% < 35 weeks, 9.1% < 37 weeks).

There were three patients with an antepartum diagnosis of intrauterine growth retardation (1%), three with sickle cell disease (1%), 14 with diabetes (5%), and 17 with hypertension or preeclampsia (6%).

Intraobserver correlation was judged to be good when 50 tracings were scored by the same observer over two separate time periods ($n = 50$, $r = 0.877$, $p < 0.001$, coefficient of variation = 10.1%). A similar correlation was noted for Doppler flow velocity measurements taken on 50 patients ($n = 50$, $r = 0.95$, $p < 0.001$, coefficient of variation = 9%).

In addition, a good correlation ($r = 0.86, p < 0.0001$) was noted between the umbilical S/D ratio and the umbilical PI (Fig. 1). In view of this, the S/D ratio will be used primarily in this article . However, the umbilical S/D ratio was slightly more skewed (skewness = 3.52, mean = 2.36 ± 0.59, median = 2.22) than was the umbilical PI (skewness = 2.69, mean = 0.85 ± 0.25, median = 0.81).

As visualized in Fig. 2, a strong negative correlation between the umbilical S/D ratio and fetal weight can be seen ($r = -0.60$, $p < 0.0001$). This remains true even when controlling for gestational age with multiple regression analysis (multiple $r = 0.69$; umbilical S/D ratio: $t = -9.8$; $p < 0.0001$; gestational age, $t = 7.5$; $p < 0.0001$). In addition, a negative correlation could be seen for gestational age and umbilical S/D ratios. There was no correlation between gestational age, fetal weight, and the initial tracing (tracing 1) or the final 30-minute tracing (tracing 2).

The initial fetal heart rate tracing and the last 30-minute tracing were correlated ($r = 0.27$, $p < 0.001$). In addition, both tracings were negatively associated with the umbilical S/D ratio ($r = -0.24$, $p < 0.001$).

Fetal compromise. Various factors were considered in the evaluation of fetal compromise. These included 5-minute Apgar score, occurrence of fetal distress, fetal heart rate tracings 1 and 2, and arterial umbilical blood gas values. Umbilical S/D ratio analysis proved to be negatively correlated with 5-minute Apgar scores (Table I). This remained true even when controlling for gestational age and fetal weight (multiple regression, 0.44; umbilical S/D ratio, $t = 6.3$, $p < 0.0001$; fetal weight, $t = 5.3$, $p < 0.0001$; gestational age, $t = 2.12$, $p < 0.04$). In addition, a higher umbilical S/D ratio was associated with fetal distress. This approached significance with the two-tailed Student t test (pooled variances, $t = 4.22$, $p < 0.0001$; separate variances, $t = 1.83$, $p < 0.08$) and achieved significance with the one-tailed Student t test ($p < 0.05$). Overall, the distribution of the umbilical S/D ratio in fetal distress was also significant with the Kolmogorov-Smirnov

Table II. Pearson product moment correlation coefficients between Doppler flow velocimetry, fetal heart rate tracings, and umbilical artery blood gases for 135 patients

	Umbilical PI	*Umbilical SD ratio*	*Fetal tracing 1*
pH	−0.03	−0.02	0.23*
Base excess	0.09	0.02	−0.30†
P_{CO_2}	−0.03	−0.02	0.02
P_{O_2}	−0.01	0.01	0.09
Bicarbonate	0.03	0.04	0.17‡
Oxygen saturation	0.01	−0.01	0.09

*$p < 0.001$.
†$p < 0.0001$.
‡$p < 0.01$.

Table III. Sensitivity, specificity, positive and negative predictive values of an abnormal outcome for fetal tracings and umbilical SD ratios

	Fetal tracing (%)	*Umbilical SD (%)*	*Tracing and SD ratio combined (%)*
Sensitivity	36	29	29
Specificity	88	92	98
Positive predictive value	23	30	56
Negative predictive value	93	92	94

two-sample test ($p < 0.05$). Finally, low scores on fetal heart rate tracing 1 were associated with fetal distress and low 5-minute Apgar scores (5-minute Apgar scores were believed to reflect fetal status more than did 1-minute Apgar scores).

Umbilical artery cord blood gases showed more variable results. Low scores on fetal tracing 1 were associated with lower umbilical arterial pH and higher base excess ($r = 0.23$, $p < 0.01$ and $r = -0.30$, $p < 0.001$, respectively). P_{CO_2}, P_{O_2}, and oxygen saturation were not correlated with fetal heart rate tracings (Table II). In addition, there appeared to be no benefit in examining fetal heart rate tracing 2 as opposed to tracing 1 with regard to arterial blood gas, fetal distress, and Apgar scores because the correlation coefficients were not any greater for tracing 2.

No correlation could be found between umbilical S/D ratios and the arterial umbilical cord pH, bicarbonate, base excess, P_{O_2}, P_{CO_2}, or oxygen saturation (Table II). In view of this lack of correlation, it was decided to look at a smaller group of at-risk pregnancies. A total of 16 fetuses with an umbilical S/D ratio ≥3 (equivalent to a PI ≥1.2, both approximately >2 SD of the mean), and with available umbilical arterial cord blood gases were analyzed (26 fetuses had a S/D ratio ≥3). In this high-risk population positive and negative correlations could be seen between umbilical S/D ratios, base excess and pH ($r = 0.86$, $p < 0.003$, and $r = -0.74$, $p < 0.05$, respectively). No correlation was seen for P_{CO_2}, P_{O_2}, oxygen saturation and bicarbonate. In this group of patients, low scores on the fetal heart rate monitor were associated with increased base excess ($r = -0.82$, $p < 0.007$). There were no other significant correlations when pH ($r = 0.34$, $p < 0.3$), P_{CO_2}, P_{CO_2}, bicarbonate, or oxygen saturation were analyzed.

The S/D ratios of the right and left sides of the uterus were also analyzed by Pearson correlation coefficients and compared with the umbilical S/D ratio, fetal heart rate tracings 1 and 2, resuscitation, fetal distress, fetal weight, gestational age, maternal age, Apgar scores, and arterial blood gas values. Except for a correlation with umbilical S/D ratios no other significant association was found ($r = 0.23$, $p < 0.001$).

The sensitivity, specificity, positive and negative predictive value for fetal heart rate tracing 1, umbilical S/D ratio, and both umbilical S/D ratio and fetal heart rate tracing are shown in Table III. A fetal heart rate tracing <7 and an umbilical S/D ratio ≥3 (>31 weeks' gestation) were considered abnormal. These values were chosen because they have been suggested in other studies.[7] Abnormal outcome was defined as fetal distress or a 5-minute Apgar score <7. For the population of patients with a normal outcome the S/D ratio was 2.31 ± 0.43 for a gestational age ≥31 weeks and 3.33 ± 1.0 for a gestational age <31 weeks. There were 39 fetal heart rate tracings that were scored <7. In 231 cases scores were ≥7 (in three cases the tracing could not be located). There were 26 umbilical artery velocimetry recordings ≥3 and 247 recordings that were <3. For a gestational age ≥31 weeks, 7% of the population had umbilical S/D ratios ≥3. There were no instances in which umbilical diastolic flow was absent.

For the population with an abnormal outcome the S/D ratio was 2.77 ± 1.22 for a gestational age ≥31

weeks and 3.16 ± 1.19 for those <31 weeks' gestation. The difference between the normal group and the abnormal group approached significance ($p = 0.06$) for a gestational age ≥31 weeks, with the two-tailed Student t test for separate variances.

The umbilical S/D ratio had a 30% positive predictive value for an abnormal outcome, whereas the fetal heart rate tracing had a positive predictive value of 23%. The sensitivity of the fetal heart rate tracing appeared to be slightly better than that of the umbilical S/D ratio (36% versus 29%, respectively). When the two modalities were combined the positive predictive value increased to 56%; however, the sensitivity remained unchanged (Table III).

Adjustment of the umbilical S/D ratio for gestational age did not change the sensitivity or the predicative value, inasmuch as only two fetuses <31 weeks' gestation had normal S/D ratios >3—one with fetal distress, the other with a normal outcome.

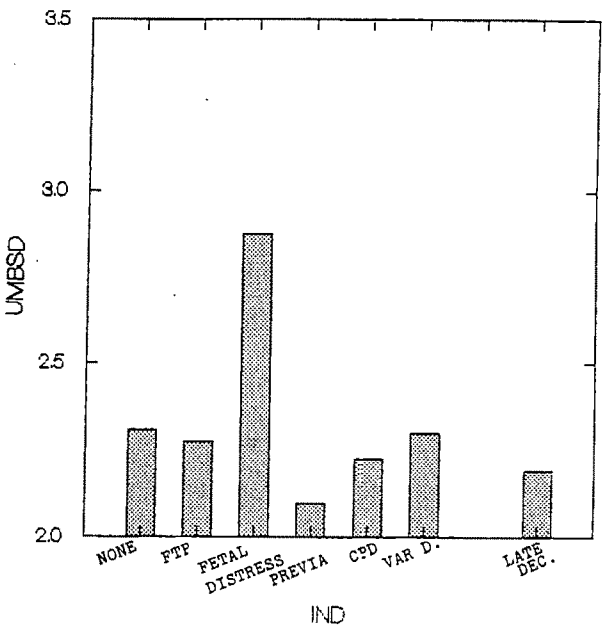


Fig. 3. Relationship between umbilical S/D ratio *(UMBSD)* and various indications *(IND)* for delivery. (*FTP* = Failure to progress, *CPD* = cephalopelvic disproportion, *VAR D.* = variable deceleration, *LATE DEC.* = late deceleration.)

Comment

There are three major angle independent indices that evaluate the flow velocity waveform—PI, resistance index, and S/D ratio. The PI and S/D ratio were strongly correlated in this report ($r = 0.86$, $p < 0.0001$). This finding confirms earlier work by Thompson[7] and others. It appears that the exact correlation depends on the manner in which the PI is calculated. In a study by Gilbert et al.,[8] a correlation coefficient of 0.95 was noted when the PI was calculated by a manual pen method, but only a correlation of 0.68 when an automated method was used.

As can be seen in this study and in others,[8, 9] there was a strong correlation between fetal weight and flow velocimetry. Several studies have suggested that flow velocimetry may be useful in the diagnosis of intrauterine growth retardation.[4, 9, 10, 11]

Other investigators have attempted to compare flow velocimetry and the ultrasonographic estimation of fetal weight.[12] Whereas there is a good correlation between fetal weight and flow velocimetry ($r = -0.6$, $p < 0.0001$), it would be erroneous to suggest that flow velocity waveform measurements are designed to estimate fetal weight. Morphometric measurements derived ultrasonographically should not be compared with measurements obtained by velocimetry.[13] Flow velocimetry will, however, identify the small-for-gestational-age fetus at increased risk.[9, 11, 14]

Biochemical data also was previously used to identify the fetus at risk. That data was obtained with a transabdominal technique to obtain amniotic fluid[15] during amniocentesis or by cordocentesis to obtain fetal blood during the antepartum period.[16, 17] Ours is the only study that has sought to compare the role of vascular impedance in the umbilical circulation during labor with biochemical data. Increased umbilical S/D ratios, a reflection of decreased umbilical blood flow, was not significantly correlated with arterial cord blood gas values when a group of 135 laboring patients who had cord blood gas measurements available were analyzed. However, when a small subset of high-risk fetuses with umbilical S/D ratios ≥3 (which corresponded to a PI of ≥1.2) were considered independently, a significant correlation could be seen. Umbilical S/D ratios (and PI) were negatively associated with pH and positively associated with base excess ($r = -0.74$, $p < 0.05$ and $r = 0.84$, $p < 0.003$, respectively). The fetus with increased placental impedance demonstrated an increased risk for acidosis and probably for excessive lactic acid accumulation. It is not surprising that in a large normal population with the additional stress of labor any relation between biochemical indices and measurements of blood flow velocity would be obscured. This was not the case when considering a small high-risk population. In addition, there may have been a tendency to administer oxygen to laboring patients who showed signs of fetal compromise. Oxygen treatment may have altered flow velocimetry and umbilical artery cord blood gases.[18] However, inasmuch as a significant relationship was present between the fetal tracing and the flow velocity measurements that were recorded simultaneously in the larger cohort, it may indicate that a correlation does exist between placental impedance and fetal oxygenation. In a report by Ferrazzi et al.[19] a significant correlation was found between the umbilical PI and pH, Pco_2, and lactate levels from umbilical cord blood obtained by cordocentesis and after elective ce-

sarean section in a small high-risk population. It is also interesting that they found a strong association between lactate concentration and PI when measurements >1.5 were reached. Above this value lactate concentration appeared to increase sharply. A critical level of blood flow reduction has been also shown to cause fetal hypoxia and acidosis in the sheep model (unpublished observation). Other data that have been presented by Soothill[20] also found an association between venous umbilical blood gas values obtained through cordocentesis and blood flow velocity measurements. All of these correlations have been noted in a select high-risk population.

In addition to biochemical data, pregnancies with increased placental impedance had lower 5-minute Apgar scores, fetal distress occurred (Fig. 3), and intubation was required more often, independent of gestational age. The fetal heart rate tracing was also significantly correlated with these outcomes and appeared to be more closely associated with arterial blood gases than was Doppler flow velocimetry. It is understandable that fetal distress would be better correlated with fetal heart rate tracings than with flow velocimetry because tracings were used in the diagnosis of fetal distress and in the management of all patients.

A comparison of the initial fetal tracing and Doppler velocimetry is revealed in Table III. Sensitivity, specificity, and positive and negative predictive values were comparable for the two modalities. When both modalities were used in conjunction, however, the positive predictive value increased to 56% and specificity increased to 98%. Therefore it appears that decreased flow velocity measurements may provide additional information in the identification of the high-risk fetus.

In summary, it appears that Doppler flow velocimetry is as sensitive as the NST in the identification of the fetus at risk for a poor perinatal outcome. However, it should be acknowledged that the sensitivity in this nonselected population was generally low, as were the majority of the correlation coefficients. Thus, it would be unwise to make firm recommendations concerning the benefits of Doppler velocimetry compared to the NST in labor without further study. Each of these modalities, though, have their own unique benefits and may be used concurrently in the laboring patient. The patient with abnormal NST results and an abnormal umbilical S/D ratio appears to be at greatest risk for a poor outcome. In addition, in a high-risk population of growth retarded infants the umbilical S/D ratio appears to be well correlated with umbilical cord pH and base excess. However, the fetal heart rate tracing reflects the dynamic changes that can occur during labor and is better correlated with umbilical arterial blood gas values at delivery in a group of low-risk patients.

REFERENCES

1. Hon E, Quilligan E. The classification of fetal heart rate. Conn Med 1967;31:779.
2. Schifrin B, Dame L. Fetal heart rate patterns. JAMA 1972;219:1322.
3. Schifrin B. The rational for antepartum fetal heart rate monitoring. J Reprod Med 1979;23:213.
4. Trudinger B, Giles W, Cook C. Fetal umbilical artery flow velocity waveforms and placental resistance-clinical significance. Br J Obstet Gynaecol 1985;92:23.
5. Lyons E, Bylsma-Howell M, Sham S, et al. A scoring system for non-stressed antepartum fetal heart rate monitoring. Am J Obtet Gynecol 1979;133:242.
6. Dubowitz L, Dubowitz V, Goldberg C. Clinical assessment of gestational age of the newborn infant. J Pediatr 1970;77:1.
7. Thompson RS, Trudinger BJ, Cook CM. A comparison of Doppler ultrasound waveform indices in the umbilical artery. I. Indices derived from the maximum velocity waveform. Ultrasound Med Biol 1986;12:835.
8. Gilbert WM, Nicolaides KH, Sel T, Campbell S. Comparison of umbilical artery flow velocity waveform indices as measured by continuous-wave Doppler ultrasound. J Ultrasound Med 1988;7:549.
9. Erskine RL, Ritchie JW. Umbilical artery blood flow characteristics in normal and growth retarded fetuses. Br J Obstet Gynaecol 1985;92:605.
10. Fleischer A, Schulman H, Farmakides G, et al. Umbilical artery velocity waveforms and intrauterine growth retardation. Am J Obtet Gynecol 1985;151:502.
11. Shulman H, Fleischer A, Stern W, et al. Umbilical velocity wave ratios in human pregnancy. Am J Obtet Gynecol 1984;148:985.
12. Divon M, Guidetti D, Braverman J, et al. Intrauterine growth retardation - a prospective study of the diagnostic value of real-time sonography combined with umbilical artery flow velocimetry. Obstet Gynecol 1988;72:611.
13. Chervenak F, Romero R, Berkowitz R, et al. Use of sonographic estimated fetal weight in the prediction of intrauterine growth retardation. Am J Perinatol 1984; 1:298.
14. Trudinger B, Giles W, Cook E. Flow velocity waveforms in the maternal uteroplacental and fetal umbilical placental circulations. Am J Obtet Gynecol 1985;152:155-63.
15. Green J, Paul R. The value of amniocentesis in prolonged pregnancy. Obstet Gynecol 1978;51:293.
16. Daffos F, Capella-Pavlovsky M, Forestier F. Fetal blood sampling during pregnancy with the use of needle guided by ultrasound; a study of 606 consecuive cases. Am J Obtet Gynecol 1985;153:655-60.
17. Pardi G, Bauscaglia M, Ferrazzi E, et al. Cord sampling for the evaluation of oxygenation and acid base balance in growth retarded fetuses. Am J Obtet Gynecol 1987;157:1221-8.
18. Arduini D, Rizzo G, Mancuso S, Romanini C. Short-term effects of maternal oxygen administration on blood flow velocity waveforms in healthy and growth retarded fetuses. Am J Obtet Gynecol 1988;159:1077-80.
19. Ferrazzi E, Pardi G, Bauscaglia M, et al. The correlation of biochemical monitoring verses umbilical flow velocity measurements of the human fetus. Am J Obtet Gynecol 1988;159:1081-7.
20. Soothill P, Nicolaides K, Rodeck C, Gamsutt. Blood gases and acid base status of the human second-trimester fetus. Obstet Gynecol 1986;68:173-6.

The effect of magnesium sulfate tocolysis on the fetal biophysical profile

Alan M. Peaceman, MD, Bruce A. Meyer, MD, James A. Thorp, MD, Valerie M. Parisi, MD, MPH, and Robert K. Creasy, MD

Houston, Texas

The biophysical profile has proved to be a valuable tool for the assessment of fetal well-being, independent of gestational age. Magnesium sulfate is commonly used as a tocolytic agent, yet relatively little is known about its effects on the biophysical activities of the fetus. To investigate the effects of magnesium sulfate on the biophysical profile, we performed serial studies on patients who received tocolytic therapy with this agent because of preterm labor. A total of 16 women with 22 fetuses at 26 to 34 weeks' gestation in spontaneous preterm labor were studied. An initial biophysical profile was performed at the time of admission, and a second examination was performed when maternal serum magnesium levels reached 6 to 8 mg/dl. On admission all fetuses had reactive nonstress test results and 21 of 22 (95%) demonstrated sustained fetal breathing movements. With magnesium sulfate tocolysis, 50% of fetuses had nonreactive nonstress test results, and only 4 of 22 (18%) demonstrated sustained fetal breathing movements. Fetal tone, gross body movements, and amniotic fluid volume were found to be unaffected by magnesium sulfate tocolysis. (AM J OBSTET GYNECOL 1989;161:771-4.)

Key words: Magnesium sulfate, preterm labor, biophysical profile

The biophysical profile is a valuable tool in the assessment of fetal well-being.[1, 2] Fetal tone, movements, reactivity, and breathing reflect acute central nervous system function. Amniotic fluid volume is a marker for fetal urine production, which may be diminished with chronic intrauterine compromise.[3] This test has proven validity in fetal assessment throughout the third trimester[4] and in twin gestations.[5] The five biophysical parameters involved can be influenced by other factors in addition to fetal distress. These include premature rupture of the membranes, normal sleep-wake cycles,[3] infection,[6] and pharmacologic intervention.[5] Interpretation of the biophysical profile results should take these other factors into account.

Magnesium sulfate is a commonly used agent for tocolysis in the management of pretern labor. It can cause maternal lethargy and depression of deep tendon reflexes when administered in doses often used for tocolysis.[7] Magnesium readily crosses the placenta, and fetal blood magnesium levels correlate well with maternal levels.[8] The higher doses of magnesium sulfate used for tocolysis may cause central nervous system depression in the fetus as well and thereby alter the biophysical profile. The purpose of this prospective study was to assess the effect of magnesium sulfate, in doses used to arrest preterm labor, on the parameters of the biophysical profile.

Material and methods

All women who were seen at the Hermann Hospital labor and delivery suite at 26 to 34 weeks' gestation with an initial episode of spontaneous preterm labor, as evidenced by regular uterine contractions and documented cervical change, were eligible for participation. Patients who had underlying hypertension or diabetes were excluded from this study. Other exclusion criteria included cervical dilation >4 cm, treatment in the preceding week with any tocolytic agent or sedative, and the presence of any significant abnormalities of the fetal heart rate tracing on admission (as judged by the managing physician). A total of 16 patients were recruited for the study, six of whom were carrying twin gestations. An initial biophysical profile was performed by the chief resident or attending physician before the initiation of tocolytic therapy. Fetal nonstress tests (NSTs) were evaluated from heart rate monitoring in the hour preceding the initial ultrasonographic examination. This recording was obtained with the patient in the semi-Fowler position with Doppler ultrasonography to record the fetal heart rate (Hewlett-Packard 8040A Cardiotocograph, Palo Alto, Calif.). The NST was considered reactive if, in any 20-minute period, there were two or more heart rate accelerations

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology and Reproductive Sciences, University of Texas Medical School at Houston.

Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.

Reprint requests: Alan M. Peaceman, MD, Department of Obstetrics and Gynecology and Reproductive Sciences, University of Texas Medical School at Houston, 6431 Fannin, Suite 3.204, Houston, TX 77030.

6/6/13718

Table I. Fetal biophysical profile

Reactive nonstress test	At least two episodes of fetal heart rate acceleration >15 beats/min and ≥15 seconds' duration associated with fetal movement in 20 minutes
Fetal breathing movements	At least one episode of sustained fetal breathing movements ≥30 seconds' duration in 30 minutes of observation
Fetal tone	At least one episode of active extension with return to flexion of fetal limbs or trunk in 30 minutes of observation
Fetal movement	At least three discrete limb or body movements in 30 minutes of observation
Amniotic fluid volume	At least one pocket of amniotic fluid that measured 3 cm in two perpendicular planes

Two points were awarded for each parameter when the above criteria were fulfilled. No points were given if the criteria were not met.

Modified from Manning et al. Fetal assessment based on fetal biophysical profile scoring: experience in 12,620 referred high-risk pregnancies. I. Perinatal mortality by frequency and etiology. AM J OBSTET GYNECOL 1985;151:345.

≥15 beats/min that lasted ≥15 seconds and that were associated with fetal movement. The test was considered nonreactive if no 20-minute interval of the 60-minute observation period contained two or more accelerations with fetal movements. Linear array real-time ultrasonographic scanning was performed with a 3.5 mHz transducer (General Electric RT 2800, Milwaukee, Wisc.). Fetal movements, tone, breathing movements, and amniotic fluid volume were assessed. Each parameter was assigned either 0 points or 2 points on the basis of a protocol modified from Manning et al.[1] (Table I). Two points were awarded for (1) reactive NST results, (2) an amniotic fluid pocket that measured ≥3.0 cm in two perpendicular planes, (3) at least three discrete body or limb movements, (4) at least one episode of limb or trunk extension with return to flexion, and (5) at least one episode of sustained fetal breathing >30 seconds. A total of 30 minutes of real-time ultrasonographic scanning was allowed for the evaluation of these parameters. The results of this initial biophysical profile were then recorded, in addition to maternal age, gestational age, and cervical dilation data.

After completion of the initial biophysical profile, the fetuses were continuously monitored, and each patient was administered an intravenous loading dose of magnesium sulfate. This preceded continuous intravenous infusion of a more concentrated solution. A total of 10 gm of magnesium sulfate (American Reagent Laboratories, Shirley, N.Y.) was diluted in 250 ml of 5% dextrose, which resulted in a 4% solution. A loading dose of 4 to 6 gm was then infused through an IMED 960 volumetric infusion pump (IMED Corporation, San Diego, Calif.) over 20 to 30 minutes. For continuous infusion, an 8% solution of magnesium sulfate in 5% dextrose was administered at 1.5 to 3.5 gm/hr. The doses used for loading and continuous infusion were determined by the attending physician to attain maternal serum magnesium concentrations in the therapeutic range of 6 to 8 mg/dl (5.0 to 6.7 mEq/l). Serum magnesium levels were measured every 4 to 6 hours, and the infusion rate was adjusted until the serum level was in the desired range. After a therapeutic level was documented, a repeat biophysical profile was performed and the results were recorded. The 60-minute interval after the magnesium level reached the therapeutic range was used for evaluation of fetal heart rate reactivity. Fetal heart rate baselines from the two NST recordings were also compared. All data are expressed as the mean ± 1 SD. Statistical analysis was performed by the Wilcoxon signed rank test and the McNemar exact test for correlated proportions. A paired Student *t* test was used for evaluation of fetal heart rate baseline changes. A p value of 0.05 was chosen to represent statistical significance. This study was approved by the institutional review board of the University of Texas Medical School at Houston.

Results

The mean maternal age was 23.4 ± 4.5 years, with mean gestational age 30.7 ± 2.2 weeks. Mean cervical dilation on initiation of tocolysis was 2.8 ± 1.5 cm. None of the study participants was delivered of infants before the second biophysical profile, and there were no maternal complications of therapy. The mean serum magnesium level at the time of the second biophysical profile was 6.6 ± 0.6 mg/dl, and the mean length of time between the two examinations was 14.1 ± 5.4 hours. Examination of the cardiotocograph recordings at the time of the second biophysical profile revealed that preterm labor had been arrested in all cases and that there were no instances of suspected fetal distress. The results of the biophysical tests are displayed in Fig. 1. All 22 fetuses were found to have reactive fetal heart rate patterns on admission, but only 11 of 22 (50%) were found to be reactive during therapeutic magnesium tocolysis ($p < 0.001$). All but one fetus demonstrated sustained breathing movements at the time of the initial biophysical profile. After a therapeutic magnesium level was achieved, however, only four fetuses demonstrated sustained breathing movements ($p < 0.0001$). All fetuses demonstrated normal tone,

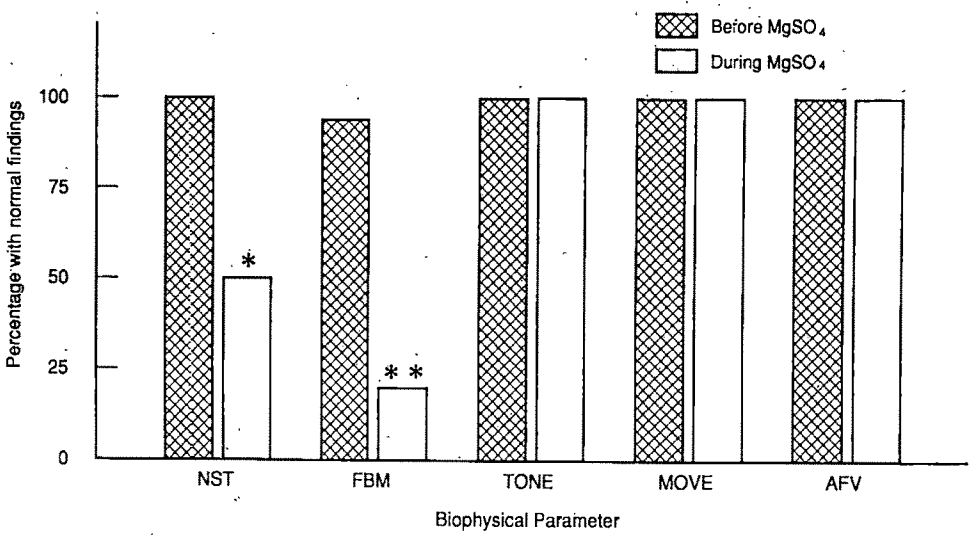


Fig. 1. Fetal biophysical profile results before and during magnesium sulfate tocolysis. Percentages of patients who fulfill criteria for each parameter of biophysical profile before and during therapy are compared (*$p < 0.001$, **$p < 0.0001$). *NST*, Nonstress test, *FBM*, fetal breathing movements, *TONE*, fetal tone, *MOVE*, fetal movement, *AFV*, amniotic fluid volume.

gross movements, and amniotic fluid volume at both the initial and follow-up biophysical profile examinations. Magnesium sulfate tocolysis resulted in a statistically significant decrease in overall biophysical profile scores ($p = 0.0002$), with a decrease of 2 or 4 points observed in 18 of 22 fetuses.

Examination of baseline fetal heart rates revealed a decrease ≥10 beats/min in 16 of 22 (73%) fetuses. The mean baseline fetal heart rate during the first biophysical profile was 144 beats/min and decreased to 131 beats/min at the time of the second biophysical profile. This decrease was statistically significant ($p < 0.0001$).

Comment

The biophysical profile has been shown to be a valuable tool for the assessment of the well-being of the fetus.[1-3] The test results reflect central nervous system function, and are independent of gestational age and fetal number.[4,5] Biophysical parameters can be altered by hypoxemia, drugs, rupture of the membranes, infection, and periodic rest cycles.[3,6] Most clinical protocols for tocolysis of preterm labor involve some form of monitoring of fetal well-being. The biophysical profile may be a useful tool in this setting, but the effects of pharmacologic intervention on this test must be taken into account before interpretation of the test results are used clinically.

Magnesium sulfate first achieved widespread use in obstetrics for the prevention of eclamptic seizures because of both its efficacy and safety.[9] Maternal complications have been seen with the use of this agent, but they are relatively infrequent when magnesium sulfate is administered by controlled infusion systems and the appropriate safeguards of monitoring urine output and deep tendon reflexes are observed. Respiratory paralysis and general anesthesia can occur at serum levels of 18 mg/dl, but are easily reversed with calcium gluconate.[10] Although clinical neurologic depression has been reported in neonates of women treated with magnesium sulfate because of preeclampsia,[11] most reports fail to identify any adverse effect on Apgar scores,[12,13] neonatal mortality rate,[14] or neonatal neurologic assessment examinations.[12] In a study of the sheep fetus, Bolan et al.[15] found that magnesium readily crossed the placenta but caused no alteration in baseline heart rate, hemodynamic parameters, or arterial blood gas parameters. Evaluation of the effects of magnesium sulfate on the human fetus to this point have included observations of changes in heart rate variability and baseline. In a study of patients with preeclampsia, Stallworth et al.[16] failed to demonstrate any significant change in fetal heart rate variability in the first 75 minutes of therapy, whereas Zuspan,[10] Lin et al.,[8] and Babaknia and Niebyl[17] all observed some decrease in fetal heart rate variability without evidence of compromise in the infant after delivery. Mean maternal and neonatal magnesium levels obtained in the above studies ranged from 3.6 to 5.7 mg/dl. The therapeutic range of magnesium in the prevention of eclamptic seizures has been reported to be 4.2 to 7.2 mg/dl.[18]

When magnesium sulfate is used as a tocolytic agent,

higher doses than are normally used in protocols for eclamptic seizure prophylaxis are often required to arrest preterm labor. Elliott[19] suggested that a maternal serum magnesium level of 5.5 to 7.5 mg/dl is necessary to inhibit uterine contractions. Only two studies have investigated the effects of magnesium sulfate therapy on the human fetus in patients treated because of preterm labor. Thiagarajah et al.[20] found a decrease in baseline fetal heart rate, whereas Brar et al.[21] did not observe such change. Neither of these studies evaluated parameters of the biophysical profile. Because magnesium passes readily to the fetus and fetal levels correlate with maternal levels, the use of magnesium sulfate for tocolysis may have more pronounced effects on the fetus than those seen with its use in seizure prophylaxis. Magnesium sulfate therapy for tocolysis is usually discontinued long before the birth of the infant. Because of placental transfer and rapid maternal excretion, neonatal magnesium levels may not be significantly elevated if maternal administration has been discontinued. Thus when magnesium sulfate is used as a tocolytic agent, evaluation of the newborn may not reflect the drug's effects on the fetus. Therefore, tests that directly assess the fetus are necessary for the evaluation of intrauterine effects of this therapy. To our knowledge, no previous studies have evaluated the effects of magnesium sulfate tocolysis on the biophysical profile.

It has been reported that breathing movements may be absent from fetuses during term[22] and preterm[23] labor. This finding was not observed in our study, as all but one fetus demonstrated breathing movements on admission. The diagnosis of preterm labor followed strict criteria of both regular uterine contractions and documented cervical change. Because arrest of preterm labor had occurred at the time of all follow-up examinations, yet breathing movements were not observed in most fetuses at that time, we must conclude that the significant reduction of fetal breathing movements seen is a pharmacologic effect rather than a result of labor.

We conclude that magnesium sulfate tocolysis (1) results in nonreactive nonstress test results and cessation of fetal breathing movements in a significant proportion of fetuses; (2) has no significant effect on fetal tone, gross body movements or amniotic fluid volume as assessed by the biophysical profile during our period of observation; (3) results in a decrease in baseline fetal heart rate in a significant number of fetuses; and (4) significantly reduces the overall biophysical profile score in the majority of fetuses. Most likely these changes do not reflect compromise of fetal well-being but rather central nervous system depression on a pharmacologic basis. These effects should be taken into account when the biophysical profile is used to assess fetal well-being in a patient who receives magnesium sulfate in high doses for tocolysis.

REFERENCES

1. Manning FA, Morrison I, Lange IR, Harman CR, Chamberlain PF. Fetal assessment based on fetal biophysical profile scoring: experience in 12,620 referred high-risk pregnancies. Am J Obstet Gynecol 1985;151:343-50.
2. Vintzileos AM, Campbell WA, Ingardia CJ, Nochimson DJ. The fetal biophysical profile and its predictive value. Obstet Gynecol 1983;62:271-8.
3. Manning FA, Platt LD, Sipos L. Antepartum fetal evaluation: development of a fetal biophysical profile. Am J Obstet Gynecol 1980;136:787-95.
4. Baskett TF. Gestational age and fetal biophysical assessment. Am J Obstet Gynecol 1988;158:332-4.
5. Lodeiro JG, Vintzileos AM, Feinstein SJ, Campbell WA, Nochimson DJ. Fetal biophysical profile in twin gestations. Obstet Gynecol 1986;67:824-7.
6. Vintzileos AM, Campbell WA, Nochimson DJ, Connolly ME, Fuenfer MM, Hoehn GJ. The fetal biophysical profile in patients with premature rupture of the membranes—an early predictor of fetal infection. Am J Obstet Gynecol 1985;152:510-6.
7. Petrie RH. Tocolysis using magnesium sulfate. Semin Perin 1981;5:266-73.
8. Lin CC, Pielet BW, Poon E, Sun G. Effect of magnesium sulfate on fetal heart rate variability in preeclamptic patients during labor. Am J Perinatal 1988;5:208-13.
9. Pritchard JA. The use of magnesium ion in the management of eclamptogenic toxemia. Surg Gynecol Obstet 1955;100:131-40.
10. Zuspan FP. Problems encountered in the treatment of pregnancy-induced hypertension. Am J Obstet Gynecol 1978;131:591-7.
11. Lipsitz PJ. The clinical and biochemical effects of excess magnesium in the newborn. Pediatrics 1971;47:501-9.
12. Green KW, Key TC, Coen R, Resnik R. The effects of maternally administered magnesium sulfate on the neonate. Am J Obstet Gynecol 1983;146:29-33.
13. Pruett KM, Kirshon B, Cotton DB, Adam K, Doody K. The effects of magnesium sulfate therapy on Apgar scores. Am J Obstet Gynecol 1988;159:1047-8.
14. Stone SR, Pritchard JA. Effect of maternally administered magnesium sulfate on the neonate. Obstet Gynecol 1970;35:574-7.
15. Bolan JC, Craft JB, Patch JH, et al. Pharmacokinetics of magnesium sulfate in the sheep model. Pediatr Pharmacol 1985;5:189-99.
16. Stallworth JC, Yeh SY, Petrie RH. The effect of magnesium sulfate on fetal heart rate variability and uterine activity. Am J Obstet Gynecol 1981;140:702-6.
17. Babaknia A, Niebyl JR. The effect of magnesium sulfate on fetal heart rate baseline variability. Obstet Gynecol 1978;51:2s-4s.
18. Pritchard JA. The use of magnesium sulfate in preeclampsia-eclampsia. J Repro Med 1979;23:107-14.
19. Elliott JP. Magnesium sulfate as a tocolytic agent. Contemp Ob Gyn 1985;25:49-61.
20. Thiagarajah S, Harbert GM, Bourgeois FJ. Magnesium sulfate and ritodrine hydrochloride: systemic and uterine hemodynamic effects. Am J Obstet Gynecol 1985; 153:666-74.
21. Brar HS, Medearis AL, DeVore GR, Platt LD. Maternal and fetal blood flow velocity waveforms in patients with preterm labor: effect of tocolytics. Obstet Gynecol 1988; 72:209-14.
22. Boylan P, Lewis PJ. Fetal breathing in labor. Obstet Gynecol 1980;56:35-8.
23. Castle BM, Turnball AC. The presence or absence of fetal breathing movements predicts outcome of preterm labor. Lancet 1983;2:471-3.

Controlled trial of hydration and bed rest versus bed rest alone in the evaluation of preterm uterine contractions

Richard A. Pircon, MD, Howard T. Strassner, MD, Donna S. Kirz, MD, and Craig V. Towers, MD

Orange and Long Beach, California, and Chicago, Illinois

Patients who are seen with uterine contractions but without documented change in cervical dilation or effacement are often treated with intravenous hydration before the initiation of intravenous tocolytic therapy. This is done with the intention of stopping uterine activity in patients with false preterm labor. A prospective randomized study was conducted to evaluate the effect of hydration on preterm uterine contractions in patients without proved preterm labor. A total of 28 patients were treated with bed rest and an intravenous bolus and subsequent continuous infusion of 5% dextrose in lactated Ringer's solution. A control group of 20 patients were treated with bed rest alone. Uterine activity and arrest of uterine contractions were compared between the two groups. Contractions stopped in 54% of the patients treated with hydration, whereas contractions stopped in 40% of the patients in the control group. This difference was not statistically significant. As a crossover study, those in the control group with contractions that continued after the initial observation period were subsequently treated with intravenous fluids. Only one patient in this group stopped contracting. Of all patients whose contractions stopped with either therapy, 18% eventually were delivered of preterm infants. This included 20% of the hydration group and 14% of the control group. The use of hydration as a pretherapy indicator to differentiate true preterm labor from false preterm labor could not be supported by this study. In addition, patients whose contractions stopped with either hydration or bed rest are at increased risk of subsequent preterm delivery. (Am J Obstet Gynecol 1989;161:775-9.)

Key words: Premature labor, premature contractions, tocolysis

Preterm delivery is a problem that affects approximately 8% of the obstetric population and is responsible for 75% of perinatal mortality,[1] thus there has been a great deal of interest in the development and use of effective tocolytic agents. Although early intervention is desired to prevent preterm delivery, the use of these agents should be limited to patients with true preterm labor (i.e., patients with uterine contractions that result in progressive cervical dilation and effacement). Many patients are first seen with uterine contractions and after observation do not demonstrate cervical change. Therefore it is difficult, based on the presence of contractions alone, to differentiate true preterm labor from frequent preterm (Braxton Hicks) contractions.

Various clinical parameters have been used to help separate these two groups. Some have used the character and frequency of uterine contractions to differentiate true labor from false labor. Perhaps the most commonly used method to distinguish true labor from false preterm labor is the response to hydration and bed rest. Although this practice is thought to be effective, no one has studied its usefulness in a prospective randomized fashion. Because of this we prospectively sought to determine the effect of hydration on the arrest of preterm uterine contractions in patients seen for evaluation of preterm labor. These patients were compared to a control group that received only bed rest and observation.

Material and methods

Patients seen with preterm uterine contractions at the labor and delivery unit at Rush Presbyterian–St. Lukes Medical Center were evaluated for enrollment in the study. Only those with a singleton pregnancy between 20 to 36 weeks' gestation and no identifiable cause of preterm contractions were included. On initial evaluation, patients who met criteria for true preterm labor as outlined by Creasy et al.[2] were excluded and treated with a tocolytic agent. Therefore only those patients who were <2 cm dilated, <80% effaced, and had intact amniotic membranes were considered for further study.

All patients who fulfilled these criteria were then continuously monitored for uterine activity and fetal heart

From the Departments of Obstetrics and Gynecology, University of California at Irvine, Rush Presbyterian, St. Lukes Medical Center, Rush Medical College, and Memorial Medical Center of Long Beach.

Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.

Reprint requests: Richard A. Pircon, MD, Department of Obstetrics and Gynecology, University of California at Irvine, 101 The City Dr., Building 25, Route 81, Orange, CA 92668.

6/6/14094

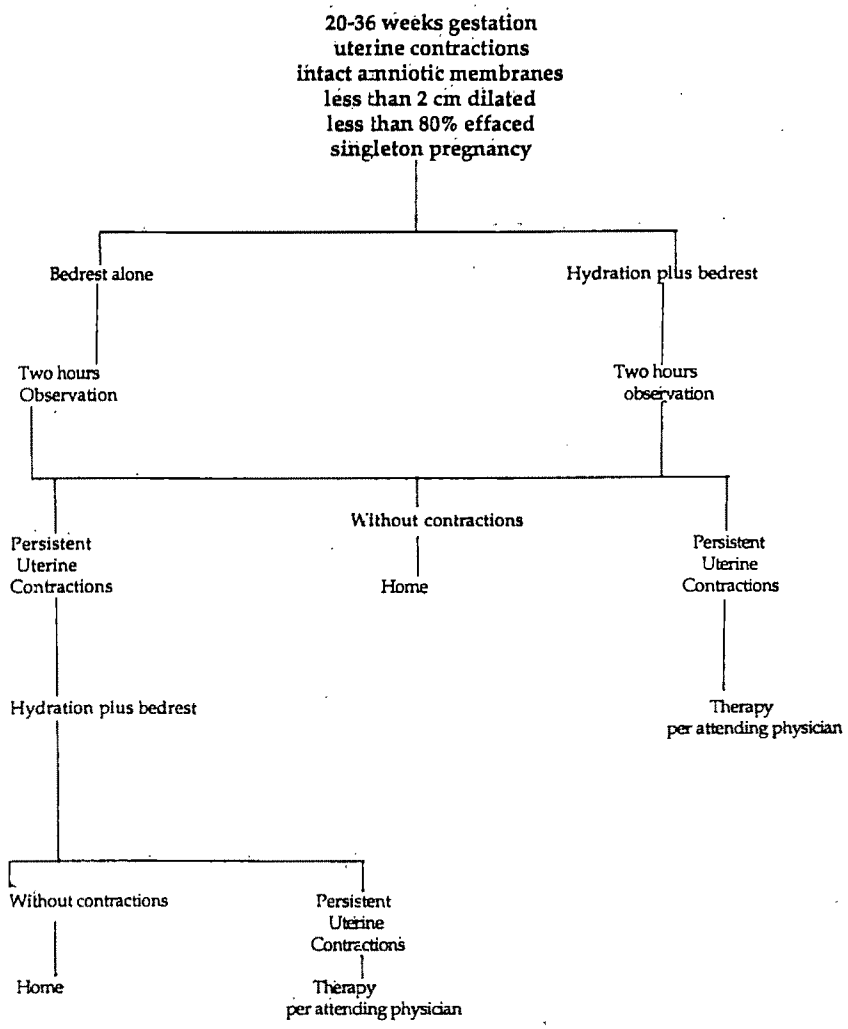


Fig. 1. Study protocol.

tones. Any patient with evidence of fetal distress was excluded from the study and treated in an appropriate fashion. At the end of 30 minutes uterine contractions were quantitated. Patients with uterine contractions more frequent than every 10 minutes or three contractions in 30 minutes were eligible for the study (Fig. 1).

These patients were randomly treated with hydration and bed rest or with bed rest only (control group). Allocation of patients to study groups was based on the day they were examined. Those treated with hydration were given an intravenous bolus of 500 ml of 5% dextrose in lactated Ringer's solution, and then 200 ml/hr continuous infusion. Patients in the control group were given ice chips only, and this was restricted to <200 ml total oral intake.

Both groups were then observed for 2 hours All patients were placed in the left lateral position during observation. Fetal heart tones and uterine contractions were continuously monitored. Sterile vaginal examinations were repeated after 1 hour of observation. If cervical change was noted by the same examiner, the patient was considered to be in preterm labor, removed from the study, and treated with intravenous tocolytic agents. After the 2-hour study period, uterine contractions were quantitated, and the patient was again examined. As before, those with cervical change were treated with intravenous tocolytic agents. After the 2 hours of observation the study was concluded for all patients who received hydration and those whose contractions stopped with bed rest. Patients in the hydration group without cervical change but with persistent contractions were treated on an individual basis by their attending physician. Patients whose contractions abated were discharged home. Each patient's disposition was recorded and outcome of therapy was noted.

Study participants who were treated with bed rest alone and who continued to contract after the initial 2-hour study period were then treated with intravenous hydration. The patients were given a 500 ml bolus of

5% dextrose in lactated Ringer's solution and then 200 ml/hr continuous infusion. These patients were observed for an additional 2 hours. The outcome of this additional therapy in this group of patients was recorded. Patients who continuted to contract were treated individually by their attending physician.

The charts of all study participants were reviewed after they were delivered of infants. Subsequent returns to the hospital and admissions because of preterm contractions were recorded. The difference between the hydration group and the control group in the abatement of uterine contractions were statistically evaluted by χ^2 with Yates correction. The two study populations were evaluated with regard to differences in age, gravidity, parity, previous preterm deliveries, estimated gestational age, uterine contraction frequency and duration, and cervical status when first seen. This comparison was done with the Mann-Whitney U test. For those patients whose contractions stopped during the study period, we reviewed any subsequent admissions because of preterm labor and compared these differences.

Results

A total of 48 patients were enrolled in the study; 28 were treated with hydration and 20 were treated with bed rest only. During the observation period, five patients had cervical change; two were in the control group and three were in the hydration group. No patient had evidence of fetal distress during the observation period.

There was no significant difference between groups with respect to age, gravidity, parity, previous preterm deliveries, or estimated gestational age when first seen. In addition, there was no difference between groups with regard to cervical dilation, effacement, station, and uterine contraction frequency and duration when first seen.

Of the 28 patients who received hydration, 15 (53.6%) stopped contracting during the observation period, whereas 7 (35%) of the 20 control patients stopped contracting. By χ^2 analysis, there was no significant difference between the two therapies of the abatement of preterm uterine contractions ($p \leq 0.33$).

Of the 13 patients in the control group who did not stop contracting during the study period, 12 were then treated with intravenous hydration. Only one patient stopped contracting with hydration; the other 11 required use of a tocolytic agent. One patient was not treated with hydration as per protocol. She was treated with further observation and no additional therapies and contractions stopped with this management.

There were three patients who were seen at the hospital more than once with preterm contractions without cervical dilation or effacement and an intact amniotic membrane. They were placed in different study groups on each visit. Both hydration and bed rest were effective in stopping contractions in two of the patients. The third patient did not stop contracting during the observation period with either treatment regimen. When she was first seen she was randomized to the control group. During that admission the contractions became less frequent during the 2-hour observation period. This is the patient who was not subsequently treated according to protocol, but was observed further without additional therapy. The contractions eventually stopped, and she was discharged. On a subsequent admission the same patient was randomized to the hydration group and again continued to contract after the 2-hour study period. She was then treated with a β-sympathomimetic tocolytic agent with subsequent tocolysis.

Of the 22 patients who stopped contracting during the initial study period, seven were seen again with uterine contractions before 36 completed weeks' gestation. Two of these patients were originally treated with bed rest alone. Five were treated with hydration and bed rest. This difference was not significant. As previously noted, three of these patients were reenrolled in the study. The other four patients had cervical dilation or effacement and could not be reenrolled.

Seventeen (35%) of the patients were delivered of preterm infants. Of these, six were in the control group and 11 were in the hydration group. The difference was not statistically significant. Of the 22 patients who stopped contracting during the study period, four (18%) eventually were delivered of preterm infants. This included three (20%) of the 15 patients in the hydration group and one (14%) of the seven patients in the control group. Of those who did not stop contracting during the study period, 13 patients (50%) were delivered of preterm infants. Eight of these patients were in the study group and five were in the control group. These differences were also not significant.

Comment

Preterm labor is defined as the onset of uterine contractions with resultant dilation and effacement of the cervix >20 and <37 weeks' gestation. Without change in the dilation or effacement of the cervix it is difficult to decide whether a patient is having true labor or preterm uterine irritability. This clinical problem is evident by the various definitions of preterm labor used by investigators who study various tocolytic agents. All agree that the patient must have regular painful uterine contractions. Some insist on documented cervical dilation or effacement.[3, 4] In our study, we found this accounted for only 10% of those patients first seen with persistent painful uterine contractions. Other investi-

gators only require uterine contractions to be of a certain frequency. Beall et al.[5] in their study that compared ritodrine, terbutaline, and magnesium sulfate required contractions to be more frequent than every 10 minutes. Elliott,[6] while studying magnesium sulfate, required a uterine contraction frequency twice that of Beall's criteria. Neither study required documented cervical change or minimal cervical dilation or effacement before entry into their studies.

Creasy et al.[2] used cervical status along with uterine contraction frequency as an indicator of preterm labor. For the diagnosis he required documented cervical change in those patients with intact membranes and a cervix <2 cm dilated or <80% effaced. However, patients contracting with ruptured membranes or a cervix >80% effaced or 2 cm dilated are considered to be in preterm labor. With the use of these criteria, one limits the use of tocolytic agents to patients with contractions who show the true sign of labor—cervical change. However, use of these criteria does not delay the use of tocolytic agents in patients first seen with an abnormally dilated or effaced cervix. For these reasons, we have adopted these criteria at our institution.

A problem, however, arises in patients who are regularly contracting without evidence of cervical change. Castle and Turnbull[7] have advocated the use of ultrasonography to demonstrate the presence or absence of fetal breathing movements. They found that the presence of fetal breathing predicted patients whose contractions would spontaneously subside. Others have not found this technique to be helpful.[8]

Ingermarsson[9] used sedation and bed rest in an effort to discriminate true preterm labor from preterm uterine irritability. The patients were given 10 mg of valium intramuscularly and were observed for abatement of the uterine contractions. Cotton et al.[10] hydrated patients by giving 1 L of 5% dextrose in lactated Ringer's solution. Valenzuela[11] used a combination of hydration and 8 to 12 mg of intramuscular morphine sulfate. Although many of these regimens are now commonly used, no one has studied their effects in a prospective randomized fashion.

The tocolytic effect of hydration is attributed to inhibition of antidiuretic hormone through the Henry-Gauer reflex.[12] This reflex, however, has only been described in dogs. Goodlin et al.[13] studied plasma volumes in patients with preterm labor. They found patients with preterm labor were hypovolemic and their plasma volumes were ≥45 ml/kg below normal. They also found significant delays in delivery in patients with preterm contractions whose plasma volumes were expanded with albumin. They noted that β-sympathomimetics, tranquilizers, and bed rest expanded plasma volume.

We studied the effect of hydration and bed rest versus bed rest alone in the abatement of preterm uterine contractions. Of patients in the control group, 40% stopped contracting with observation alone (seven during the 2-hour observation period and one who was treated with continued observation beyond the completion of the study), whereas 54% of the study participants who received hydration stopped contracting. The difference did not reach statistical significance. Although there is a trend, the efficacy of hydration in the differentiation of true labor from false labor is doubted further when patients who did not stop contracting after treatment with bed rest alone are followed up. All except one of these patients were subsequently treated with hydration. Only one patient stopped contracting, whereas the other 10 patients had persistent uterine activity. Furthermore, no difference was seen between therapies in patients who were seen more than once and who were treated with different therapies with each visit.

One other study that evaluated the efficacy of treating threatened preterm labor is that of Valenzuela et al.[8] As previously noted, they used hydration in combination with sedation. This was a prospective study; however, it was not randomized with a control group. They found that 55% of the patients responded to this therapy. This was similar to our data in which 54% responded to hydration and 40% responded to bed rest alone.

The data of Valenzuela et al.[8] is similar to ours with regard to preterm delivery rates. Their study revealed an overall preterm delivery rate of 26% with a 15% rate in patients who stopped contracting with hydration and sedation. Our study had an overall preterm delivery rate of 35%. Of the patients who stopped contracting during the study period, 14% of the control group and 20% of the hydration group were delivered of preterm infants. Finally, of those who did not stop contracting during the study period, 50% of our patients were delivered of preterm infants, as compared to 37% in the study of Valenzuela et al.[8]

The benefit of hydration was not demonstrated by this study. The potential risks, however, have been documented in previous articles. Of those who continue to contract and are subsequently treated with intravenous tocolytic agents, the risk of pulmonary edema is increased by the use of large amounts of fluid. This has been demonstrated with use of both β-sympathomimetics and magnesium sulfate.[6, 14] The use of sedatives such as valium or morphine sulfate in the treatment of patients who could potentially be delivered of preterm infants is also questioned. The depressive effects of these drugs on the preterm neonate can be seen at delivery and up to 2 to 4 days after their use.[15] Therefore we cannot advocate the use of either hydration or other agents until their benefit can be proved through prospective randomized studies.

REFERENCES

1. Fuchs F. Prevention of prematurity. AM J OBSTET GYNECOL 1976;126:809-17.
2. Creasy RK, Herron MA. Prevention of preterm birth. Semin Perinatol 1981;5:295-302.
3. Barden TP, Peter JB, Merkatz IR. Ritodrine hydrochloride: a betamimetic agent for use in preterm labor. Obstet Gynecol 1980;56:1-6.
4. Wesselius-Decasparis A, Thiery M, Yo Le Sian A, et al. Results of double-blind, multicentre study with ritodrine in premature labour. Brit Med J 1971;3:144-7.
5. Beall MH, Edgar BW, Paul RH, Smith-Wallace T. A comparison of ritodrine, terbutaline, and magnesium sulfate for the suppression of preterm labor. AM J OBSTET GYNECOL 1985;153:854-9.
6. Elliott JP. Magnesium sulfate as a tocolytic agent. AM J OBSTET GYNECOL 1983;174:277-84.
7. Castle BM, Turnbull AC. The presence or absence of fetal breathing movements predicts the outcome of preterm labour. Lancet 1983;2:471-2.
8. Goodlin RC. Identifying true premature labour. Lancet 1984;1:793.
9. Ingermarsson I. Effect of terbutaline on premature labor. AM J OBSTET GYNECOL 1976;125:520-4.
10. Cotton DB, Strassner HT, Hill LM, Schifrin BS, Paul RH. Comparison of magnesium sulfate, terbutaline and a placebo for inhibition of preterm labor. J Repro Med 1984; 26:92-7.
11. Valenzuela G, Cline S, Hayashi RH. Follow-up of hydration and sedation in the pretherapy of premature labor. AM J OBSTET GYNECOL 1983;147:396-8.
12. Bieniarz J, Burd L, Motew M, Scommegna A. Inhibition of uterine contractility in labor. AM J OBSTET GYNECOL 1971;111:874-85.
13. Goodlin RC, Quaife MA, Dirksen WJ. The significance and diagnosis of maternal hypovolemia. Semin Perinatol 1981;5:163-74.
14. Hawker F. Pulmonary oedema associated with beta 2-sympathomimetic treatment of premature labour. Anaesth Intensive Care 1984;12:143-51.
15. Stechler G. Newborn attention as affected by medications during labor. Science 1964;144:315-7.

Fetal heart rate patterns in the small-for-gestational-age human fetus

Robert Gagnon, MD, Cora Hunse, RN, and Alan D. Bocking, MD

London, Ontario, Canada

A total of 24 pregnant women with growth-retarded fetuses were studied to examine the distribution of fetal heart rate accelerations between 30 and 40 weeks' gestation, as compared with those of fetuses of normal growth that were matched for gestational age and length of fetal heart rate tracings. Growth-retarded fetuses had significantly lower Po_2 levels in the umbilical artery at birth (3 mm Hg less) than did healthy fetuses ($p < 0.05$), but without metabolic acidosis. There was a larger proportion of small amplitude (<10 beats/min) and a smaller proportion of large amplitude (>20 beats/min) fetal heart rate accelerations in the small-for-gestational-age fetuses than in the fetuses of normal growth. Although the number of accelerations was significantly reduced (50% less) in growth-retarded fetuses compared with healthy fetuses, there was no significant difference in the mean basal fetal heart rate and the mean number of decelerations between the two groups. Currently used definition of an acceleration as ≥15 beats/min for ≥15 seconds was applicable only in fetuses of normal growth. We hypothesized that a decrease in absolute acceleration frequency might be a useful index to detect the chronically hypoxemic fetus before severe metabolic acidosis and irreversible damage occurred. (AM J OBSTET GYNECOL 1989;161:779-84.)

Key words: Nonstress tests, intrauterine growth retardation, computerized fetal heart rate analysis

From the Departments of Obstetrics and Gynecology and Physiology, Lawson Research Institute, St. Joseph's Health Centre, University of Western Ontario.
Supported by the Physician's Services Incorporated and the Canadian Medical Research Council.
Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.
Reprint requests: Robert Gagnon, MD, Department of Obstetrics and Gynecology, St. Joseph's Health Centre, 268 Grosvenor St., London, Ontario N6A 4V2 Canada.
6/6/13676

The association between the presence of fetal heart rate (FHR) accelerations and fetal health[1] has led to the development and clinical use of antepartum FHR tests.[2] Currently used criteria for nonstress tests (NST) are based on the detection of FHR accelerations above the baseline of a predefined amplitude and duration.[3] To provide a better understanding of antepartum FHR tests in high-risk pregnancies, the aims of this study were to analyze the distribution of FHR accelerations

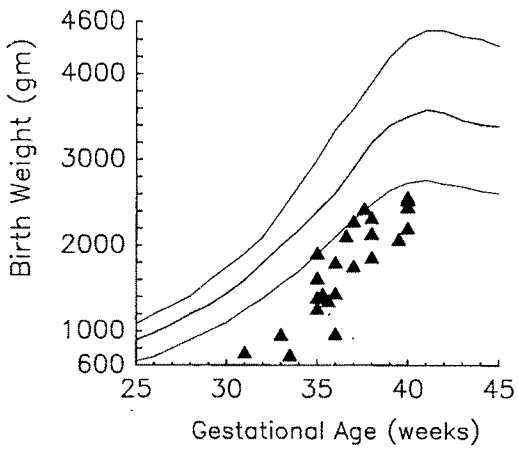


Fig. 1. Birth weight distribution of neonates. Normal range (mean ± 2 SD) is shown.

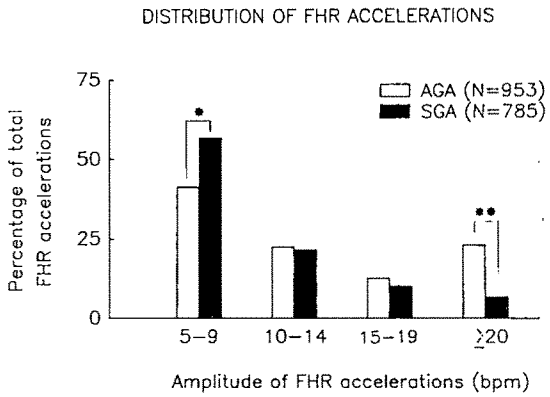


Fig. 2. Frequency histogram of 1738 FHR accelerations ≥15 seconds duration was plotted in successive increments of 5 beats/min to define distribution of acceleration amplitudes in SGA and AGA fetuses. (* $p < 0.05$; ** $p < 0.01$.)

in small-for-gestational-age (SGA) fetuses from 30 weeks' gestation to term and to determine whether current criteria used for nonstress tests are applicable in SGA fetuses.

Material and methods

Experimental design. The study participants received a standardized 800 kcal breakfast or lunch at 8 AM or at noon. All studies began 1 hour later and were conducted in a quiet room with patients either sitting upright in bed or resting in a lateral recumbent position. Participants were asked not to smoke ≥4 hours before the studies. Smoking or food intake by patients or observers was not permitted during the studies. This protocol, although not done for routine NSTs, was designed to minimize the potential effect of maternal glycemia on FHR.[4] In the SGA group, participants were studied continuously for 30 minutes (n = 10) or 1 hour (n = 30) and were matched for length of observation and gestational age with patients with appropriate for-gestational-age (AGA) fetuses. Seven patients in the SGA group and two in the AGA group participated in more than one study, separated by ≥7 days. Six studies were done at 30 to 32 weeks' gestation, 19 at 33 to 35 weeks' gestation, and 15 at 36 to 40 weeks' gestation in each group for a total observation time of 70 hours.

FHR measurements. FHR was recorded with a Hewlett-Packard 8040A (Hewlett-Packard GMBH, Boeblinger, Federal Republic of Germany) external monitor by use of a compact transducer and was analyzed on-line by a Sage II microprocessor (Sage Computer Technology, Reno, Nevada) as previously described.[5,6] The FHR intervals were examined by means of an artifact detection algorithm, and valid pulse intervals were averaged over 3.75 seconds. During on-line analysis a baseline was fitted to the heart rate record.[7] FHR accelerations ≥10 beats/min for ≥15 seconds above the baseline and FHR decelerations of ≥10 beats/min for ≥60 seconds below the baseline were recognized automatically.

For each 10-minute interval the basal FHR (beats/min), the number of FHR accelerations, and the number of FHR decelerations were calculated. Fetal breathing movements and gross fetal body movements were measured with a real-time ultrasound scanner (Model 2130, Advanced Diagnostic Research Corp., Tempe, Arizona) and will be reported elsewhere.

Data analysis. The basal FHR, the number of FHR accelerations ≥10 beats/min for ≥15 seconds above the baseline, the number of FHR decelerations ≥10 beats/min for ≥60 seconds below the baseline, and amplitude of individual FHR accelerations were determined on-line in successive 10-minute intervals. The number of FHR accelerations ≥15 seconds and FHR decelerations ≥60 seconds were also automatically determined off-line for acceleration and deceleration amplitudes of 5 beats/min and in successive increments of 5 beats/min to a maximum amplitude of 60 beats/min.

Portions of records that could not be analyzed because of FHR signal loss of >30% or patient interruption were not included and made up 1.2% of total study time. The average signal loss of the remaining 69.2 hours included in this analysis was 3.3% ± 0.5% in the SGA group and 4.5% ± 0.5% in the AGA group.

Results are presented as grouped mean and SEM. The significance of difference in the means was examined by unpaired Student t test and χ^2 for proportions. Standard regression analysis with Pearson product-moment correlations (r) were determined.

Results

Patients. Informed consent was obtained from 46 pregnant women with singleton pregnancies between 30 and 40 weeks' gestation with clinical or ultrasono-

Table I. Patient characteristics

	SGA (n = 24)		AGA (n = 36)	
	Mean ± SEM	Range	Mean ± SEM	Range
Gestational age at test (wk)	35.3 ± 0.4	31-40	35.4 ± 0.4	31-40
Gestational age at delivery (wk)	36.6 ± 0.5*	31-40	39.2 ± 0.2	37-42
Birth weight (gm)	1762 ± 116*	720-2540	3365 ± 68	2750-4540
5-minute Apgar score	8.7 ± 0.3	5-10	9.2 ± 0.1	7-10
Length of stay in neonatal intensive care unit (days) (n = 18)	17.8 ± 3.9	2-66	0	

*$p < 0.001$ compared with AGA.

Table II. Umbilical cord blood gas values

	SGA (n = 24)		AGA (n = 26)	
Value	Mean ± SEM	Range	Mean ± SEM	Range
P_{O_2} (mm Hg)				
Artery	15.1 ± 1.0*	5.8-21.7	18.0 ± 0.8	10.9-24.9
Vein	25.1 ± 1.5	10.2-45.2	27.8 ± 0.9	17.2-37.2
P_{CO_2} (mm Hg)				
Artery	49.8 ± 1.9	30.2-65.1	48.9 ± 2.1	30.4-83.4
Vein	43.0 ± 1.6	30.1-55.5	39.5 ± 1.2	26.3-55.2
pH				
Artery	7.26 ± 0.01	7.09-7.36	7.27 ± 0.01	7.15-7.36
Vein	7.31 ± 0.01†	7.16-7.41	7.35 ± 0.01	7.25-7.42

*$p < 0.05$ compared with AGA.
†$p < 0.01$ compared with AGA.

graphic findings suspicious for intrauterine growth retardation (IUGR) (SGA fetus) and in 36 low-risk pregnant women (AGA fetus) matched for gestational age. All AGA infants subsequently had normal neonatal outcomes. All patients either had reliable menstrual dates or underwent ultrasonographic examination during the second trimester. Usher and McLean[8] charts were used to classify the birth weights at sea level of the fetuses studied. Growth retardation was diagnosed when birth weight was less than the 3rd percentile for gestational age. A total of 22 (48%) of the 46 participants with fetuses thought to be SGA had normal term deliveries with normal birth weights and were excluded from subsequent analysis. All other participants were delivered of infants with a birth weight less than the 3rd percentile (Fig. 1). The 3rd percentile at sea level is similar to the 10th percentile for birth weights of the Denver standards.[9]

Patients who subsequently were delivered of SGA infants ($n = 24$) were matched for gestational age and length of heart rate tracings with healthy pregnant women (AGA; $n = 36$). Table I illustrates the characteristics of the fetuses studied. Umbilical arterial and venous blood gas values were determined in all SGA fetuses and in 26 AGA fetuses. The mean umbilical artery P_{O_2} level at birth was 15.1 ± 1.0 mm Hg in SGA neonates, which was significantly lower than 18.0 ± 0.8 mm Hg in AGA neonates ($p < 0.05$; Table II), which suggests the presence of chronic hypoxemia in SGA fetuses. There was no significant difference in the umbilical artery P_{CO_2} level and pH at birth between SGA and AGA neonates (Table II). One neonatal death of a 40-week SGA infant occurred because of bilateral diaphragmatic hernia (umbilical arterial cord blood pH of 7.30 and P_{O_2} level of 14.8 mm Hg). There was no other congenital anomaly or neonatal death. Twelve (50%) patients in the SGA group were delivered of infants by cesarean section: two because of intrapartum fetal distress; two because of prolonged nonreactive NSTs; two because of footling breech presentation at the onset of labor; two because of severe pregnancy-induced hypertension; one because of prolapsed cord; and three were repeat cesarean sections.

Distribution of FHR accelerations. Fig. 2 is a histogram plot of 1738 FHR accelerations ≥15 seconds above the baseline in the 60 fetuses studied. The percentage of total FHR accelerations was calculated in successive increments of 5 beats/min to define the distribution of acceleration amplitudes. In SGA fetuses, 56.8% of FHR accelerations were <10 beats/min am-

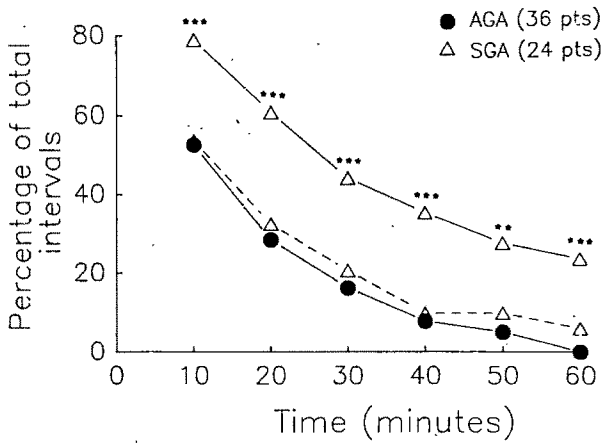


Fig. 3. Percentage of time intervals during which fewer than two FHR accelerations of ≥15 beats/min for ≥15 seconds occurred was plotted for all fetuses (——). (** $p < 0.01$ compared with AGA, *** $p < 0.001$ compared with AGA.) Percentage of time intervals during which fewer than two FHR accelerations of ≥10 beats/min for ≥15 seconds occurred was also plotted for SGA fetuses on same time scale (- - - -).

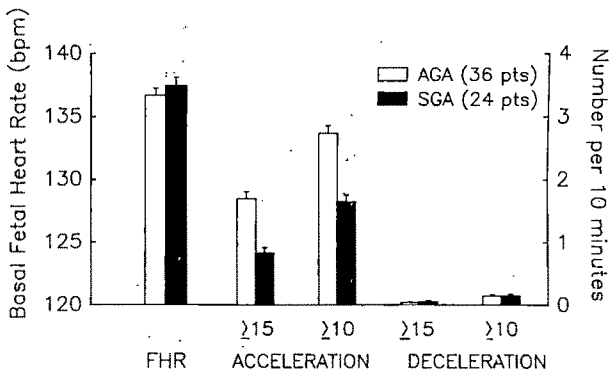


Fig. 4. Mean basal FHR, mean number of FHR accelerations per 10 minutes of ≥15 beats/min and ≥10 beats/min for ≥15 seconds, and mean number of FHR decelerations per 10 minutes of ≥15 beats/min and ≥10 beats/min for ≥60 seconds were plotted for each FHR tracing of AGA and SGA fetuses.

plitude, which was significantly higher than the 41.3% in AGA fetuses ($p < 0.05$). In contrast, SGA fetuses had fewer accelerations of large amplitude (≥20 beats/min) than did AGA fetuses (6.8% and 23%, respectively, $p < 0.01$).

The percentage of time intervals during which fewer than two FHR accelerations ≥15 beats/min for ≥15 seconds occurred is plotted in 10-minute increments (Fig. 3). In the AGA group, 52.6% of tracings had fewer than two FHR accelerations at 10 minutes and 1.3% at 60 minutes. However, in SGA fetuses 79% of the recordings had fewer than two FHR accelerations at 10 minutes, 35.2% at 40 minutes, and 24% at 60 minutes, which was significantly higher than in the AGA fetuses (all $p < 0.001$). With a minimum amplitude of 10 beats/min for ≥15 seconds to define an acceleration in SGA fetuses, the length of time from the beginning of recordings until two FHR accelerations occurred was similar to that of the AGA group (Fig. 3).

Relationship between basal FHR and amplitude of FHR accelerations. Fig. 4 summarizes characteristics of FHR accelerations and decelerations for each of the 426 10-minute intervals. The mean number of FHR accelerations per 10 minutes of ≥15 beats/min amplitude was 0.8 ± 0.1 in the SGA group, compared with 1.7 ± 0.1 in AGA fetuses ($p < 0.001$). With the use of a definition of 10 beats/min for acceleration amplitude in SGA fetuses, the mean number of FHR accelerations per 10 minutes was similar to that of the AGA group with the use of 15 beats/min for acceleration amplitude (1.6 ± 0.1 and 1.7 ± 0.1, respectively, Fig. 4). No significant difference was seen between AGA and SGA fetuses in the mean basal FHR and the mean number of FHR decelerations ≥10 beats/min for ≥15 beats/min for ≥60 seconds (Fig. 4).

There was a significant negative correlation between the mean amplitude of FHR accelerations of ≥10 beats/min for ≥15 seconds and the mean basal FHR in AGA fetuses ($r = -0.43$, $p < 0.01$), which demonstrated that in AGA fetuses at 30 to 40 weeks' gestation the higher the mean basal heart rate, the lower the height of individual FHR acceleration. No such correlation was found in SGA fetuses ($r = 0.02$, NS).

Comment

Analysis of the distribution of FHR acceleration amplitude in a highly selected population of SGA fetuses from 30 to 40 weeks' gestation demonstrated a larger proportion of small amplitude (<10 beats/min) and a smaller proportion of large amplitude (>20 beats/min) FHR accelerations than did the AGA fetuses. With 10 beats/min to define the minimum amplitude of an acceleration in SGA fetuses, the length of time necessary to have a reactive nonstress test will follow the same pattern as that in AGA fetuses with the use of 15 beats/min.

Although there was a significant negative correlation between the mean amplitude of FHR accelerations and the mean basal FHR in AGA fetuses, this relationship was not demonstrated in SGA fetuses. No significant difference in the mean basal FHR and the mean number of FHR decelerations was seen between SGA and AGA fetuses.

Creasy et al.[10] produced IUGR in the fetal lamb by reduction of the normal maternal uteroplacental blood flow. This was done by gradual embolization of the uterine vascular bed of the pregnant sheep with nonradioactive microspheres. The fetal lambs with growth retardation were chronically hypoxemic for the last 2 weeks of embolization and on average the P_{O_2} level into

the descending aorta was 3 mm Hg lower than that of fetuses of normal growth. Our data also demonstrated that SGA fetuses had, on average, Po_2 levels in the umbilical artery at birth 3 mm Hg lower than did AGA fetuses without significant metabolic acidosis (Table II), which suggests that SGA fetuses were chronically hypoxemic but not acidotic before birth.

It was previously reported[11] that human fetuses with severe but late-onset (>32 weeks' gestation) IUGR (birth weight less than the 3rd percentile) have a reduction in long-term FHR variability (25% less), incidence of gross fetal body movements (40% less), and time spent in FHR accelerations (50% less). Similar observations have been made in human fetuses with early-onset (<32 weeks' gestation) IUGR.[12] We have made use of computerized FHR analysis to define the distribution of acceleration amplitudes, which is the major component of long-term FHR variability.[5] Our data demonstrated that the decrease in FHR variability previously described in SGA fetuses[11-13] could be a result of a shift in the distribution of FHR acceleration amplitude, the small amplitude (<10 beats/min) being predominant with IUGR. A similar pattern is seen in healthy fetuses before 30 weeks' gestation[11] which suggests that severely growth-retarded (birth weight less than the 3rd percentile) human fetuses may have an "immature pattern" in the distribution of acceleration amplitude. This observation might indicate a delay in the functional maturation of the autonomic control of the FHR due to chronic nutritional deprivation and hypoxemia.

Current data, like others,[12, 13, 15] demonstrated a significant decrease in the number of FHR accelerations (50% less) in compromised SGA fetuses compared with AGA fetuses with the use of 10 or 15 beats/min as a definition for minimum FHR acceleration amplitude. With 10 beats/min as a minimum amplitude, the detection of FHR accelerations was improved in SGA fetuses at a level comparable to AGA fetuses with the use of 15 beats/min. It is therefore possible to speculate that currently used criteria for nonstress tests (FHR accelerations ≥15 beats/min for ≥15 seconds) could lead to classification of a high proportion of SGA fetuses as nonreactive (24% at 60 minutes, Fig. 3) and premature delivery.

We previously described in healthy human fetuses of >30 weeks' gestation, a significant negative correlation between the basal FHR and the mean amplitude of FHR accelerations.[14] It is believed that individual fetuses >30 weeks' gestation have a set "gain" for FHR accelerations that is related to the basal heart rate.[14] This relationship between basal heart rate and amplitude of FHR accelerations is not present <30 weeks' gestation in healthy human fetuses.[14] In SGA fetuses of >30 weeks' gestation such correlation could not be demonstrated, which suggests an impairment of the cardioacceleratory (β-sympathetic) system in the presence of chronic fetal hypoxemia. FHR decelerations ≥60 seconds did not occur more frequently in SGA fetuses than in AGA fetuses, which suggests that the parasympathetic (cardioinhibitory) system is probably not affected to the same extent as is the sympathetic tone during mild chronic fetal hypoxemia and nutritional deprivation. However, this remains to be determined.

The results indicated that in the presence of chronic hypoxemia without acidosis associated with IUGR, the earliest change in FHR patterns might be a decrease in the number of FHR accelerations rather than an increase in the number of FHR decelerations. Furthermore, the baseline heart rate was not significantly altered in the presence of IUGR. Use of an amplitude ≥10 beats/min to define an acceleration could be a strategy to increase the chance to detect FHR accelerations at >30 weeks' gestational age in SGA fetuses. The data also support the concept, as recently suggested,[16-17] that the presence of a decline in absolute acceleration frequency may be a sensitive indicator of chronic fetal hypoxemia before severe metabolic acidosis and irreversible damage occurs.

We thank Dr. J. Patrick for his interest in this work and Mrs. M. de Sousa for her technical assistance.

REFERENCES

1. Hammacher K. The clinical significance of cardiotocography. In: Huntingford PJ, Hunter EA, Saling E, eds. Perinatal medicine. New York: Academic Press, 1969:80.
2. Rochard F, Schifrin BS, Gaupil F, et al. Non-stressed fetal heart rate monitoring in the antepartum period. Am J Obstet Gynecol 1976;126:699.
3. Paul RH. The evaluation of antepartum fetal well-being using the nonstress test. Clinics in Perinat 1982;9:253.
4. Bocking AD, Adamson L, Carmichael L, Patrick J. Effect of intravenous glucose injection on human maternal and fetal heart rate at term. Am J Obstet Gynecol 1984; 148:414.
5. Dawes GS, Redman CWG, Smith JH. Improvements in the registration and analysis of fetal heart rate records at the bedside. Br J Obstet Gynaecol 1985;92:317.
6. Gagnon R, Hunse C, Carmichael L, et al. External vibratory acoustic stimulation near term: fetal heart rate and heart rate variability responses. Am J Obstet Gynecol 1987;156:323.
7. Dawes GS, Houghton CRS, Redman CWG. Baseline in human fetal heart rate records. Br J Obstet Gynaecol 1982;89:270.
8. Usher R, McLean F. Intrauterine growth of liveborn caucasian infants at sea level. J Pediatr 1969;74:901.
9. Lubchenco LO, Hansman C, Boyd E. Intrauterine growth in length and head circumference as estimated from live births at gestational ages from 26 to 42 weeks. Pediatrics 1966;37:403.
10. Creasy RK, Barrett CT, de Swiet M, et al. Experimental intrauterine growth retardation in the sheep. Am J Obstet Gynecol 1972;112:566.
11. Gagnon R, Hunse C, Fellows F, et al. Fetal heart rate and activity patterns in growth-retarded fetuses: changes after

vibratory acoustic stimulation. AM J OBSTET GYNECOL 1988;158:265.
12. Gagnon R, Hunse C, Carmichael L, et al. Vibratory acoustic stimulation in 26-to-32-week, small-for-gestational-age fetus. AM J OBSTET GYNECOL 1989;160:160-5.
13. Henson G, Dawes GS, Redman CWG. Characterization of the reduced heart rate variation in growth-retarded fetus. Br J Obstet Gynaecol 1984;91:751.
14. Gagnon R, Campbell K, Hunse C, et al. Patterns of human fetal heart rate accelerations from 26 weeks to term. AM J OBSTET GYNECOL 1987;157:743.
15. Bekedam DJ, Visser GHA, Mulder EJH, et al. Heart rate variation and movement incidence in growth-retarded fetuses: the significance of antenatal late heart rate decelerations. AM J OBSTET GYNECOL 1987;157:126.
16. Devoe LD, Castillo R, Saad S, et al. Percent acceleration time: a new method of fetal assessment. Obstet Gynecol 1986;67:191.
17. Devoe LD, Castillo R, McKenzie J, et al. Sequential nonstress testing with use of each fetus as its own control. AM J OBSTET GYNECOL 1986;154:931.

Impact of residual amniotic fluid volume in patients receiving parenteral tocolysis after premature rupture of the membranes

Richard K. Silver, MD, Scott N. MacGregor, DO, and Edward D. Hobart
Evanston, Illinois

The inhibition of labor subsequent to premature rupture of the membranes remains controversial. To evaluate the possibility that diminished amniotic fluid volume impacts on the latent period in patients receiving tocolytic therapy after premature rupture of the membranes, we reviewed our recent clinical experience with premature rupture of the membranes and the use of tocolytic agents. Between January 1985 and June 1988, 70 singleton pregnancies with premature rupture of the membranes between 25 and 34 weeks of gestation without prior tocolytic therapy were included. Ritodrine ($n = 22$), magnesium sulfate ($n = 37$), or both agents ($n = 11$) were administered and followed by oral terbutaline if tocolysis was achieved. Ultrasonographic examination was performed after admission and patients were grouped on the basis of estimates of residual amniotic fluid volume. Fourteen patients had adquate fluid volume; 20 had diminished fluid volume, and 36 had oligohydramnios. Patients with adequate fluid volume had a significantly longer interval between tocolytic therapy and delivery when compared with the other two categories (analysis of variance, $p < 0.001$), and proportionately more patients remained undelivered for >1 week (adequate 8 of 14 vs. diminished 3 of 20 and oligohydramnios 3 of 36; $p < 0.001$). In the 18 patients with premature rupture of the membranes before 30 weeks' gestation, adequate fluid volume was associated with the longest latent period (adequate 14.0 days; diminished 12.6 days, and oligohydramnios 2.8 days; analysis of variance, $p < 0.05$). We conclude that tocolytic therapy is of little benefit in the presence of oligohydramnios. If additional prospective studies of tocolytic efficacy in pregnancies complicated by premature rupture of the membranes are undertaken, inclusion of residual amniotic fluid volume as an independent variable before randomization should be considered. (AM J OBSTET GYNECOL 1989;161:784-7.)

Key words: Premature membrane rupture, tocolysis, amniotic fluid volume

The inhibition of labor subsequent to premature rupture of the membranes remains controversial. Although tocolytic therapy may forestall delivery in some patients,[1, 2] an improvement in perinatal outcome has not been identified.[3, 4] The inclination to use tocolytic agents is greatest when the diagnosis is made before 30 weeks' gestation because those fetuses may best benefit from pregnancy prolongation.[5] Other rationales for tocolytic therapy include delay of delivery to accomplish maternal transport or to administer antenatal steroids (the latter rationale is also controversial).

Two prospective, randomized trials evaluating the efficacy of tocolytic therapy in patients with premature rupture of the membranes were recently reported.[3, 4] Neither study identified an impact of tocolytic use on perinatal outcome, although one trial observed a sig-

From the Division of Maternal-Fetal Medicine at Evanston Hospital and the Department of Obstetrics and Gynecology, Northwestern University Medical School.
Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.
Reprint requests: Richard K. Silver, MD, Evanston Hospital, G435, 2650 Ridge Ave., Evanston, IL 60201.
6/6/13675

nificant delay in delivery among patients diagnosed before 28 weeks' gestation.[4] Although both studies reached similar conclusions, the influence of diminished amniotic fluid volume was not considered in either study design. In patients with premature rupture of the membranes, reduced amniotic fluid is predictive of subclinical infection[6] and shorter interval to delivery.[7] Because they did not consider amniotic fluid volume before randomization, or control for its effects during analyses, both studies may have failed to identify significant differences between control and treatment groups. In clinical terms, patients with low amniotic fluid volume and preterm contractions may respond differently to tocolytic therapy when compared with quiescent patients with adequate amniotic fluid volume. To evaluate the possibility that diminished amniotic fluid volume impacts on the latent period in patients receiving tocolytic therapy after premature rupture of the membranes, we reviewed our recent clinical experience with premature rupture of the membranes and the use of tocolytic agents.

Material and methods

Between January 1985 and June 1988, all pregnancies complicated by preterm premature rupture of the membranes that were treated by tocolysis were considered for this study. Only singleton pregnancies between 25 and 34 weeks of gestation that had not received tocolytic therapy before premature rupture of the membranes were included. Of the 92 cases identified during this interval, 22 were excluded from analysis (twins, $n = 9$; prior tocolytic treatment, $n = 13$). The remaining 70 patients composed the study population.

The diagnosis of membrane rupture was made by sterile speculum examination in all cases; ferning of amniotic fluid was considered confirmatory. Reconfirmation of the diagnosis was mandatory for patients transferred from referring hospitals with the diagnosis of premature rupture of the membranes. Parenteral therapy with either ritodrine or magnesium sulfate was administered at the onset of regular contractions (or on admission, if contractions began before presentation). The decision to use tocolytics was made independently of amniotic fluid estimates. Ritodrine was administered at an initial dose of 100 μg/min, and was increased by 50 μg/min every 10 minutes until either an effective dose or 450 μg/min was reached. Magnesium sulfate was initially given as a 6 gm loading dose over 20 minutes followed by a 2 gm/hr maintenance dose (with a 4 gm/hr infusion limit). Measurements of magnesium ion blood levels were obtained when clinically indicated, and adjustment of either drug's infusion rate was predicated on achieving a uterine response of fewer than four contractions per hour. Oral terbutaline was administered to patients after intravenous treatment. The dose and frequency were adjusted to maintain maternal tachycardia (pulse≥100 beats/min). Tocolytics were withdrawn for any of the following reasons: if the diagnosis of intraamniotic infection was made (by virtue of elevated maternal temperature, fetal tachycardia, uterine tenderness, or positive Gram stain of amniotic fuid); if a mature lecithin/sphingomyelin ratio was identified; if labor progressed despite maximal therapy (cervical dilatation ≥5 cm); or if a change in fetal assessment (e.g., significant heart rate abnormality) prompted delivery.

An ultrasonographic examination was performed shortly after admission to assess fetal growth parameters and evaluate amniotic fluid volume. The largest pocket of amniotic fluid was identified and its vertical diameter was measured and recorded. Amniotic fluid assessment was repeated weekly if the patient remained undelivered.

Obstetric data were collected and coded and ultrasonographic results were also coded without knowledge of corresponding outcomes. Statistical methods included analysis of variance for continuous data, and χ^2 for categoric variables (3 × 2 contingency tables).

Results

Forty-one of the 70 patients were transported from referring hospitals and tocolytic therapy was instituted in 36 cases before transfer. Although 10 patients had a history of prior preterm birth, 40 (57.1%) were nulliparous. The mean gestational age on admission was 31.2 weeks; 18 patients were <30 weeks of gestation when premature rupture of the membranes occurred. The time from membrane rupture to hospital admission varied (range, 1 to 80 hours), with a mean interval of 13 hours. Tocolytic therapy was begun within 24 hours of membrane rupture in 59 (84.3%) patients. Magnesium sulfate was used most often (37 of 70, 52.9%) and 11 patients received simultaneous treatment with both magnesium sulfate and ritodrine. Forty-one patients (58.6%) were undelivered 48 hours after premature rupture of the membranes and 14 (20%) had a latent period >1 week. The most common indication for withdrawal of tocolysis was progressive labor despite maximum therapy ($n = 37$). Suspected intraamniotic infection prompted delivery in 16 cases. Of the 34 patients who had successful sampling by amniocentesis, positive Gram stains were identified in four cases. A mature lecithin/sphingomyelin ratio (obtained by amniocentesis) permitted withdrawal of tocolytic therapy in an additional six patients, and fetal heart rate abnormalities led to emergent delivery in six patients.

The initial ultrasonographic evaluation permitted categorization of patients on the basis of vertical measurement of the largest amniotic fluid pocket. Fourteen

Table I. Selected peripartum variables grouped acccrding to residual amniotic fluid volume after premature rupture of the membranes

	Amniotic fluid volume		
	Adequate (N = 14)	*Diminished (N = 20)*	*Oligohydramnios (N = 36)*
Gestational age at premature rupture of the membranes (wk, mean ± SD)	30.9 ± 2.0	31.2 ± 3.1	31.4 ± 2.9
Tocolysis instituted within 24 hr of premature rupture of the membranes (No. and %)	13 (92.8)	16 (80.0)	30 (83.3)
Magnesium sulfate only (No. and %)	6 (46.2)	12 (60.0)	19 (52.8)
Ritodrine only (No. and %)	7 (50.0)	2 (10.0)	13 (36.1)
Magnesium sulfate plus ritodrine (No. and %)	1 (7.1)	6 (30.0)	4 (11.1)
Indication for delivery (No. and %)			
Failed tocolysis	7 (50.0)	12 (60.0)	22 (61.1)
Intraamniotic infection	3 (21.4)	4 (20.0)	10 (27.7)
Abnormal fetal heart rate	0 (−)	4 (20.0)	2 (5.6)
Birth weight (gm, mean ± SD)	2018 ± 603	1707 ± 556	1776 ± 534

No significant differences by analysis of variance (continuous data) or 3 × 2 contingency tables (discrete variables).

Table II. Comparison of selected measures of latency between patients grouped according to residual amniotic fluid volume

	Amniotic fluid volume			
	Adequate (N = 14)	*Diminished (N = 20)*	*Oligohydramnios (N = 36)*	*p*
Increase in gestational age (days, mean ± SD)	10.5 ± 8.4	6.3 ± 6.3	1.4 ± 1.2	<0.0001*
Tocolytic therapy—delivery >48 hr (No. and %)	10 (71.4)	14 (70.0)	7 (19.4)	<0.001†
Tocolytic therapy—delivery >1 wk (No. and %)	8 (57.1)	3 (15.0)	3 (8.3)	<0.001†

*Analysis of variance.

†3 × 2 contingency tables.

Table III. Comparison of selected measures of latency among patients with premature rupture of the membranes before 30 weeks' gestation grouped according to residual amniotic fluid volume

	Amniotic fluid volume			
	Adequate (N = 3)	*Diminished (N = 6)*	*Oligohydramnios (N = 9)*	*p*
Gestational age at diagnosis (wk, mean ± SD)	28.0 ± 0.7	26.9 ± 1.9	26.9 ± 1.3	NS
Membrane rupture to tocolysis <24 hr (No. and %)	2 (66.7)	3 (50.0)	6 (66.7)	NS
Increase in gestational age (days, mean ± SD)	14.0 ± 9.8	12.6 ± 9.8	2.8 ± 3.5	<0.05
Tocolytic therapy—delivery >48 hr (No. and %)	3 (100.0)	6 (100.0)	4 (44.4)	=0.061
Tocolytic therapy—delivery >1 wk (No. and %)	2 (66.7)	3 (50.0)	2 (22.2)	NS
Birth weight (gm, mean ± SD)	1488 ± 444	1061 ± 296	1072 ± 168	=0.072

Analysis of variance for continuous data; 3 × 2 contingency tables for discrete variables.

patients had a measurement >3 cm and were considered to have adequate fluid volume; 20 had diminished fluid (vertical diameter between 1 and 3 cm), and 36 had oligohydramnios (vertical diameter <1 cm). Selected obstetric variables were compared between cases grouped according to amniotic fluid volume (Table I).

As shown in Table II, patients with adequate amniotic fluid volume had a significantly longer interval between institution of tocolytic therapy and delivery. This resulted in a greater proportion of undelivered patients at 48 hours of latency, a difference that persisted beyond 1 week of expectant management. Tocolytic failure was highest in patients with oligohydramnios (n = 22; 61.1%). This finding could not be attributed to a difference in the incidence of intraamniotic infection.

The impact of amniotic fluid volume was also examined in the subset of pregnancies admitted before 30 weeks' gestation (Table III). The presence of adequate fluid volume was again associated with the longest latent period and a significant increase in the proportion of undelivered patients beyond 48 hours from membrane rupture. In this same comparison the net increase in gestational age was highest in the adequate and diminished fluid groups (14.0 and 12.6 days; respectively) when compared with the patients with oligohydramnios (2.8 days; $p < 0.05$).

Perinatal outcome was favorable in this series with only four neonatal deaths, all occurring in patients with decreased amniotic fluid. The majority of significant morbidity also occurred in patients with decrased fluid. Ten of 11 cases of respiratory distress syndrome and 4 of the 5 cases of intraventricular hemorrhage were observed in these patients. Neonatal sepsis (e.g., bacteremia, pneumonia, or both) was observed in 3 of 14 infants in the adequate fluid group compared with 2 of 20 and 5 of 36 neonates associated with diminished fluid or oligohydramnios, respectively. Major maternal complications related to tocolytic treatment were not observed, nor were tocolytics withdrawn because of patient intolerance.

Comment

The persistence of amniotic fluid after premature rupture of the membranes appears to have a favorable impact on the latent period among patients receiving parenteral tocolytic therapy. Even patients with diminished fluid volume (maximum vertical measurement between 1 and 3 cm) experienced a significant increase in latency when compared with patients with oligohydramnios. The extent that tocolytic therapy itself influenced outcome cannot be ascertained from these data. However, one observation regarding tocolytic efficacy can be made despite the limitations of this uncontrolled study. Of the 36 patients with oligohydramnios, only 7 had a latent period >2 days. That 80% of these patients were delivered within 48 hours underscores the relative futility of tocolytic use in the absence of residual amniotic fluid. However, one potential justification would be to delay delivery long enough to complete a course of maternal steroid therapy, a controversial approach in patients with ruptured membranes.[8-10]

Many theories have been proposed to explain why patients without fluid are delivered shortly after membrane rupture. For example, a larger opening in the amniotic membrane, associated with excessive leakage, may permit a significant inoculum of genital tract bacteria to gain access to the amniotic cavity early in a patient's course.[7] Alternatively, the antibacterial properties attributed to amniotic fluid may be diminished in patients with the least residual fluid volume, leading to prompt bacterial colonization, subclinical infection and labor.[11] Oligohydramnios might result instead from increased uterine tone, which could interfere with reaccumulation and retention of fluid after membrane rupture. Although this last theory might provide a physiologic justification for tocolytic use, it is unlikely that any tocolytic treatment currently available will modify the clinical course in patients with oligohydramnios.

In conclusion, if additional prospective studies of tocolytic efficacy in pregnacies complicated by premature rupture of the membranes are undertaken, inclusion of residual amniotic fluid volume as an independent variable before randomization should be considered. A disproportionate representation of patients with oligohydramnios in either the treatment or control group might inadvertently bias the results. Finally, in those patients with oligohydramnios, tocolytic therapy is of no measurable benefit and should probably be withheld.

REFERENCES

1. Levy DL, Warsof SL. Oral ritodrine and preterm premature rupture of membranes. Obstet Gynecol 1985; 66:621.
2. Christensen KK, Ingemarsson I, Leidman T, Solum H, Svenningsen N. Effect of ritodrine in labor after premature rupture of the membranes. Obstet Gynecol 1980;55:187.
3. Garite TJ, Keegan KA, Freeman RK, Nageotte MP. A randomized trial of ritodrine tocolysis versus expectant management in patients with premature rupture of membranes at 25 to 30 weeks of gestation. Am J Obstet Gynecol 1987;157:388.
4. Weiner CP, Renk K, Klugman M. The therapeutic efficacy and cost effectiveness of aggressive tocolysis for premature labor associated with premature rupture of the membranes. Am J Obstet Gynecol 1988;159:216.
5. Goldenberg RL, Nelson KG, Davis RO, Koski J. Delay in delivery: influence of gestational age and the duration of delay on perinatal outcome. Obstet Gynecol 1984;64:480.
6. Gonik B, Bottoms SP, Cotton DB. Amniotic fluid volume as a risk factor in preterm premature rupture of the membranes. Obstet Gynecol 1985;65:456.
7. Vintzeleos AM, Campbell WA, Nochimson DJ, Weinbaum PJ. Degree of oligohydramnios and pregnancy outcome in patients with premature rupture of the membranes. Obstet Gynecol 1985;66:162.
8. Garite TJ, Freeman RK, Dorchester WL. Prospective randomized study of corticosteroids in the management of premature rupture of the membranes and the preterm gestation. Am J Obstet Gynecol 1981;141:508.
9. Simpson GF, Harbert GM. Use of betamethasone in management of premature gestation with premature rupture of the membranes. Obstet Gynecol 1985;66:168.
10. Morales WJ, Diebel D, Lazar AJ, Zadrozny D. The effect of antenatal dexamethasone administration on the prevention of respiratory distress syndrome in preterm gestations with premature rupture of the membranes. Am J Obstet Gynecol 1986;154:591.
11. Galask RP, Snyder IR. Antimicrobial factors in amniotic fluid. Am J Obstet Gynecol 1970;106:59.

Cardiovascular and metabolic effects associated with nifedipine and ritodrine tocolysis

James E. Ferguson II, MD, Donald C. Dyson, MD,[a] R. Harold Holbrook, Jr, MD, Thomas Schutz, BA, and David K. Stevenson, MD[b]

Santa Clara and Stanford, California

Recent investigations have indicated that nifedipine, a calcium channel entry blocker, may be useful in the treatment of preterm labor. This prospective, randomized study compares cardiovascular and metabolic effects measured in association with sublingual and oral administration of nifedipine with those noted with the intravenous and oral administration of the β-adrenergic agent ritodrine. Serial measurements of cardiovascular parameters, hematocrit, electrolytes, glucose, blood urea nitrogen, creatinine, calcium, and serum glutamic-oxaloacetic and glutamic-pyruvic transaminase were compared between groups. Sublingual and oral nifedipine caused minimal cardiovascular alterations. At doses sufficient to achieve tocolysis, ritodrine caused more pronounced cardiovascular changes than nifedipine. Both agents had a hemodilutional effect, but nifedipine was not associated with alterations in serum electrolytes or a dramatic hyperglycemia. On the basis of this study, it appears that the use of nifedipine for preterm labor management is associated with hemodilutional changes but not the adverse cardiovascular or metabolic effects often associated with ritodrine tocolysis. (AM J OBSTET GYNECOL 1989;161:788-95.)

Key words: Nifedipine, ritodrine, premature labor, tocolysis

Currently a variety of pharmacologic agents are used to treat preterm labor, but only the β-adrenergic agent ritodrine has a Food and Drug Administration–labeled indication for such therapy.[1] The use of β-adrenergic agents, however, can be associated with undesirable cardiovascular and metabolic side effects.[1,2] These potential side effects prompted evaluation of an alternate tocolytic agent.

Because of their relaxing effects on the myometrium, calcium channel entry blockers show promise. Preliminary studies with nifedipine have suggested that it can inhibit labor while producing few apparent side effects.[3-5] However, knowledge of possible cardiovascular or metabolic effects associated with the sublingual and oral routes of administration of nifedipine in the pregnant patient is limited. We conducted this study to evaluate cardiovascular and metabolic responses to nifedipine, and to compare them with changes noted with the β-adrenergic agent ritodrine.

Material and methods

Between July 1984 and June 1986, patients with preterm labor occurring between 24 and 36 completed weeks of gestation were eligible for enrollment into the study. Informed consent as approved by the Stanford University Committee for Protection of Human Subjects in Research and the Kaiser Permanente Human Subject's Committee was obtained from each patient. Patients with the following maternal factors were excluded: tocolytic exposure during the current pregnancy, diabetes, hyperthyroidism, cardiac disease, severe preeclampsia-eclampsia, abruptio placentae, chorioamnionitis, multiple gestation, hydramnios, and cervical dilatation >4 cm. Fetal distress, severe intrauterine growth retardation, dead fetus, or fetal anomaly incompatible with life constituted fetal factors for exclusion.

Preterm labor was defined as regular uterine contractions with a documented change in cervical dilatation or effacement before 36 completed weeks' gestation. Once preterm labor was diagnosed and written consent had been obtained, the assignment group was determined by opening a sequentially numbered sealed envelope, randomized by the use of a random number table, with the patients stratified according to cervical dilatation (≤2 cm or >2 but ≤4 cm) and whether the patient had intact or ruptured membranes. An intravenous infusion of 5% dextrose in lactated Ringer's solution at 100 ml/hour was administered to all patients after an initial 200 ml fluid bolus. An electrocardiogram was obtained from each patient before and at 12 hours of treatment. Fetal heart rate and uterine activity were monitored continuously during the study. Cervical cultures for group B β-hemolytic streptococcus and *Neisseria gonorrhoeae* were obtained. The use of amniocentesis and steroid administration followed our standard

From the Department of Obstetrics and Gynecology,[a] Kaiser Permanente Hospital, and the Departments of Gynecology and Obstetrics and Pediatrics,[b] Stanford University School of Medicine.

Supported in part by a Mellon Foundation Fellowship Award and National Intitutes of Health grants RR-81 and HD-14426.

Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.

Reprint requests: J. E. Ferguson II, MD, Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of Virginia School of Medicine Charlottesville, VA 22908.

6/6/13712

practice as previously described.[6] Tocolysis was attempted in each group of patients for 48 hours in patients with ruptured membranes and through 36 completed weeks of gestation in patients whose membranes were intact.

Nifedipine tocolysis was initiated with a 10 mg capsule given sublingually. Patients were instructed to bite the capsule between their molars and, if possible, to avoid swallowing to facilitate sublingual absorption. If uterine contractions persisted after 20 minutes, a similar dose was repeated at intervals of 20 minutes, up to a maximal total dose of 40 mg during the first hour of treatment. If sublingual tocolysis stopped the uterine activity, then oral therapy with 20 mg of nifedipine was initiated 6 hours after the last sublingual capsule. This dose was repeated at 4 to 6-hour intervals, or if clinically indicated to continue tocolysis, occasionally at more frequent intervals. During treatment in the ritodrine group the infusion rate was begun at 50 μg/min and increased by 50 μg/min every 15 to 30 minutes in response to continued uterine activity until uterine contractions were inhibited, 350 μg/min was reached, or unacceptable side effects developed. Thereafter the infusion rate was decreased by 50 μg/min and was then maintained at that rate for 12 hours. After 12 hours the infusion rate was decreased by 50 μg/min every 2 hours until 100 μg/min was reached. If uterine contractions recurred, the sequence was repeated. Oral therapy (10 to 20 mg every 4 to 6 hours) was started 30 minutes before discontinuation of intravenous ritodrine. Tocolysis was considered to have been achieved when the treating physician determined that uterine contractions were stopped. At that time no further sublingual nifedipine therapy was given or the infusion rate of ritodrine was decreased to the maintenance rate.

In both study groups, fluid intake was limited to 2400 ml/24 hr, and volume of total intake and urine output was recorded. Patients were allowed no oral intake except when sips of water were permitted to swallow oral medications. Blood urea nitrogen, creatinine, calcium, serum glutamic-oxaloacetic and glutamic-pyruvic transaminase levels were measured before therapy and after 24 hours of treatment. Hematocrit, glucose, sodium, potassium, chloride, and bicarbonate levels were measured before therapy and after 8, 16, and 24 hours of treatment. The anion gap was determined for these intervals from the following formula: (sodium + potassium) − (chloride + bicarbonate). A baseline hemogram also was obtained for all patients.

Patients were positioned in left lateral recumbency for cardiovascular measurements. The superior arm was used and Korotkoff phase 4 documented the diastolic pressure. Mean arterial pressure was calculated with the standard formula. The pulse rate was determined by counting the pulse for 1 full minute. Patients in each group had a minimum of two pretreatment blood pressure and pulse rate determinations. In the patients who received nifedipine, systolic and diastolic blood pressures and pulse rate were measured 5, 10, and 20 minutes after each sublingual dose, then hourly until the first oral dose. During oral administration these parameters were measured before successive doses and at 5, 10, and 20 minutes, and 2, 4, and 6 hours after each dose during the first day. In the patients who received ritodrine, similar parameters were determined at the time of and 10 minutes, 20 minutes, 2 hours, and 4 hours after each change in the intravenous infusion rate and every hour once a stable infusion rate was determined.

Statistical analyses were performed with the use of an unpaired t test for comparison between the two treatment groups at specific time points (baseline and interval values), paired t test and assessment of difference patterns for comparison of serial interval values to baseline values within a treatment group, and χ^2 analysis with Yates correction for continuity. Additionally, a two-way analysis of variance with comparison of least-square means to identify specific differences was used to evaluate the effects of time and treatment, and the interaction of time and treatment. Linear regression was used to determine if there was any correlation between cumulative nifedipine dose over 24 hours on a milligram per kilogram basis and changes from baseline in the 24-hour metabolic parameters.

Results

Twenty-seven patients were randomly assigned to the nifedipine group and 26 to the ritodrine group. Cardiovascular data were not available for analysis from one of the nifedipine patients, and metabolic data were unavailable from one patient in the ritodrine group. During oral therapy, only patients who received 20 mg doses of nifedipine at 6-hour intervals in the initial 24 hours are reported. To evaluate possible differences between the two treatment groups at the same clinical end point (i.e., after uterine contractions had stopped), we compared cardiovascular effects at similar time intervals in both groups. Comparisons were made between cardiovascular values recorded at 20 minutes and between averaged values recorded from 2 to 4 hours after the last sublingual dose or the intravenous infusion rate necessary to stop uterine contractions in patients in the nifedipine and ritodrine groups, respectively.

Various factors that could have affected the study outcome are shown in Table I and indicate that the groups are comparable.

Pretreatment values and the cardiovascular responses to sublingual nifedipine administration for the 26 patients in whom sublingual tocolysis was evaluated are shown in Fig. 1, *A*. Twenty patients required four sublingual doses, four required three doses, and two patients required only two sublingual doses. When compared with pretreatment values, the systolic blood

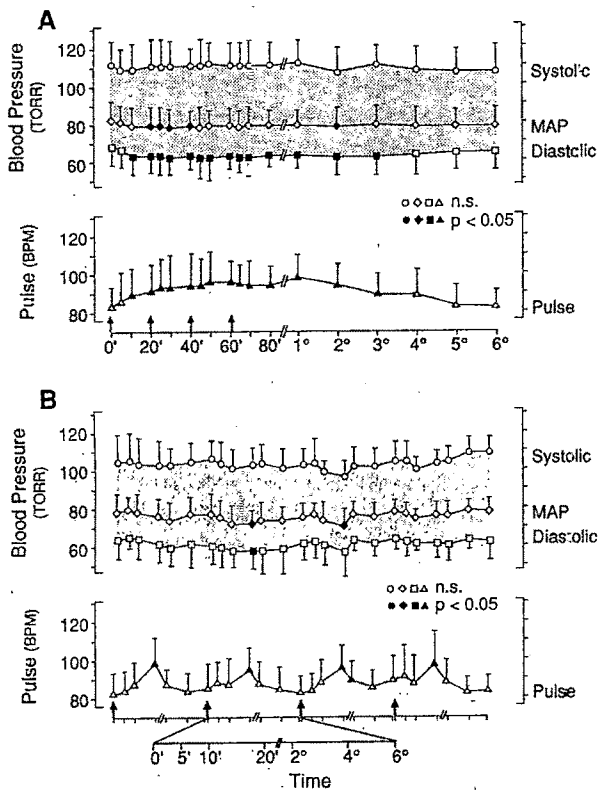


Fig. 1. A, Effects of sublingual nifedipine on systolic and diastolic blood pressure, mean arterial pressure *(MAP)*, and pulse rate. *Vertical arrows* indicate timing of administration of 10 mg of nifedipine. *Solid symbols* represent $p < 0.05$, compared with baseline values. **B,** Effects of oral nifedipine on systolic and diastolic blood pressure, mean arterial pressure *(MAP)*, and pulse rate. *Vertical arrows* indicate timing of administration of 20 mg of nifedipine. *Solid symbols* represent $p < 0.05$ compared with baseline values. Values expressed as mean ± SD.

pressure statistically did not change during sublingual dosing or during the 6-hour inteval after the last sublingual dose. However, diastolic blood pressure was decreased by 4 torr 10 minutes after the initial dose ($p = 0.018$). Additional doses caused little further decrease in diastolic blood pressure when compared with diastolic values recorded just before subsequent sublingual doses (i.e., at 20, 40, or 60 minutes after the initiation of treatment). The lowest mean diastolic pressure was 62.2 torr. The diastolic blood pressure remained decreased through 3 hours after the last sublingual dose ($p = 0.044$) and then returned toward pretreatment values. Changes in the mean arterial pressure occurred during the interval when diastolic blood pressure was decreased; however, because systolic blood pressure did not change significantly, the decreases in diastolic blood pressure were not always associated with statistically significant decreases in mean arterial pressure. The pulse rate increased 10 minutes after the initial dose ($p = 0.002$) when compared with baseline values and remained elevated through 3 hours after the last sublingual dose. When compared with pulse values recorded just before subsequent sublingual doses, only the increased value recorded 20 minutes after the third sublingual dose was statistically greater ($p = 0.018$). The maximal mean pulse rate was 98 beats/min and occurred 1 hour after the last sublingual dose.

Table I. Maternal factors of study patients randomly selected to receive nifedipine or ritodrine tocolysis

Classification	*Nifedipine*	*Ritodrine*
Age (yr)*	26.6 ± 5.2	25.9 ± 5.5
Gestational age (wk)*	30.7 ± 3.0	30.8 ± 2.3
White blood cell count*	11.1 ± 3.8	12.7 ± 3.8
Parity		
0	14/27	16/26
>0	13/27	10/26
Cervical dilatation (cm)		
≤2	22/27	20/26
>2, ≤4	5/27	6/26
Premature rupture of membranes	6/27	6/26
Betamethasone use	23/27	23/26

*Mean ± SD; none of these was significantly different by Student's t test or χ^2 analysis, where appropriate.

The cardiovascular responses of patients who received 20 mg of nifedipine orally at exactly 6-hour intervals are shown in Fig. 1, *B*. Values at 5, 10, and 20 minutes, and 2, 4, and 6 hours after each oral dose are compared with those measured just before each dose. Systolic blood pressure did not change significantly during oral therapy. Whereas diastolic blood pressure did decrease somewhat after each oral dose, when compared with predose values, only the decrease at 20 minutes after the second oral dose was statistically significant ($p = 0.03$). The mean arterial pressure demonstrated a similar pattern of change. After each oral dose, the pulse rate increased when compared with values measured before each dose. Statistically significant increases in pulse rate were consistently noted 20 minutes after each oral dose. Maximal pulse increases and diastolic blood pressure and mean arterial pressure decreases were similar in magnitude to those measured during sublingual administration.

Fig. 2 shows the cardiovascular response to stepwise increases with equal doses in the infusion rate of ritodrine in the 26 patients who were evaluated. The cardiovascular parameters depicted were obtained 15 to 30 minutes after the particular infusion rate was initiated. There was no significant change in systolic blood pressure, but diastolic and consequently mean arterial pressures decreased. Pulse rate increased significantly.

These changes were progressive until the 200 μg/min infusion rate was reached. Thereafter they changed little, but remained significantly different from baseline values.

In Table II, cardiovascular parameters measured in patients in both the nifedipine and ritodrine groups at the specified intervals after tocolysis was achieved are compared to baseline values and to each other. In the nifedipine patients, when interval values were compared with baseline values, systolic and mean arterial blood pressure did not change statistically, whereas the decreased diastolic blood pressure and the increased pulse rate were statistically significant at each interval. In the ritodrine patients in whom tocolysis was achieved, the labor-inhibiting rate was 221 ± 74 μg/min(mean ± SD), and the mean maintenance rate was 165 ± 53 μg/min. Systolic blood pressure did not change statistically when values from the ritodrine patients at each interval were compared to baseline; however, the decreased diastolic blood pressure and mean arterial pressure and the increased pulse rate were statistically significant at both intervals. When the nifedipine and ritodrine groups were compared, baseline values did not differ statistically for any of the cardiovascular parameters, nor did systolic blood pressure values differ when compared at either interval. Comparison between groups revealed that diastolic blood pressure and mean arterial pressure were statistically lower and the pulse rate statistically higher in the ritodrine group at both intervals after tocolysis.

Values for metabolic parameters that were obtained at baseline and after 24 hours of treatment are shown in Table III. In patients who received nifedipine, there was a decrease in blood urea nitrogen and serum glutamic-pyruvic transaminase values at 24 hours when compared to baseline ($p = 0.0042$ and $p = 0.004$, respectively). Although the absolute differences between baseline and 24-hour values were minimal, the decreased calcium values in both the nifedipine and ritodrine groups were statistically significant ($p = 0.0004$ and $p = 0.0058$, respectively). When values from patients in the two treatment groups were compared at baseline and 24 hours of treatment, no significant differences existed.

Glucose values after 8, 16, and 24 hours of treatment were significantly increased in both groups when compared with their respective baseline values (Fig. 3, *A*). Values in the ritodrine group also were statistically greater than values in the nifedipine group at 8 and 16 hours of therapy. Decreases in hematocrit were noted in both groups over time; the comparisons of interval to baseline values in each treatment group are shown in Fig. 3, *B*. Only the values at 8 hours of treatment differed significantly when interval values were compared between groups.

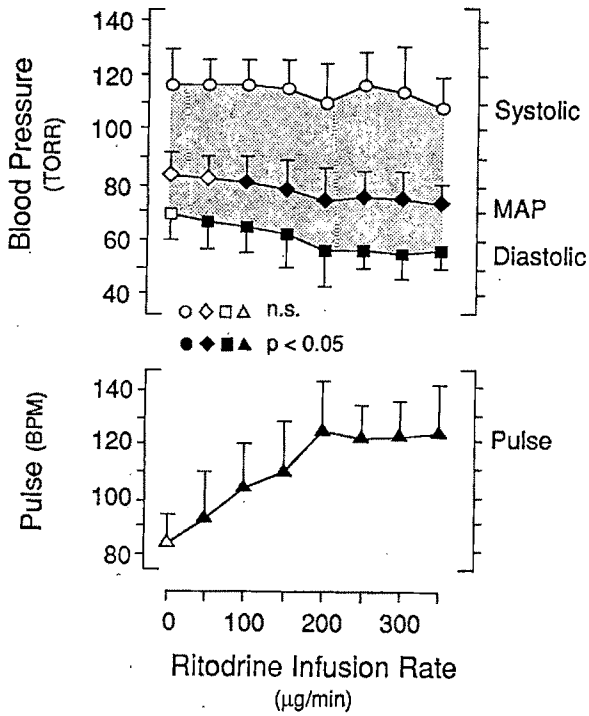


Fig. 2. Systolic and diastolic blood pressure, mean arterial pressure *(MAP)*, and pulse rate measured after stepwise increases with equal doses in the infusion rate of ritodrine. *Solid symbols* represent $p < 0.05$, compared with baseline values. Values expressed as mean ± SD.

Serum electrolyte values are tabulated in Table IV. Sodium values did not change with time in either group. During therapy, potassium values remained unchanged in the nifedipine group, but fell significantly in the ritodrine group. When comparisons were made between groups at the 8-, 16-, and 24-hour intervals of therapy, ritodrine values were lower ($p = 0.0001$, $p = 0.0001$, and $p = 0.0001$, respectively). Chloride values were unchanged over time in the nifedipine group, but increased significantly in the ritodrine group at 8 and 16 hours of treatment. Only the values at 24 hours of therapy differed between groups ($p = 0.004$). As shown in Table IV, interval values for bicarbonate did not differ from baseline in the nifedipine group; however, they decreased in the ritodrine group. When comparisons were made at the 8-, 16-, and 24-hour intervals of therapy between groups, the lower values in the ritodrine patients were only significant at the 8- and 16-hour comparisons ($p = 0.005$, $p = 0.001$, respectively). The anion gap did not change significantly over time in either group, but the anion gap was greater in the ritodrine group at the 8-hour determination when compared with the nifedipine group ($p = 0.0001$) and at the 16-hour comparison ($p = 0.025$).

Table II. Comparison of cardiovascular parameters at 20 minutes and 2 to 4 hours after the last sublingual dose or intravenous infusion rate necessary to achieve tocolysis

*Value**	*Group*	*Baseline (pretreatment)*	*20 min*	*2-4 hr*
Systolic pressure (torr)	Nif	111.3 ± 12.6 (n = 21)	111.1 ± 11.2 (n = 21)	110.6 ± 11.5 (n = 21)
	Rit	112.0 ± 8.7 (n = 23)	113.8 ± 14.6 (n = 23)	109.6 ± 10.7 (n = 23)
Diastolic pressure	Nif	68.5 ± 8.7 (n = 21)	64.5 ± 8.3 (n = 21)†,‡	63.5 ± 9.4 (n = 21)†,‡
(torr)	Rit	68.3 ± 7.4 (n = 23)	53.3 ± 11.9 (n = 23)†	51.8 ± 8.0 (n = 23)†
Mean arterial pressure	Nif	82.8 ± 9.3 (n = 21)	80.0 ± 8.3 (n = 21)‡	79.2 ± 9.5 (n = 21)‡
(torr)	Rit	82.9 ± 7.1 (n = 23)	73.5 ± 11.0 (n = 23)†	71.0 ± 7.6 (n = 23)†
Pulse (beats/min)	Nif	83.2 ± 10.5 (n = 21)	95.2 ± 13.2 (n = 21)†,‡	90.1 ± 9.3 (n = 21)†,‡
	Rit	85.4 ± 9.0 (n = 23)	122.1 ± 16.7 (n = 23)†	123.2 ± 13.1 (n = 23)†

Nif., Nifedipine; *Rit.*, ritodrine.

*Values are mean ± SD; number of subjects in parentheses.

†$p < 0.05$ (compared with baseline value).

‡$p < 0.05$ (comparison between values in nifedipine and ritodrine patients at specified interval).

Table III. Comparison of maternal baseline and 24-hour values for blood urea nitrogen, creatinine, calcium, and serum glutamic-oxaloacetic and glutamic-pyruvic transaminase

*Value**	*Group*	*Baseline (pretreatment)*	*24 hr*
Blood urea nitrogen	Nif	7.1 ± 2.1 (n = 23)	4.7 ± 1.4 (n = 14)†
(mg/100 ml)	Rit	7.5 ± 2.8 (n = 24)	6.6 ± 2.9 (n = 11)
Creatinine (mg/100 ml)	Nif	0.63 ± 0.13 (n = 25)	0.61 ± 0.14 (n = 14)
	Rit	0.68 ± 0.17 (n = 23)	0.64 ± 0.15 (n = 11)
Calcium (mg/100 ml)	Nif	8.9 ± 0.4 (n = 25)	8.4 ± 0.4 (n = 14)†
	Rit	8.8 ± 0.4 (n = 23)	8.4 ± 0.4 (n = 10)†
Serum glutamic-oxaloacetic	Nif	20.3 ± 9.5 (n = 26)	14.6 ± 10.9 (n = 14)
transaminase (IU)	Rit	18.0 ± 8.9 (n = 24)	17.8 ± 10.0 (n = 11)
Serum glutamic-pyruvic	Nif	18.0 ± 9.0 (n = 26)	10.7 ± 4.7 (n = 14)†
transaminase (IU)	Rit	15.4 ± 7.2 (n = 23)	12.9 ± 5.8 (n = 11)

Nif., Nifedipine; *Rit.*, ritodrine.

*Values are mean ± SD; number of subjects in parentheses.

†$p < 0.05$ (compared with baseline value).

Table IV. Comparison of maternal baseline values with interval values for serum levels of sodium, potassium, chloride, and bicarbonate and anion gap

*Value**	*Group*	*Baseline (pretreatment)*	*Testing interval*		
			8 hr	*16 hr*	*24 hr*
Sodium	Nif	137.0 ± 2.5 (n = 24)	136.1 ± 1.6 (n = 22)†	136.2 ± 2.2 (n = 18)	137.4 ± 1.7 (n = 20)
(mEq/L)	Rit	137.5 ± 2.4 (n = 22)	137.9 ± 2.4 (n = 20)	137.5 ± 2.4 (n = 18)	138.4 ± 2.2 (n = 17)
Potassium	Nif	3.8 ± 0.3 (n = 26)	3.9 ± 0.3 (n = 22)†	3.8 ± 0.3 (n = 21)†	3.8 ± 0.3 (n = 21)†
(mEq/L)	Rit	3.9 ± 0.2 (n = 25)	3.2 ± 0.3 (n = 22)‡	3.4 ± 0.4 (n = 21)‡	3.3 ± 0.4 (n = 21)‡
Chloride	Nif	108.9 ± 2.8 (n = 24)	109.7 ± 2.3 (n = 22)	109.0 ± 2.9 (n = 18)	109.3 ± 2.8 (n = 20)†
(mEq/L)	Rit	107.6 ± 3.5 (n = 22)	109.1 ± 2.3 (n = 20)	110.4 ± 2.1 (n = 18)‡	111.9 ± 3.0 (n = 17)‡
Bicarbonate	Nif	20.5 ± 2.7 (n = 24)	20.3 ± 2.4 (n = 22)†	20.9 ± 2.2 (n = 18)†	19.9 ± 2.2 (n = 20)
(mEq/L)	Rit	21.3 ± 2.6 (n = 22)	17.9 ± 3.3 (n = 20)‡	18.0 ± 2.7 (n = 18)‡	18.5 ± 3.5 (n = 17)‡
Anion gap	Nif	11.5 ± 3.9 (n = 24)	10.1 ± 2.1 (n = 22)†	10.1 ± 2.0 (n = 21)†	12.0 ± 2.4 (n = 20)
(mEq/L)	Rit	12.6 ± 3.5 (n = 22)	14.0 ± 3.8 (n = 20)	12.5 ± 3.3 (n = 18)	11.2 ± 4.1 (n = 17)

Nif., Nifedipine; *Rit.*, ritodrine.

*Values are mean ± SD; number of subjects in parentheses.

†$p < 0.05$ comparison between values in the nifedipine and ritodrine patients at the specified interval.

‡$p < 0.05$ compared with baseline value.

The 24-hour values for blood urea nitrogen, creatinine, serum glutamic-oxaloacetic and glutamic-pyruvic transaminase, hematocrit, glucose, sodium, potassium, chloride, bicarbonate, and anion gap were compared with their respective baseline values and compared with the cumulative nifedipine dosage over 24 hours expressed on a milligram per kilogram basis. There were no significant correlations.

Comment

Nifedipine has been used with increasing frequency for the treatment of preterm labor, but the possibility that sublingual and oral nifedipine may cause cardiovascular or metabolic alterations had not been intensively investigated. Neither nifedipine nor ritodrine caused a significant change in systolic blood pressure during treatment. However, dramatically different results were noted between the two agents when diastolic and mean arterial pressure changes were evaluated (Figs. 1, *A* and *B*, and 2). Although statistically significant declines in diastolic blood pressure occurred after sublingual nifedipine administration, values at the time of maximum mean change still represented 92% of control and are unlikely to be of physiologic significance. During oral nifedipine administration, diastolic pressure also fell, but the decrease was significant in only one instance; the value represented 89% of control. Diastolic blood pressure decreases during ritodrine administration were more dramatic, and the magnitude and timing of the decreases approximated values previously reported when β-adrenergic agents were used.[7, 8] The maximum mean decrease (16 torr) occurred after the 300 μg/min infusion rate; the diastolic blood pressure at that time was only 77% of control. In addition, the pattern of change of diastolic blood pressure was different after nifedipine and ritodrine tocolysis. During nifedipine therapy, the diastolic pressure decreased initially but then remained relatively stable during additional doses. Conversely, increasing infusion rates of ritodrine were associated with progressive decreases in diastolic and mean arterial pressure until the 200 μg/min infusion rate was reached, at which time they tended to plateau. Increased pulse rates were noted in both patient groups. The magnitude of change was markedly different, however, probably reflecting the differences in diastolic and mean arterial pressure changes.

The exaggerated response to ritodrine and minimal alterations with nifedipine treatment are apparent (Table II). Similar differences between tocolytic treatment groups have previously been reported when insignificant cardiovascular changes were found after magnesium sulfate administration, compared with dramatic differences found during ritodrine infusion.[7] Since magnesium sulfate is functionally a calcium channel entry blocker, the similar significant differences between treatment groups we report in cardiovascular responses should perhaps have been expected.

When sublingual and oral nifedipine therapy are compared, it is interesting to note that pulse increases occurred 10 minutes after the first sublingual dose, but only 20 minutes after successive oral doses. In addition, although few significant changes in diastolic or mean arterial pressure occurred during oral therapy, when they did occur they were not noted before 20 minutes. These findings are similar to those noted by Raemsch and Sommer[9] and suggest a more rapid absorption and distribution of nifedipine after sublingual administration. Our findings support the contention of McAllister[10] that biting the capsule facilitates absorption of the liquid drug and lessens the time until the pharmacodynamic effects are observed when compared with oral administration of an intact capsule with the time delay before the capsule dissolves in the stomach and the drug is absorbed.

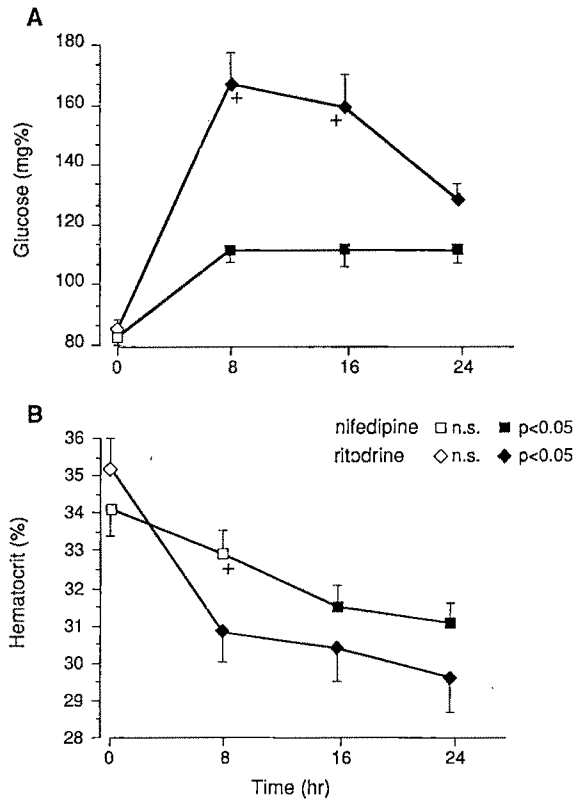


Fig. 3. Glucose values (**A**) and hematocrit values (**B**) (mean ± SEM) before and during nifedipine or ritodrine tocolysis. *Solid symbols* represent $p < 0.05$ compared with baseline values. *Cross*, $p < 0.05$ (comparison between values in nifedipine and ritodrine patients at specified interval).

This report provides the initial documentation of the cardiovascular changes of increased pulse rate and decreased diastolic pressure after the sublingual administration of nifedipine to treat preterm labor. Similar cardiovascular changes were noted with oral administration. The changes after oral administration are consistent with those reported by Ulmsten,[3] who used 30 mg as the initial dose, but discrepant from results of Read and Wellby,[4] who, using the same dosage schedule, did not note significant changes in the pulse rate or diastolic blood pressure. This discrepancy may be related to differences in the timing of measurement of the cardiovascular parameters.

Our study also contrasts the effects on metabolic parameters produced by the tocolytic agents nifedipine and ritodrine. It is well recognized that nifedipine ad-

ministration in nonpregnant patients causes vasodilatation and a resultant increase in plasma and extracellular volume.[11] The fall in hematocrit, calcium, and blood urea nitrogen levels recorded in patients in this study is consistent with the recognized vasodilatation and increased plasma volume. Ritodrine also affects fluid balance. Hemodilutional decreases in hematocrit and colloid osmotic pressure regularly accompany the clinical use of β-adrenergic tocolytic agents.[1, 2] In this study ritodrine was noted to cause a fall in calcium and a decrease in hematocrit during therapy. The decrease in hematocrit exceeded that noted with nifedipine at 8 hours of therapy.

We were especially interested in potassium values after nifedipine administration because hypokalemia has been documented previously in nonpregnant patients receiving nifedipine.[12] In the patients studied in the nifedipine group, potassium levels remained unchanged, as did the remainder of the electrolytes studied and the anion gap. Conversely, the ritodrine patients exhibited the same changes in electrolyte concentrations that have been previously documented and discussed.[2, 13, 14] Therefore differences between treatment groups in these parameters, when present, were the result of alterations of values in the ritodrine patients. Because of the lack of change of the bicarbonate and anion gap in the nifedipine group of patients it thus seems likely that this form of tocolysis is not associated with the increases in serum lactate and pyruvate that are commonly seen and responsible for the increase anion gap in patients receiving ritodrine.[13, 14]

There were marked differences in the magnitude of response demonstrated between groups for serum glucose concentrations. Patients in the ritodrine group exhibited the classic hyperglycemic response, with maximum values at 8 hours after initiation of infusion, with subsequent return toward baseline. Nifedipine patients demonstrated a modest degree of hyperglycemia. It was important to evaluate nifedipine's effect on glucose levels, because although most studies have documented that calcium channel entry blockers do not alter glucose homeostasis to a clinically relevant degree, some studies, (especially those with glucose loading and nifedipine doses >30 mg/day) have shown increased blood glucose and decreased insulin levels.[15] The mechanism is thought to involve blockade by nifedipine of a voltage-dependent "late" calcium channel of the pancreatic β-cell, which normally allows the further ingress of calcium and subsequent release of insulin.[15] Because all patients received a continuous intravenous infusion of a 5% glucose solution, we cannot discern if the increased glucose levels in the nifedipine patients were the direct result of nifedipine administration through the mechanism outlined above, a result of glucose-containing intravenous fluids, or both. The hyperglycemia noted with ritodrine was certainly much more dramatic when compared with that found with nifedipine administration, suggesting that nifedipine may be preferable if glucose homeostasis is a concern.

We have provided the initial documentation of cardiovascular responses after the sublingual use of nifedipine for tocolysis and evaluated possible metabolic changes. On the basis of this study, it appears that the use of nifedipine for preterm labor tocolysis is associated with hemodilutional changes but not the untoward and unwanted cadiovascular or metabolic effects reported with ritodrine tocolysis. Since animal studies with nifedipine have resulted in discrepant findings related to uteroplacental perfusion,[16, 17] a concern that presently limits the clinical use of calcium channel–blocking drugs is their possible effect on uteroplacental blood flow in the human. Carefully controlled human investigations that evaluate fetal response to nifedipine tocolysis are necessary before nifedipine is considered for routine clinical use in human pregnancies.

REFERENCES

1. Benedetti TJ. Maternal complications of parenteral beta-sympathomimetic therapy for premature labor. AM J OBSTET GYNECOL 1983;145:1-6.
2. Ferguson II JE, Holbrook RH Jr, Stevenson DK, Hensleigh PA, Kredentser D. Adjunctive magnesium sulfate does not alter metabolic changes associated with ritodrine tocolysis. AM J OBSTET GYNECOL 1987;156:103-7.
3. Ulmsten U. Treatment of normotensive and hypertensive patients with preterm labor using oral nifedipine, a calcium antagonist. Arch Gynecol 1984;236:69-72.
4. Read MD, Wellby DE. The use of a calcium antagonist (nifedipine) to suppress preterm labour. Br J Obstet Gynaecol 1986;93:933-937.
5. Kaul AF, Osathanondh R, Safon LE, Frigoletto FD, Friedman PA. The management of preterm labor with the calcium channel-blocking agent nifedipine combined with the beta-mimetic terbutaline. Drug Intell Clin Pharm 1985;19:369-71.
6. Ferguson II JE, Hensleigh PA, Gill P. Effects of betamethasone on white blood cells in patients with premature rupture of the membranes and preterm labor. AM J OBSTET GYNECOL 1984;150:439-41.
7. Thiagarajah S, Harbert GM, Bourgeois FJ. Magnesium sulfate and ritodrine hydrochloride: systemic and uterine hemodynamic effects. AM J OBSTET GYNECOL 1985; 153:666-74.
8. Wager J, Fredholm B, Lunell N-O, Persson B. Metabolic and circulatory effects of intravenous and oral salbutamol in late pregnancy in diabetic and nondiabetic women. Acta Obstet Gynecol Scand [Suppl] 1982;108;41-6.
9. Raemsch KD, Sommer J. Pharmacokinetics and metabolism of nifedipine. Hypertension 1983;5(suppl II): II-18–II-24.
10. McAllister RG Jr. Kinetics and dynamics of nifedipine after oral and sublingual doses. Am J Med 1986;81(suppl 6A):2-5.
11. Kleinbloesem CH. General introduction and aim of the investigations. In: Nifedipine: clinical pharmacokinetics and haemodynamic effects. Den-Haag, Netherlands: JH Pasman's, 1985:13.
12. Lederballe Pedersen O, Mikkelsen E, Christensen NJ, Kornerup HJ, Pedersen EB. Effects of nifedipine on plasma renin, aldosterone and catecholamines in arterial hypertension. Eur J Clin Pharmacol 1979;15:235-40.

13. Richards SR, Change FE, Stempel LE. Hyperlactacidemia associated with acute ritodrine infusion. AM J OBSTET GYNECOL 1983;146:1-5.
14. Kirkpatrick C, Quenon M, Desir D. Blood anions and electrolytes during ritodrine infusion in preterm labor. AM J OBSTET GYNECOL 1980;138:523-7.
15. Trost BN, Weidmann P. Effects of nitrendipine and other calcium antagonists on glucose metabolism in man. J Cardiovasc Pharmacol 1984;6:986-95.
16. Veille JC, Bissonnette JM, Hohimer AR. The effect of a calcium channel blocker (nifedipine) on uterine blood flow in the pregnant goat. AM J OBSTET GYNECOL 1986;154:1160-3.
17. Harake B, Gilbert RD, Ashwal S, Power GG. Nifedipine: effects on fetal and maternal hemodynamics in pregnant sheep. AM J OBSTET GYNECOL 1987;157:1003-8.

Prolactin levels in umbilical cord blood of human infants: Relation to gestational age, maternal complications, and neonatal lung function

C. Richard Parker, Jr., PhD,[a] Paul C. MacDonald, MD,[b] David S. Guzick, MD,[b] John C. Porter, PhD,[b] Charles R. Rosenfeld, MD,[b] and John C. Hauth, MD[a]
Birmingham, Alabama, and Dallas, Texas

The ontogeny of serum prolactin and its relation to several variables, especially lung function, in 543 neonates was studied. Umbilical cord serum prolactin levels rose between 24 and 42 weeks' gestation, correlating significantly ($p < 0.001$) with gestational age ($r = 0.44$) and birth weight ($r = 0.32$). Among infants of similar ages, however, there was no variation in serum prolactin level as a function of birth weight, sex, Apgar scores, or delivery method. Infants of women with pregnancy-induced hypertension had higher than normal prolactin levels; infants of diabetic women had normal prolactin levels. At 31.5 to 37 weeks' gestation, infants who developed respiratory distress syndrome had lower serum prolactin levels than those whose lung function was normal or else was abnormal from causes other than respiratory distress syndrome. The risk for respiratory distress syndrome was higher in newborns whose prolactin level was low (10th percentile) than in infants whose prolactin level was high (90th percentile). These results are suggestive that prolactin may play a role in fetal lung maturation. (AM J OBSTET GYNECOL 1989;161:795-802.)

Key words: Prolactin, respiratory distress syndrome (RDS), hyaline membrane disease, human fetal lung, pregnancy

The role of prolactin in lactation and the factors that serve to regulate pituitary prolactin production in adult humans are reasonably well characterized. On the other hand, the determinants of fetal pituitary prolactin production and the role circulating prolactin serves in the developing human are less well established. Two tissues in the fetus that may be regulated by prolactin are the lung and adrenal cortex. Serum levels of prolactin and the weight of the adrenals increase in concert during fetal development,[1] before the rise in the lecithin/sphingomyelin ratio in amniotic fluid.[2] Moreover, an augmentation of surfactant lipid synthesis in lung tissue by prolactin has been reported by some, but not all, investigators (see reference 3 for review). It also has been proposed that prolactin may, along with adrenocorticotropin, participate in regulation of adrenal steroid production in human adults[4] and fetuses.[5] If prolactin does, indeed, stimulate steroid production in the fetal adrenals, then prolactin also could play an indirect role in fetal lung maturation by means of inducing adrenal secretion of glucocorticoids and/or androgens that

From the Department of Obstetrics and Gynecology, University of Alabama at Birmingham,[a] and the Green Center for Reproductive Biology Sciences and Departments of Obstetrics and Gynecology and Pediatrics, University of Texas Southwestern Medical School.[b]
These studies were supported by grants HD22969 and HD13912 from the National Institutes of Health.
Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.
Reprint requests: C. Richard Parker, Jr., PhD, Department of Obstetrics and Gynecology, University of Alabama at Birmingham, UAB Station, Birmingham, AL 35294.
6/6/14095

are aromatized to estrogens in the placenta; glucocorticoids and estrogens have been shown, in turn, to stimulate surfactant lipid formation in fetal lung tissue.[6,7]

Several investigators[8-11] have provided evidence in favor of prolactin serving a role in fetal lung maturation by virtue of the observation that serum prolactin levels at birth were lower in infants who developed respiratory distress syndrome (RDS) than in infants of similar ages who did not develop RDS. More recently, however, it has been reported that umbilical cord serum levels of prolactin were not lower in newborns who developed RDS than in newborns who did not develop respiratory distress.[12] Therefore, we have continued to address the relation between prolactin in the developing fetus and several outcome variables and now provide our findings in 543 newborn infants, 361 of whom were delivered between 24 and 37 weeks of gestation.

Material and methods

Mixed umbilical arterial and venous cord blood was collected at the time of delivery over a 10-year period from newborns selected on the basis of gestational age and the presence or absence of maternal pregnancy complications. Since exogenous glucocorticosteroids can apparently affect fetal lung maturation and the fetal endocrine milieu, we excluded any newborns that had been exposed to synthetic glucocorticosteroids in utero. After clot formation, the serum was removed and stored frozen at −20° C. Serum prolactin was quantified by radioimmunoassay procedures described previously[8] that used reagents provided by the National Institute of Arthritis, Metabolism, and Digestive Diseases. The reference preparation was standardized against Research Standard A for human prolactin, 71/222, provided by the Division of Biological Standards, National Institute for Medical Research, London. Several assays were conducted to analyze all of the specimens, with each assay containing samples that were unselected with respect to any maternal or newborn parameter. The interassay coefficient of variation for the prolactin assay was 13%.

Of the 543 newborns included in this study, 228 were delivered of women whose pregnancy was considered to be uncomplicated, with the exception of premature delivery or prolonged rupture of the membranes in some; the remaining infants were delivered of women whose pregnancy was complicated by pregnancy-induced hypertension ($n = 144$), chronic hypertension ($n = 22$), diabetes ($n = 43$), or an assortment of other complications ($n = 106$). One hundred twenty-one of the newborns were the products of multiple gestations. Gestational ages of the 543 newborns ranged from 24 to 42 weeks since the onset of the last menstrual period and were determined by use of multiple clinical obstetric and fetal ultrasonographic criteria. Clinical obstetric criteria included a combination of the onset of the last normal menses, an early estimation of uterine size, fetal heart tones detected with a fetoscope at 18 to 20 weeks' gestation, quickening at 16 to 20 weeks' gestation, and concordance of uterine fundal height measurements between 20 and 30 weeks' gestation. Lung function of the newborns was determined by attending neonatologists who had no knowledge of the outcome of serum prolactin analyses. Lung function was normal in 370 of the infants whereas RDS was diagnosed in 90 infants and transient tachypnea or other respiratory abnormalities were diagnosed in 55 infants. The criteria used for establishing the diagnosis of RDS included the presence of grunting respirations and retractions, an increased oxygen requirement ($Fio_2 > 0.4$), a chest radiograph and laboratory findings consistent with a diagnosis of RDS, and exclusion of other causes of respiratory distress, especially pneumonia and/or sepsis.

Data pertaining to indices of lung function were inconclusive in 28 infants; serum prolactin levels of these infants were therefore excluded from analyses concerning lung function. By means of retrospective chart review, we also compiled data concerning Apgar scores (at 1 and 5 minutes), sex, birth weight, and delivery method (vaginal or cesarean section) on most (≥88% for each category above) of the newborns. The relationships between serum prolactin and various newborn outcome variables, maternal complications, and gestational age were evaluated by means of several statistical methods, including analysis of variance, multiple range t test, Student's t test, linear regression analysis, and logistic regression analysis. The protocol was reviewed and approved by the Institutional Review Board of the University of Texas Health Science Center at Dallas.

Results

Ontogeny of prolactin in newborn serum. When individual umbilical cord serum levels of prolactin were analyzed as a function of gestational age at delivery, a highly significant positive correlation was noted ($r = 0.44$, $p < 0.001$). A highly significant positive correlation was also observed between umbilical cord serum levels of prolactin and birth weight ($r = 0.32$, $p < 0.001$). When serum prolactin levels were subdivided according to gestational age groups (Table I), we found a highly significant variation as a function of gestational age ($F = 29.5$, $p < 0.001$). Serum levels of prolactin were significantly lower in infants whose gestational age was ≤28 weeks than in older newborns. Levels were significantly higher in infants having a gestational age that exceeded 34 weeks than in those delivered between 24 and 34 weeks of gestation. Whereas there was a significant correlation between birth weight

Table I. Developmental changes in umbilical cord serum levels of prolactin

	Gestational age group				
	24-28 wk	*28.5-31 wk*	*31.5-34 wk*	*34.5-37 wk*	*37.5-42 wk*
Prolactin (ng/ml)	93 ± 11[a]	195 ± 24[b]	215 ± 13[b]	267 ± 10[c]	313 ± 10[d]
Birth weight (gm)	837 ± 35	1326 ± 38	1782 ± 34	2370 ± 41	3011 ± 51
n	37	43	117	164	182

Data are presented as the mean ± SE. Prolactin levels having different superscripts are significantly different ($p < 0.05$).

Table II. Relation of umbilical cord serum levels of prolactin to maternal health

	Gestational age group				
	24-28 wk	*28.5-31 wk*	*31.5-34 wk*	*34.5-37 wk*	*37.5-42 wk*
Uncomplicated pregnancy	92 ± 1 ($n = 19$)	158 ± 19 ($n = 26$)	188 ± 15 ($n = 58$)	247 ± 17 ($n = 63$)	279 ± 15 ($n = 62$)
Pregnancy-induced hypertension	108 ± 14 ($n = 3$)	364 ± 89* ($n = 8$)	241 ± 28* ($n = 33$)	291 ± 18* ($n = 56$)	343 ± 20† ($n = 44$)
Chronic hypertension	—	—	289 ± 37 ($n = 3$)	317 ± 91 ($n = 5$)	345 ± 33 ($n = 14$)
Diabetes	—	132 ± 71 ($n = 2$)	205 ± 83 ($n = 2$)	241 ± 25 ($n = 19$)	288 ± 20 ($n = 20$)

Data are presented as the mean ± SE. The number of infants in each group is indicated in parentheses.
*$p < 0.05$ compared with value for uncomplicated pregnancy.
†$p < 0.01$ compared with value for uncomplicated pregnancy.

and serum prolactin levels for the entire group, the relation between these two variables among individuals within the same gestational age group was not significant.

Relation of umbilical cord serum levels of prolactin to maternal pregnancy complications. Umbilical cord serum levels of prolactin in newborns of women having no pregnancy complications are compared with those in infants of women with hypertension (chronic or pregnancy-induced) or diabetes in Table II. Within each gestational age group, cord prolactin levels from newborns of women with pregnancy-induced hypertension exceeded those from infants of women with normal pregnancies; these differences were statistically significant at all gestational periods except for infants delivered before 28.5 weeks of gestation. Serum levels of prolactin from newborns of women with chronic hypertension tended to be higher than those in infants delivered of women with a normal pregnancy; because of the small number of infants of women with chronic hypertension, however, the differences were not significant. Serum prolactin levels from infants of women with diabetes were similar to those from infants delivered of women with uncomplicated pregnancies.

Relation of umbilical cord serum levels of prolactin to intrauterine growth, Apgar scores, newborn sex, and delivery method. As shown in Table III, small-for-gestational-age infants had serum prolactin levels that were similar to those in appropriately grown infants. Also prolactin levels in newborns having Apgar scores <7 at 1 or 5 minutes were similar to those in infants with Apgar scores ≥7. Within each gestational age group, male and female infants had similar levels of prolactin in the umbilical cord serum. With the exception of infants delivered at or before 28 weeks of gestation, serum prolactin levels also were similar in those delivered vaginally and in those delivered by cesarean section.

Relation of umbilical cord serum levels of prolactin to newborn lung function. As shown in Fig. 1, umbilical cord serum levels of prolactin tended to be lower in infants who subsequently developed RDS than in newborns who did not develop RDS. The deficiency in serum prolactin levels among infants that developed respiratory problems was specific to RDS since newborns who had other causes of respiratory distress, such as transient tachypnea, were not significantly different from those in whom lung function was normal. Umbilical cord serum levels of prolactin were significantly lower in the RDS group than in newborns who did not develop RDS between 31.5 and 37 weeks of gestation.

During the intervals of 31.5 to 34 and 34.5 to 37 weeks of gestation, there were no significant differences in birth weights of the newborns as a function of their respiratory status (Table IV). On the other hand, the mean gestational age of newborns who developed RDS was slightly lower than that of infants whose lung function was normal (Table IV). Nevertheless, the deficiency in serum prolactin levels among the newborns who developed RDS was not attributable to the fact that

Table III. Umbilical cord serum levels of prolactin as a function of Apgar scores, newborn sex, and delivery method

	Gestational age group				
	24-28 wk	*28.5-31 wk*	*31.5-34 wk*	*34.5-37 wk*	*37.5-42 wk*
Growth					
AGA	97 ± 12 (n = 30)	191 ± 23 (n = 39)	213 ± 14 (n = 98)	261 ± 11 (n = 123)	322 ± 12 (n = 121)
SGA	83 (n = 1)	237 ± 143 (n = 4)	218 ± 28 (n = 20)	290 ± 21 (n = 42)	309 ± 22 (n = 54)
1 min Apgar score					
≥7	109 ± 27 (n = 4)	170 ± 29 (n = 14)	255 ± 18 (n = 63)	276 ± 11 (n = 118)	313 ± 10 (n = 150)
<7	101 ± 14 (n = 23)	214 ± 38 (n = 23)	221 ± 28 (n = 27)	312 ± 28 (n = 26)	315 ± 27 (n = 27)
5 min Apgar score					
≥7	96 ± 20 (n = 7)	206 ± 30 (n = 26)	253 ± 16 (n = 81)	280 ± 10 (n = 137)	316 ± 10 (n = 169)
<7	105 ± 16 (n = 20)	178 ± 51 (n = 11)	166 ± 28 (n = 9)	323 ± 82 (n = 7)	259 ± 33 (n = 8)
Sex					
Male	109 ± 19 (n = 16)	159 ± 18 (n = 18)	240 ± 20 (n = 51)	268 ± 13 (n = 82)	318 ± 14 (n = 84)
Female	79 ± 15 (n = 14)	238 ± 43 (n = 21)	232 ± 22 (n = 45)	287 ± 16 (n = 65)	311 ± 13 (n = 96)
Delivery method					
Vaginal	80 ± 8 (n = 32)	165 ± 20 (n = 27)	217 ± 16 (n = 83)	277 ± 13 (n = 104)	313 ± 13 (n = 113)
Cesarean section	143 ± 42* (n = 4)	246 ± 53 (n = 16)	212 ± 22 (n = 33)	250 ± 15 (n = 60)	312 ± 14 (n = 68)

Data are presented as the mean ± SE. The number of infants in each group is indicated in parentheses. *AGA*, Appropriate for gestational age (≥10th percentile); *SGA*, small for gestational age (<10th percentile according to Brenner et al.[13]).

*Serum prolactin levels are higher in infants delivered by cesarean section than in those delivered vaginally at 24 to 28 weeks ($p < 0.05$). All other comparisons at each gestational period are not significantly different ($p > 0.05$).

their gestational age was somewhat less than that of the other two groups of newborns. This obtains because the weekly rate of increase in serum prolactin levels for all newborns during the gestational period of study was computed to be 13.1 ng/ml, a value much lower than the mean difference between serum prolactin levels in the RDS group and levels in either the normal group or the abnormal, non-RDS group (Fig. 1). At these two gestational periods, the proportion of small-for-gestational-age infants was not greater in the normal lung function group than in the RDS group (Table IV).

Since it has been suggested that maternal pregnancy-induced hypertension and/or impaired intrauterine growth might be somewhat protective against the development of RDS, we sought to determine whether such might be the case in our cohort of infants. During the interval of 31.5 to 34 weeks, the proportion of newborns of normal women having RDS was 30.7% (23 of 75), and that of newborns of women with pregnancy-induced hypertension was 29.4% (10 of 34). During the interval of 34.5 to 37 weeks, again the proportion of newborns having RDS in both groups was similar: normal women, 5.1% (4 of 79); women with pregnancy-induced hypertension, 4.2% (2 of 48). On the other hand, newborns of women having pregnancy-induced hypertension were more prone to be small-for-gestational-age [31.5 to 34 weeks, 44.1% (15 of 34); 34.5 to 37 weeks, 35.4% (17 of 48)] than were those of women having uncomplicated pregnancies [31.5 to 34 weeks, 6.7% (5 of 75); 34.5 to 37 weeks, 24.1% (19 of 79)]. Among small-for-gestational-age infants, the proportion of those having RDS was similar among newborns delivered of women having normal pregnancies (6 of 24) and those delivered of women with pregnancy-induced hypertension (5 of 32).

On the basis of our observation of an obvious tendency of premature newborns who developed RDS to have the lowest serum levels of prolactin, we subjected the data for prolactin and lung function to logistic regression analysis. As shown in Fig. 2, we found that the estimated probability of RDS developing in a newborn having a prolactin level at the 10th percentile for our population at any given gestational age was greater than that for an infant whose prolactic level is equal to the 50th percentile.

On the other hand, the probability of developing

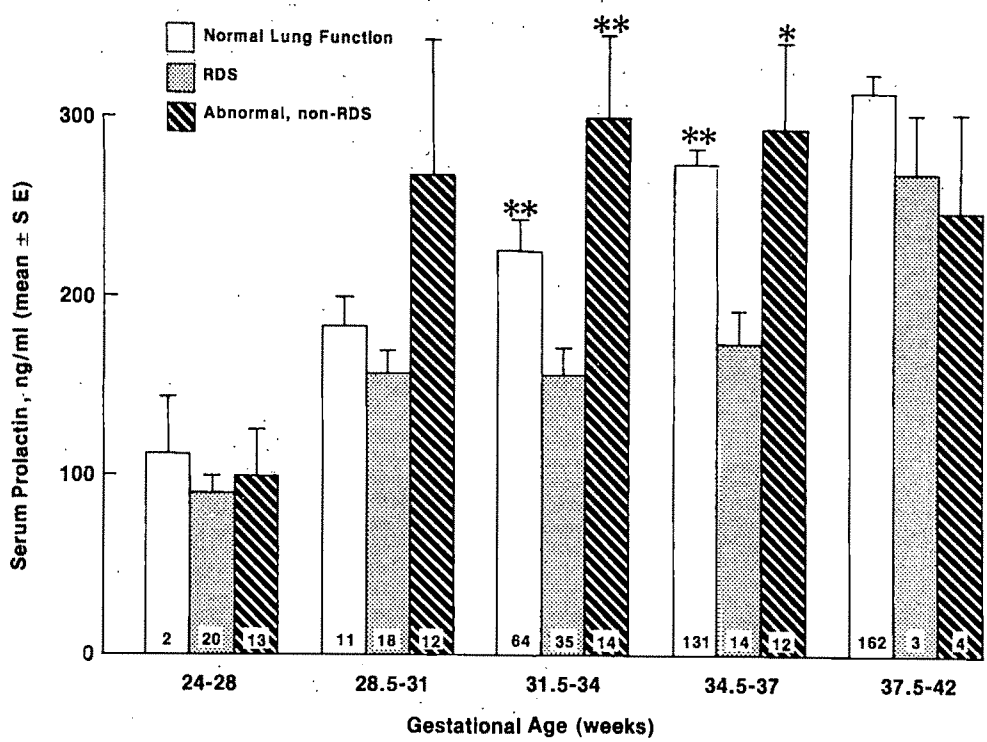


Fig. 1. Relationship between umbilical cord serum levels of prolactin and lung function in the neonate. Serum prolactin levels were subdivided according to gestational age and to lung function. Number of infants in each group is indicated at bottom of each bar. The data are presented as the mean ± SE. *Double asterisk,* $p < 0.01$ compared with RDS group; *single asterisk,* $p < 0.05$ compared with RDS group.

Table IV. Relation of birth weight and gestational age to neonatal lung function in newborns delivered at 31.5 to 37 weeks of gestation

	Newborn lung function		
Gestational age group	*Normal*	*RDS*	*Abnormal, non-RDS*
31.5-34 wk			
Gestational age (wk)	33.3 ± 0.73	32.5 ± 0.77*	32.7 ± 0.67
Birth weight (gm)	1854 ± 388	1663 ± 319	1755 ± 315
SGA (%)	14	20	13
34.5-37 wk			
Gestational age (wk)	36.0 ± 0.81	35.6 ± 0.64	35.9 ± 0.71
Birth weight (gm)	2358 ± 488	2512 ± 735	2160 ± 650
SGA (%)	20	28	39

Data are presented as the mean ± SD. *SGA,* Small for gestational age (<10th percentile according to Brenner et al.[13]).
*$p < 0.05$ compared with value for newborns with normal lung function.

respiratory distress syndrome is lower in a newborn having a high prolactin level (90th percentile) than in an infant whose prolactin level is equal to the 50th percentile. For example, at 30.5 weeks of gestation, the probability of RDS developing in an infant whose prolactin level is at the 10th percentile was computed to be 79%, that of an infant having a prolactin level at the 50th percentile was 66%, and that of an infant having a prolactin level at the 90th percentile was only 30.5%. At 34 weeks of gestation, the estimated probabilities of developing RDS at the 10th, 50th, and 90th percentiles for prolactin are 37%, 20%, and 6%, respectively.

Comment

In this study we found that the concentrations of prolactin in umbilical cord serum rose approximately threefold during the last trimester of gestation. The qualitative and quantitative aspects of these data

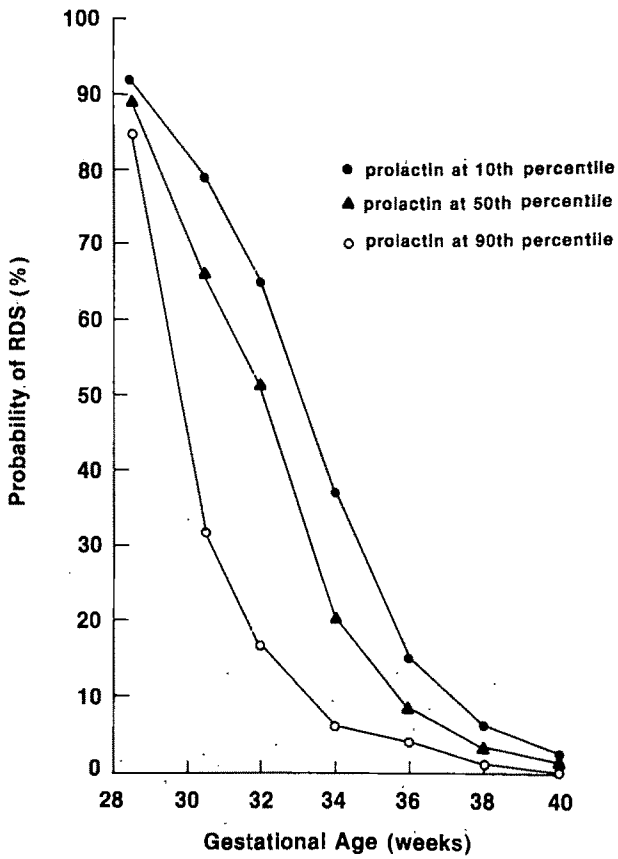


Fig. 2. Relationship between serum prolactin levels at birth and probability of developing RDS. Data for individual serum prolactin levels and for presence or absence of RDS were subjected to logistic regression analysis. Estimated probability of an infant developing RDS is plotted as a function of gestational age for newborns whose umbilical cord serum level of prolactin is equal to the 10th, 50th, and 90th percentile for the population that we studied.

are in general agreement with those reported previously.[1,3,8-12] Although there was a significant correlation throughout the entire gestational period of study between umbilical cord serum levels of prolactin and birth weight, we found no correlation between these variables among infants of similar gestational ages. Thus serum prolactin levels appear to correspond generally to developmental stage but are not appreciably influenced by the factor(s) that contribute to the extent of fetal growth at any given period of gestation. Also, whereas serum prolactin levels are believed to be influenced by acute stress,[14] we found no relation between umbilical cord serum prolactin levels and Apgar scores of the newborn infants. On the other hand, we (reference 15 and the present study) and others[11] have noted that serum prolactin levels in newborns of women with pregnancy-induced hypertension were significantly increased over those of newborns of women with normal pregnancy. A trend toward relative hyperprolactinemia also was noted in newborns of women with chronic hypertension. Such findings are suggestive that chronic intrauterine stress associated with various forms of maternal hypertension may lead to increased fetal prolactin production. In contrast to the recent report of Saltzman et al.,[16] however, we found no inhibitory effect of maternal diabetes on newborn prolactin levels either at or before term. The reasons for these discrepant findings are unclear at present but may be related to possible differences in the diabetic classes represented in the two studies.

In agreement with the results of our prior study[8] and those of several other investigators,[9-11] we find that newborns who subsequently develop RDS have a relative deficiency in serum prolactin levels at birth when compared with infants of similar ages who have normal lung function. The differences among these two groups of newborns are most apparent during the interval of 31.5 to 37 weeks of gestation. Because of the large number of premature infants in our study, we were able to perform logistic regression analysis on the relationship between umbilical cord serum levels of prolactin and lung function. On the basis of the results of these analyses, it seems reasonably clear that during the latter third of pregnancy the risk that RDS will develop is considerably less in a newborn who has a high serum prolactin level than in a newborn of similar age who has a low serum prolactin level.

Our current data also are suggestive that the prolactin deficit is specific to the occurrence of RDS; newborns who developed other types of respiratory problems had prolactin levels that were similar to those from newborns with normal lung function, but were significantly higher, at least between 31.5 and 37 weeks of gestation, than those from newborns who developed RDS. We are cognizant of the difficulty in differentiating between mild RDS and other respiratory abnormalities. Therefore, if some of the infants diagnosed with respiratory distress from causes other than RDS (who also tended to have normal or even elevated prolactin levels) actually did have RDS, then the deficiency in prolactin levels associated with RDS might be less apparent. On the other hand, if the RDS group contained infants who had respiratory distress from non-RDS causes, then the prolactin levels associated with true RDS might be even lower than we observed. It is likely that any errors in diagnosis would tend to be balanced in such a manner that our data are representative of meaningful physiologic differences between infants who develop RDS and those who develop other causes of respiratory distress. Among our selected group of newborns, those who were small-for-gestational-age were not found to be protected against RDS; the incidence of small-for-gestational-age was somewhat higher in those with RDS than among infants having normal lung function. Also, among this selected population, the incidence of RDS in newborns of women with pregnancy-

induced hypertension was not lower than that in similar-aged infants of normal women.

In addition to the relationship between low serum prolactin levels in the newborn and the development of RDS that has been demonstrated in this study, as well as others,[8-11] there also are reports that are suggestive of stimulatory effects of prolactin on the fetal lung, particularly in the production of surfactant lipids (see reference 3). Some investigators, however, have failed to detect any effect of prolactin on the developing lung (see reference 3). Other hormones also appear to participate in the induction of fetal lung maturation.[6, 7] Moreover, since the effects of prolactin on human fetal lung tissue have been evident in vitro only in the presence of other hormones (see reference 3), it seems likely that fetal lung maturation is subject to multifactorial regulation.

We believe that the human fetal adrenals play a role in the timing of the onset of surfactant production and fetal lung maturation. The human fetal adrenals produce glucocorticosteroids and large quantities of dehydroepiandrosterone sulfate,[17] which serves as precursor to placentally formed estrogens. Prolactin production in the pituitary of adult humans is stimulated by estrogens.[18] Thus at least three substances that have been suggested to participate in fetal lung maturation, i.e., glucocorticosteroids, estrogens, and prolactin, are produced directly or indirectly as a result of fetal adrenal activation. During the latter third of gestation, the human fetal lungs are exposed to ever-increasing quantities of estrogens, particularly estriol,[19] glucocorticosteroids,[20] and prolactin.[1, 3, 8-12] Coordinated production of all of these hormones and other factors, such as thyroid hormones,[6] may be required for the orderly maturation of the fetal respiratory apparatus. Disruption in the timely production of all or perhaps only some such substances may lead to delays in the maturation of the fetal lungs. We have found subnormal lecithin/sphingomyelin ratios in amniotic fluid of infants with hypoplastic adrenals caused by anencephaly[21]; Naeye et al.[22] have found morphologic evidence for lung immaturity in such infants. Moreover, deficiencies of estriol[19, 23] and cortisol[20] have been observed in infants who subsequently develop RDS. On the other hand, we have found that high umbilical cord serum levels of estriol (>90th percentile) are correlated with a low incidence of RDS in premature infants.[19]

On the basis of the findings of this study and the above considerations, we propose that prolactin, in addition to other factors, may play a role in lung maturation in the developing human fetus. We further suggest that pharmacologic induction of lung maturation in utero might be more successful if the agent(s) administered also possibly lead to increases in serum levels of prolactin and other hormones, such as estrogens (rather than reduced estrogen formation that occurs after glucocorticosteroid administration[24]).

We thank Kim Rivera and Jodie Roberts for excellent technical assistance and Barbara Quinnelly for editorial help.

REFERENCES

1. Winters AJ, Colston C, MacDonald PC, Porter JC. Fetal plasma prolactin levels. J Clin Endocrinol Metab 1975;41:626.
2. Gluck L, Kulovich MV. Lecithin/sphingomyelin ratios in amniotic fluid of normal and abnormal pregnancy. Am J Obstet Gynecol 1973;115:539.
3. Quirk JG, MacDonald PC, Johnston JM. Role of fetal pituitary prolactin in fetal lung maturation. Semin Perinatol 1982;6:238.
4. Schiebinger RJ, Chrousos GP, Cutler GB Jr, Loriaux DL. The effect of serum prolactin on plasma adrenal androgens and the production and metabolic clearance rate of dehydroepiandrosterone sulfate in normal and hyperprolactinemic subjects. J Clin Endocrinol Metab 1986; 62:202.
5. Brown TJ, Glinz B, Milne CM, Oakey RE. Stimulation by polypeptides of dehydroepiandrosterone sulfate synthesis in human foetal adrenal slices. J Endocrinol 1981;9:111.
6. Hitchcock KR. Lung development and the pulmonary surfactant system: Hormonal influences. Anat Rec 1980; 198:3.
7. Khosla SS, Gobran LI, Rooney SA. Stimulation of phosphatidylcholine synthesis by 17β-estradiol in fetal rabbit lung. Biochem Biophys Acta 1980;617:282.
8. Hauth JC, Parker CR, MacDonald PC, Porter JC, Johnston JM. A role of fetal prolactin in lung maturation. Obstet Gynecol 1978;51:81.
9. Gluckman PD, Ballard PL, Kaplan SL, Liggins GC, Grumbach MM. Prolactin in umbilical cord blood and the respiratory distress syndrome. J Pediatr 1978;93:1011.
10. Smith YF, Mullon DK, Hamosh M, Scanlon JW, Hamosh P. Serum prolactin and respiratory distress syndrome in the newborn. Pediatr Res 1979;14:93.
11. Grosso DS, MacDonald CP, Thomasson JE, Christian CD. Relationship of newborn serum prolactin levels to the respiratory distress syndrome and maternal hypertension. Am J Obstet Gynecol 1980;137:569.
12. Ho Yuen B, Phillips WDP, Cannon W, Sy L, Redford D, Burch P. Prolactin, estradiol, and thyroid hormones in umbilical cord blood of neonates with and without hyaline membrane disease: a study of 405 neonates from midpregnancy to term. Am J Obstet Gynecol 1982;142: 698.
13. Brenner WE, Edelman DA, Hendricks CH. A standard of fetal growth for the United States of America. Am J Obstet Gynecol 1986;126:555.
14. Noel GL, Suh HK, Stone G, Frantz AG. Human prolactin and growth hormone release during surgery and other conditions of stress. J Clin Endocrinol Metab 1972;35:840.
15. Parker CR Jr, Hankins GDV, Carr BR, Gant NF, MacDonald PC, Porter JC. Prolactin levels in umbilical cord serum and its relation to fetal adrenal activity in newborns of women with pregnancy-induced hypertension. Pediatr Res 1986;20:876.
16. Saltzman DH, Barbieri RL, Frigoletto FD Jr. Decreased fetal cord prolactin concentration in diabetic pregnancies. Am J Obstet Gynecol 1986;154:1035.
17. Parker CR Jr, Leveno K, Carr BR, Hauth J, MacDonald PC. Umbilical cord plasma levels of dehydroepiandrosterone sulfate during human gestation. J Clin Endocrinol Metab 1982;54:1216.
18. Franks S. Regulation of prolactin secretion by oestrogens: physiological and pathological significance. Clin Sci 1983; 65:457.

19. Parker CR Jr, Hankins GDV, Guzick DS, Rosenfeld CR, MacDonald PC. Ontogeny of unconjugated estriol in fetal blood and the relation of estriol levels at birth to the development of respiratory distress syndrome. Pediatr Res 1987;21:386.
20. Murphy BEP. Cortisol and cortisone levels in the cord blood at delivery of infants with and without the respiratory distress syndrome. Am J Obstet Gynecol 1974; 119:1112.
21. Parker CR Jr, Leveno KJ, Milewich L, MacDonald PC. Lecithin/sphingomyelin ratios in amniotic fluid of pregnancies with an anencephalic fetus. Obstet Gynecol 1986;64:546.
22. Naeye RL, Harcke HT Jr, Blanc WA. Adrenal gland structure and the development of hyaline membrane disease. Pediatrics 1971;45:650.
23. Conly PW, Lemaire WJ, Monkus EF, Cleveland WW. Plasma estriol concentration in infants with the respiratory distress syndrome. J Pediatr 1973;83:851.
24. Simmer HH, Tulchinsky D, Gold EM, Frankland M, Greipel M, Gold AS. On the regulation of estrogen production by cortisol and ACTH in human pregnancy at term. Am J Obstet Gynecol 1974;119:283.

Amniotic fluid leukotaxis assay as an early indicator of chorioamnionitis

Glenn A. Pankuch, BS, Peter H. Cherouny, MD, John J. Botti, MD, and Peter C. Appelbaum, MD, PhD
Hershey, Pennsylvania

A characteristic feature of histologic chorioamnionitis caused by ascending infection is the amniotropism displayed by polymorphonuclear leukocytes in the placental membranes. We tested the hypothesis that amniotic fluid from patients with histologic chorioamnionitis and intact membranes without clinical infection contains leukoattractants detectable by an in vitro leukotactic assay. Leukoattractants were detected in 13 cases (87%) and absent in 2 cases (13%) with histologic chorioamnionitis ($p < 0.005$). The two cases with histologic chorioamnionitis that had negative leukotaxis were classified as stage I/mild severity. A positive leukotactic response was a better predictor of histologic chorioamnionitis (87%) than positive Gram stain (33%), amniotic fluid culture (53%), gas-liquid chromatography (40%), or a combination of these three methods (60%). These results suggest that demonstration of leukoattractants in amniotic fluid is an earlier and more sensitive predictor of chorioamnionitis than is presently available. (Am J Obstet Gynecol 1989;161:802-7.)

Key words: Amniotic fluid, leukoattractant, histologic chorioamnionitis

Preterm birth is the major cause of perinatal morbidity and mortality. A predisposing cause cannot be found in most cases, thus making prevention difficult. Abundant research suggests that subclinical intrauterine infection is the cause of preterm labor.[1] However, clear understanding of the role of infection is hampered by traditional diagnostic tests, which are often insensitive and nonspecific.[2]

From the Departments of Pathology and Obstetrics and Gynecology, The Milton S. Hershey Medical Center, The Pennsylvania State University.
Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.
Reprint requests: Glenn A. Pankuch, BS, Department of Pathology, The Milton S. Hershey Medical Center, P.O.Box 850, Hershey, PA 17033.
6/6/14097

Histologic examination of the placenta for chorioamnionitis is often used to exclude intrauterine exposure of the fetus to infection. Unfortunately, this information is only available after delivery. Histologic chorioamnionitis is an inflammatory lesion of the placental and extraplacental fetal membranes that is frequently found after antenatal death and preterm birth.[3,4] The cause of chorioamnionitis has long been considered to be infectious. This view has not been universally accepted because correlation with clinical infection is poor and investigators have often failed to recover bacteria from the majority of inflamed placentas.[5] However, more recent studies using techniques that optimally culture for aerobes, anaerobes, chlamydia, and genital mycoplasmas have been able to demonstrate microorganisms by culture or serology in most cases of histologic chorioamnionitis.[5-7]

A characteristic feature of histologic chorioamnionitis is the amniotropism displayed by polymorphonuclear leukocytes in the extraplacental membranes, placenta, and umbilical cord. This suggests that diffusible leukotactic factors are released into the amniotic fluid or at membrane surfaces as part of the inflammatory response.[4] Although it is true that the placental inflammation that characterizes chorioamnionitis is nonspecific for a particular microorganism, in the majority of cases it is caused by intrauterine exposure to infectious agents in general. Therefore, an assay system designed to detect a broad range of leukoattractants in amniotic fluid should reflect chorioamnionitis and, indirectly, infection. To develop this system we have investigated use of a leukotactic assay as modified from Nelson et al.[8] The assay is based on the migration of polymorphonuclear leukocytes beneath an agarose gel in a random or directed manner in response to a leukotactic gradient, and is sensitive to a wide range and concentration of leukotactic factors.[9]

The purpose of this study is to evaluate the ability of the leukotaxis assay to predict histologic chorioamnionitis in asymptomatic patients with intact membranes. The assay is also compared with traditional microbiologic tests for infection.

Material and methods

Patients. Thirty-two amniotic fluid specimens were collected from 32 patients by transabdominal amniocentesis or needle amniotomy at cesarean section. Specimens were obtained after informed consent from patients who were delivered at The Milton S. Hershey Medical Center of The Pennsylvania State University. The study protocol was approved by the university committee on clinical investigation.

All patients had intact membranes and were without evidence of clinical infection[10] when fluid was obtained. Each patient was delivered within 48 hours of amniocentesis (mean ± SD) (gestational age, interval between fluid collection and delivery 32 ± 4 weeks' gestation and 8 ± 11 hours, respectively). Excluded from study were all cases of multiple gestation, polyhydramnios, and patients receiving antibiotics or immunosuppressants within 3 weeks of admission. Preterm labor was defined as four or more uterine contractions in 20 minutes associated with cervical change in patients with gestations of <37 weeks' gestation.

Initial processing and culture of amniotic fluid. Amniotic fluid was processed within 1 hour of collection. It was anticipated that some specimens would arrive at night. When delays of >1 hour but <12 hours occurred, a portion of the sample was reserved for bacterial culture and Gram stain by inoculating a Port-A-Cul vial (BBL Microbiology Systems, Cockeysville, Md.). Vials were kept at room temperature until cultured. Specimens stored ≥12 hours were not used. The remaining fluid was then refrigerated (4° C) until it could be processed as described below for gas-liquid chromatography (GLC), leukotaxis assay, and *Mycoplasma* culture.

After Gram staining,[11] each amniotic fluid was cultured as described below for aerobic and anaerobic bacteria. After culture, 1 ml of fluid was removed for GLC (details described below). The remaining fluid was then centrifuged for 10 minutes at 1000 *g*. Supernatants were removed and frozen (−70° C) for the leukotaxis assay. The resultant pellet was then examined for polymorphonuclear leukocytes and red blood cells after Wright's staining and cultured for *Mycoplasma hominis* and *Ureaplasma urealyticum*. For genital mycoplasma culture, 10 μl of the pellet was inoculated into urease color test broth U9C. Three tenfold serial dilutions were then prepared in U9C broth (incubated aerobically at 37° C for 21 days).[12] Ten microliters of the pellet and 10 μl from each serial broth dilution were spread onto A8 differential agar medium (incubated anaerobically in a glove box) (Coy Laboratory Products, Ann Arbor, Mich.) at 37° C for 21 days. Blind passage of negative broth cultures was performed on days 3 and 7 of incubation. Both U9C broth and A8 agar[12] were prepared from quality-controlled components.

Aerobic and anaerobic bacteria were cultured by spreading 0.1, 0.01, and 0.001 ml aliquots of uncentrifuged amniotic fluid in duplicate on the following media: trypticase soy agar with 5% sheep blood (BBL Microbiology Systems) (incubated aerobically at 37° C for 72 hours); chocolate agar (BBL Microbiology Systems) (incubated in 5% to 10% carbon dioxide at 37° C for 72 hours); prereduced chopped meat glucose broth (Scott Laboratories, Fiskeville, R.I.); enriched brucella blood and laked blood agars[11] (incubated anaerobically in a Coy glove box at 37° C for 21 days) (Coy Laboratory Products). The number of polymorphonuclear leukocytes, red blood cells, and bacteria in stained smears was examined by trained technologists and recorded as follows: few = <1 cell per oil immersion field; + = 1 cell per oil immersion field; + + = 2 to 4 cells per oil immersion field; + + + = 5 to 30 cells per oil immersion field; + + + + = >30 cells per oil immersion field. Smears were considered positive for polymorphonuclear leukocytes if ≥1 polymorphonuclear leukocyte/oil immersion field was found in the absence of red blood cells. Microorganisms were identified as described in standard texts.[11, 12]

Because a previous study at our institution failed to detect *Chlamydia trachomatis* by culture and serologic testing in our patient population,[5] these organisms were not studied. Viruses were not examined because no correlation has been found between acute chorioamnionitis and elevated maternal antibody titers during

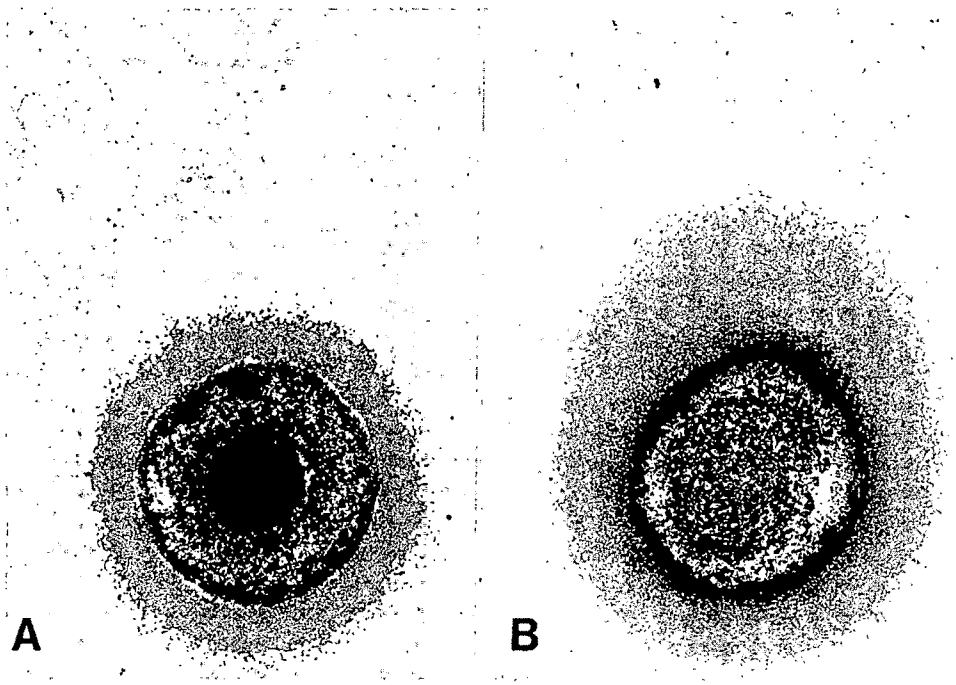


Fig. 1. Migration patterns of neutrophils obtained by incubating polymorphonuclear leukocytes in the central well. The well containing amniotic fluid is at the top, and the well containing basal medium Eagle is off the bottom of the photograph. **A,** Negative leukotaxis; **B,** positive leukotaxis. (Original magnification ×12.)

pregnancy against those viruses most frequently infecting the placenta and fetus.[5]

GLC of amniotic fluid. Specimens were extracted and analyzed for volatile and nonvolatile organic acids using standard procedures.[11] Uninoculated controls treated the same as test samples were run with each analysis. Extracts were analyzed on a CAPCO model 700 gas chromatograph (DODECA, Fremont, Calif.) equipped with a thermal conductivity detector.[11]

Histology. After delivery sections of the central and peripheral area of the placenta, the umbilical cord, and rolled section of fetal membranes wre fixed in buffered formalin and stained with hematoxylin and eosin. Histologic chorioamnionitis was defined as the accumulation of polymorphonuclear leukocytes in the intervillous space immediately below the chorionic plate. The inflammatory process was classified both by stage and severity as described elsewhere.[13]

Leukotaxis assay. Details of the assay appear in Chenoweth et al.[14] Briefly, glass microscopic slides (Fisher Scientific, Springfield, N.J.) were coated with gelatin (Difco Laboratories, Detroit) and air dried. Five ml of agarose (Sigma Chemical Co., St. Louis) containing gelatin (Difco) and basal medium Eagle (GIBCO Laboratories, Grand Island, N.Y.) was layered onto each slide and allowed to solidify. Three precisely spaced circular wells were then cut into agarose gel with the aid of a specially made punch and template.[8] Ten microliters of the test amniotic fluid were placed into one of the outer wells and basal medium Eagle (control) in the other. Finally, 10 μl of human polymorphonuclear leukocytes suspended in basal medium Eagle (1×10^5 polymorphonuclear leukocytes per milliliters) were placed into the central well. The slide was incubated at 37° C in an atmosphere containing 5% carbon dioxide in air. After a 2-hour incubation period the slide was removed and fixed in anhydrous methanol. After fixation the gel was removed from the slide intact. The fixed cells on the slide surface were stained with Wrights stain and air dried. The cost and time required to complete the assay are approximately $2 per test and 8 hours, respectively.

Measurements of the distance polymorphonuclear leukocytes migrated toward the well containing amniotic fluid compared with the distance migrated in response to the control (neat basal medium Eagle) were assessed qualitatively and semiquantitatively (Fig. 1). Qualitative assessment of the migratory pattern (+ or −) was performed under a hand lens (×4 magnification). Migratory distances were semiquantified with a microscope (American Optical Corporation, Buffalo, N.Y.) equipped with a calibrated ocular micrometer (×100). Direct migration (A) was defined as the furthest point (linear distance) that three neutrophils in the same plane moved from the margin of the central well toward the outer well containing the test amniotic

Table I. Comparison between placental histology and laboratory tests of amniotic fluid from patients who were delivered within 48 hours of amniocentesis*

Histologic chorioamnionitis	*No. of fluids*	*No. of positive laboratory tests (%)*					
		Leukotaxis assay	*Polymorphonuclear leukocytes†*	*Culture*	*GLC*	*Gram stain*	*Combined microbiologic tests‡*
Present	15	13 (87)§	11 (73)§	8 (53)§	6 (40)‖	5 (33)¶	9 (60)§
Absent	17	1 (6)	0	0	0	0	0

*Amniotic fluid from patients with intact membranes, without clinical infection.
†Considered positive if ≥1 polymorphonuclear leukocyte per oil immersion field in fluid pellet.
‡≥1 test(s) (culture, Gram stain, GLC) positive.
§$p < 0.005$.
‖$p < 0.01$.
¶$p < 0.025$.

fluid. Similarly, spontaneous migration (B) was defined as the distance toward the outer well containing the control basal medium Eagle. From these measurements the chemotactic index (CI) was calculated (CI = A/B).[8] Experiments were run in triplicate. The calculated chemotaxic index for each amniotic fluid was the mean of all three experiments. Fluids with a chemotaxic index of ≥1.2 were considered positive. Positive and negative controls were included with each experiment as a check on assay performance. The positive control was an amniotic fluid from a patient whose placenta showed evidence of histologic chorioamnionitis (stage III–severe) and the negative control was amniotic fluid from a patient without histologic chorioamnionitis. To test the effects of storage, five positive and five leukotaxis-negative fluids were stored at −70° C for 2 months. Fluid testing was performed without prior knowledge of results from histologic examination or clinical features of the case.

Statistical comparisons. Statistical comparisons were tested at the 5% level of significance ($p = 0.05$) using the Fisher's exact test. The diagnostic accuracy of laboratory tests was defined by sensitivity, specificity, and efficiency.[15]

Results

A comparison between placental histology and laboratory tests of amniotic fluid is shown in Table I. Histologic chorioamnionitis was found in 15 placentas, and correlated well with leukoattractants in amniotic fluid and, to a lesser extent (in decreasing order) with polymorphonuclear leukocytes in fluid pellets, culture, GLC, and Gram stain. The breakdown by histologic stage/severity was as follows: stage I–mild (two placentas), stage I–moderate (three placentas), stage II–mild (one placenta), stage II–moderate (two placentas), and stage III–severe (seven placentas). The leukotaxis assay was positive in 13 cases (87%) and negative in two cases (13%) with histologic chorioamnionitis ($p < 0.005$). The two cases with histologic chorioamnionitis that had negative leukotaxis were classified as stage I/mild severity.

Amniotic fluid cultures were positive in eight (53%) of the cases with histologic chorioamnionitis. Each fluid yielded a single isolate. Organisms recovered included *Fusobacterium nucleatum* (six fluids), microaerophilic streptococci (one fluid), and *U. urealyticum* (one fluid). The leukotaxis assay was positive in each of the culture-positive fluids. In every case in which positive polymorphonuclear leukocytes were found in fluid pellets, leukotaxis assays and histologic chorioamnionitis were positive. However, in two cases leukoattractants and histologic chorioamnionitis were found without significant numbers of polymorphonuclear leukocytes in fluids.

Leukoattractants were found in four fluids in which the combined results of culture, Gram stain, and GLC were negative (Table I). Fluid pellets of all four were positive for polymorphonuclear leukocytes and all matching placentas had histologic chorioamnionitis. The breakdown by histologic stage-severity was as follows: stage I–moderate (one placenta), stage II–mild (one placenta), and stage III–severe (two placentas).

Histologic chorioamnionitis was not detected in association with one leukotaxis-positive fluid from a patient who was delivered preterm (Table I). All other laboratory tests on this fluid were negative. This patient was not in labor at the time of amniocentesis, which was performed for fetal distress. After delivery histologic examination of the placenta revealed recent infarction. A comparison of the diagnostic accuracy of each laboratory test as a predictor of histologic chorioamnionitis is shown in Table II.

A positive leukotaxis result was associated with delivery within 48 hours in every case. Thirteen (93%) of the leukotaxis-positive fluids were from patients with preterm labor at amniocentesis. One leukotaxis-positive fluid was from a patient not in labor; infarction was

Table II. Sensitivity, specificity, and efficiency of laboratory tests of amniotic fluid for histologic chorioamnionitis*

	Leukotaxis assay	*Polymorphonuclear leukocytes†*	*Culture*	*GLC*	*Gram stain*	*Combined microbiologic tests‡*
Sensitivity (%)	87	73	53	40	33	60
Specificity (%)	94	100	100	100	100	100
Efficiency (%)	91	88	78	72	69	81

*Amniotic fluid from patients with intact membranes without clinical infection, and delivering within 48 hours of amniocentesis.
†Considered positive if ≥1 polymorphonuclear leukocyte per oil immersion field in fluid pellet.
‡≥1 test(s) (culture, Gram stain, GLC) positive.

found in this patient's placenta (see Table I). Of 18 leukotaxis-negative fluids from patients delivering within 48 hours, 10 were from patients in labor at amniocentesis. Placentas from two of these patients had histologic chorioamnionitis (stage I–mild severity). There were no complications related to tocolytic therapy in any patient.

One patient in this study developed clinical infection after amniocentesis and another developed postpartum endometritis. Fluid cultures yielded *Fusobacterium nucleatum* and microaerophilic streptococci, respectively. Both fluids were leukotaxis-positive. Neonatal sepsis strictly indicated by positive cultures of blood or cerebrospinal fluid was not found in any patient. No significant difference was found between qualitative and semiquantitative measurement of leukotaxis. No change in leukotaxis was found in fluids stored frozen at −70° C for 2 months.

Comment

It has been suggested that ascending intrauterine infection is a significant underlying cause of preterm labor and premature rupture of the membranes. This hypothesis is supported by histologic data that indicate an association between chorioamnionitis and these common clinical entities.[1] Because histologic information is not available before delivery, other criteria are used to define infection during the perinatal period. Signs and symptoms of clinical infection are typically used for diagnosis. Unfortunately, criteria for clinical infection are nonspecific, insensitive, and frequently only indicate advanced amniotic fluid infection. Other criteria used to define infection include amniotic fluid culture, Gram stain, GLC, and bacterial endotoxin assay.[2, 16]

In this study histologic chorioamnionitis was used as evidence of exposure to intrauterine infection. Amniotic fluid culture, Gram stain, and GLC were found to be less sensitive tests for intrauterine infection (as determined by histology) than the leukotaxis assay. The higher sensitivity of the leukotaxis assay may be attributed to detection of extraamniotic fluid infection. The above three microbiologic assays require microorganisms, or their products, to reach a detectable level in amniotic fluid. Although very specific for intraamniotic fluid infection, these tests may fail to detect the initial stage of an ascending infection, which is believed to begin within the intact membranes, spreading secondarily to the amniotic fluid.[17] Furthermore, the microbiologic approach of attempting to identify the etiologic agent is limited because a wide variety of microorganisms have been implicated,[1] thus making exhaustive detection difficult. The inability of microbiologic tests to detect the initial stages of an ascending infection may limit an understanding of the role played by infection in premature labor.

Another criterion occasionally used for detection of infection is assay of polymorphonuclear leukocytes in amniotic fluid, either by direct microscopic examination or indirectly by leukocyte esterase activity. The significance, origin, and role played by amniotic fluid polymorphonuclear leukocytes in perinatal infection is largely unknown. In our study the association found between amniotic fluid polymorphonuclear leukocytes, leukoattractants, and histologic chorioamnionitis supports the theory that polymorphonuclear leukocytes can derive from inflamed membranes.[4] These findings suggest that polymorphonuclear leukocytes in increased numbers (≥1 polymorphonuclear leukocytes/oil immersion field/pellet) may reflect histologic chorioamnionitis and, indirectly, exposure to intrauterine infection. The advantage of the leukotaxis assay is that leukoattractants must precede the arrival of polymorphonuclear leukocytes in amniotic fluid. Measurement of C-reactive protein in maternal blood has also been suggested as an early indicator of histologic chorioamnionitis. However, conflicting conclusions have been reached regarding its potential clinical application.[18]

Findings in this study raise several questions. (1) Leukoattractants and polymorphonuclear leukocytes were found in four fluids in which the combined results of culture, Gram stain, and GLC were negative. These patients' placentas had marked histologic chorioamnionitis. Do these four cases represent extraamniotic infection? Despite exhaustive microbiologic techniques

there remains the possibility that some microorganisms in amniotic fluid were not detected. (2) Can leukoattractants present in amniotic fluid be unrelated to infection? This is not likely, because with the exception of the patient described below, all leukotaxis-positive fluids were associated with histologic chorioamnionitis. Other than infection, no satisfactory explanation for chorioamnionitis has been demonstrated.[3] Leukoattractants (but not polymorphonuclear leukocytes) detected in one fluid from a patient without chorioamnionitis may have been related to placental infarction. (3) Did measurement of leukoattractants in amniotic fluid correlate with severity of chorioamnionitis? The lowest histologic stage and severity of choriomnionitis was found in two placentas, both of which were accompanied by fluid without leukoattractants. This suggests that leukoattractants did not reach detectable levels. We lack sufficient data to draw conclusions regarding measurement of leukotaxis with more severe grades of chorioamnionitis.

Leukoattractants were not characterized in this study because the goal was to determine whether leukoattractants detected by the assay reflect histologic chorioamnionitis. Products of bacteria, complement, degenerating cells, leukotrienes, and reactive oxygen species, all of which are present during an inflammatory response, represent a partial list of leukoattractants. In vivo any of these factors may act alone or in concert with others to cause polymorphonuclear leukocyte migration. Regardless of whether all instances of chorioamnionitis result from infection, it is clear that inflammation can contribute to,[19] and perhaps initiate, labor.

The importance of *Fusobacterium* as a cause of occult amniotic fluid infection in patients with intact membranes has been emphasized.[20] Although life-threatening *Fusobacterium* chorioamnionitis does occur, most morbidity associated with this organism is attributed to prematurity. This view is supported by our study in which *F. nucleatum* accounted for 75% of positive cultures. Each of these patients was delivered preterm, and neonatal cultures of blood and cerebrospinal fluid were uniformly sterile.

In summary, the leukotaxis assay provides a simple direct method of determining placental inflammation and indirectly, exposure to intrauterine infection before delivery. This assay contributes to ongoing efforts designed to determine the relationship between preterm delivery and infection. We plan to continue to study these tests for their ability to detect intrauterine infection at an earlier stage than is presently possible. This may permit earlier intervention in the form of delivery of a healthier neonate, or successful intrauterine treatment of infections with the option of continuation of preterm pregnancy.

We thank Alan Pankuch for making the punch and template used in preparing the agarose gel slides.

REFERENCES

1. Minkoff H. Prematurity: infection as an etiologic factor. Obstet Gynecol 1983;62:137-44.
2. Gravett MG, Eschenbach DA, Speigel-Brown CA, Holmes KK. Rapid diagnosis of amniotic fluid infection by gas-liquid chromatography. N Engl J Med 1982;306:725-8.
3. Fox H, Elston CW. Pathology of the placenta. Philadelphia: WB Saunders, 1978:286-325.
4. Blanc WA. Pathology of the placenta, membranes, and umbilical cord in bacterial, fungal, and viral infections in man. In: Naeye RL, Kissane JM, Kaufman N, eds. Perinatal diseases. Baltimore: Williams & Wilkins, 1981:67-132.
5. Pankuch GA, Appelbaum PC, Lorenz RP, Botti JJ, Schachter J, Naeye RL. Placental microbiology and histology and the pathogenesis of chorioamnionitis. Obstet Gynecol 1984;64:802-6.
6. Quinn PA, Butany J, Taylor J, Hannah W. Chorioamnionitis: its association with pregnancy outcome and microbial infection. Am J Obstet Gynecol 1987;156:379-87.
7. Hillier SL, Martius J, Krohn M, Kiviat N, Holmes KK, Eschenbach DA. A case-control study of chorioamnionic infection and histologic chorioamnionitis in prematurity. N Engl J Med 1983;319:972-8.
8. Nelson RD, Quie PG, Simmons RL. Chemotaxis under agarose: a new and simple method for measuring chemotaxis and spontaneous migration of human polymorphonuclear leukocytes and monocytes. J Immunol 1975; 115:1650-6.
9. Orr W, Ward PA. Quantitation of leukotaxis in agarose by three different methods. J Immunol Methods 1978; 20:95-107.
10. Gibbs RS, Blanco JD, St. Clair PJ, Castaneda YS. Quantitative bacteriology of amniotic fluid from women with clinical intraamniotic infection at term. J Infect Dis 1982;145:1-8.
11. Holdeman LV, Cato EP, Moore WEC, eds. Anaerobe laboratory manual. 4th ed. Blacksburg: Virginia Polytechnic Institute and State Univesity, 1977.
12. Razin S, Tully JG, eds. Methods in mycoplasmology. New York: Academic Press, 1983 vol 1.
13. Naeye RL, Maisels J, Lorenz RP, Botti JJ. The clinical significance of placental villous edema. Pediatrics 1983; 71:588-94.
14. Chenoweth DE, Rowe JG, Hugli TE. Modified method for chemotaxis under agarose. J Immunol Methods 1979;25:337-353.
15. Galen RS, Gambino SR. Beyond normality: the predictive value and efficiency of medical diagnoses. New York: John Wiley & Sons, 1975.
16. Romero R, Kadar N, Hobbins JC, Duff GW. Infection and labor: the detection of endotoxin in amniotic fluid. Am J Obstet Gynecol 1987;157:815-9.
17. Evaldson GR, Malmborg A-S, Nord CE. Premature rupture of the membranes and ascending infection. Br J Obstet Gynaecol 1982;89:793-801.
18. Ernest JM, Swain M, Block SM, Nelson LH, Hatjis CG, Meis PJ. C-reactive protein: a limited test for managing patients with preterm labor or premature rupture of membranes? Am J Obstet Gynecol 1987;156:449-54.
19. López Bernal A, Hansell DJ, Cañete Soler R, Keeling JW, Turnbull AC. Prostaglandins, chorioamnionitis, and preterm labor. Br J Obstet Gynaecol 1987;94:1156-8.
20. Easterling TR, Garite TJ. *Fusobacterium*: anaerobic occult amnionitis and premature labor. Obstet Gynecol 1985; 66:825-8.

Evidence relating bacterial vaginosis to intraamniotic infection

Helayne M. Silver, MD, Rhoda S. Sperling, MD, Patricia J. St. Clair, BA, and Ronald S. Gibbs, MD

San Antonio, Texas

We performed a two-part study to determine relationships of bacterial vaginosis and intraamniotic infection. In the first part of the study, we determined whether bacterial vaginosis organisms (*Gardnerella vaginalis, Mycoplasma hominis,* and anaerobes) were associated with each other in the amniotic fluid of 408 cases of intraamniotic infection. In the second part, we determined the association of bacterial vaginosis itself with intraamniotic infection in 125 cases at high risk for intraamniotic infection. Strong associations were observed among the bacterial vaginosis organisms in amniotic fluid ($p < 0.01$ to $p < 0.001$). Women with bacterial vaginosis were more likely to develop intraamniotic infection than those without bacterial vaginosis (69% vs. 46%, $p = 0.03$). Women with bacterial vaginosis were more likely to have *G. vaginalis* and *M. hominis* in the amniotic fluid ($p < 0.01$ and 0.04, respectively). These observations implicate bacterial vaginosis as a cause of intraamniotic infection. (AM J OBSTET GYNECOL 1989;161:808-12.)

Key words: Bacterial vaginosis, intraamniotic infection, amniotic fluid, *Gardnerella vaginalis, Mycoplasma hominis*

Bacterial vaginosis (also called nonspecific vaginitis and *Gardnerella vaginalis* vaginitis) is a common cause of vaginal symptoms.[1] Although bacterial vaginosis has a complex and at times controversial microbial etiology, evidence now supports a major role for anaerobes.[2, 3] *G. vaginalis* and *Mycoplasma hominis* are also found more commonly in vaginal secretions of affected women.[4, 5]

Other investigators have implicated bacterial vaginosis in preterm labor, preterm birth, preterm premature rupture of membranes, and amniotic fluid infection.[6-8] The purposes of this study were to (1) identify significant associations of organisms in amniotic fluid (especially of anaerobes, *G. vaginalis,* and *M. hominis*) from a series of cases of intraamniotic infection, (2) determine characteristics of intraamniotic infection associated with these organisms, and (3) determine whether bacterial vaginosis was associated with intraamniotic infection.

Methods

Data were gathered in two consecutive groups of women. In both groups intraamniotic infection was defined on the basis of a temperature ≥37.8° C and rupture of the membranes plus two or more of the following: maternal tachycardia (>100 beats/min), fetal tachycardia (>160 beats/min), uterine tenderness, foul odor of the amniotic fluid, or peripheral leukocytosis (>12,000/mm³).[9] Asymptomatic women had membrane rupture but no temperature ≥37.8° C and no other signs or symptoms of infection. Neonatal sepsis was defined as typical signs plus isolation from the blood of a pathogenic organism. Neonatal pneumonia was defined as respiratory symptoms with a patchy infiltrate on chest x-ray film.

Amniotic fluid was obtained by aspiration of an intrauterine pressure catheter that had been inserted for obstetric indications. Because of the possibility of contamination, the first 7 ml (equivalent to the volume of the catheter) was discarded, and the next 5 to 20 ml was collected in a separate plastic syringe. After air was expressed and the syringe was tightly capped, the specimen was transported to the laboratory where it was plated quantitatively within 60 minutes on a variety of media for aerobes and anaerobes.[9] After 1983, specimens were also plated on V-selective agar (Remel, Lenexa, Kan.) as a selective media for *G. vaginalis.* Otherwise, bacteriologic methods were similar throughout. Maternal and neonatal blood samples were collected by sterile venipuncture and inoculated into a set of two bottles, each containing 100 ml trypticase soy broth with 3% carbon dioxide and 0.03% sodium polyanethol sulfonate (BBL, Cockeysville, Md.). Amniotic fluid and blood isolates were identified by standard techniques. Amniotic fluid specimens were qualitatively cultured for genital mycoplasmas with a modification of the method of Kenny.[10] Blood was not cultured for mycoplasmas.

From the Department of Obstetrics and Gynecology, The University of Texas Health Science Center at San Antonio.
Supported in part by National Institutes of Health grant HD12292, National Institute of Child Health and Human Development.
Dr. Sperling was a Visiting Fellow from the Department of Obstetrics and Gynecology, Mt. Sinai School of Medicine, New York, New York.
Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.
Reprints not available.
6/6/13674

After collection of specimens, infected mothers were treated with penicillin G (5 million units intravenously every 6 hours) and gentamicin (1.5 mg/kg intravenously every 8 hours), usually beginning during labor. After 1982, for women with cesarean delivery, clindamycin was usually added after cord clamping for improved activity against anaerobes. In neonates, blood cultures and a chest radiograph were performed, collection of cerebrospinal fluid was performed only in infants with central nervous system signs. Neonates were treated within 2 hours of birth with ampicillin (75 mg/kg intravenously every 12 hours) and gentamicin (2.5 mg/kg intravenously every 12 hours) for at least 3 days. If results of the sepsis evaluation were negative, antibiotics were discontinued after 72 hours. For sepsis or pneumonia, infants were treated for approximately 10 days with appropriate therapy. Clinical data were collected on 29 variables.

Specific methods of each part of the study follow. In the first part, amniotic fluid was collected from patients with intraamniotic infection between July 1979 and October 1986. In determining associations and characteristics of intraamniotic infection with an index organism, we compared those cases containing the index organism (regardless of any other organism isolated) with cases not containing the index organism by the χ^2 goodness of fit test for discrete variables and a two-tailed Student's t test for continuous variables. A value of $p < 0.05$ was considered significant.

After completion of the first part of the study, we then enrolled a group of women at high risk for intraamniotic infection on the basis of rupture of membranes greater than 10 hours. We collected a vaginal Gram stain and amniotic fluid specimen (as above) and clinical data from these women. The vaginal Gram stain was scored from 0 to 10 by a single examiner (who was unaware of the clinical diagnosis) on the basis of semiquantitative estimates of *G. vaginalis*, *Lactobacillus*, and *Mobiluncus* morphotypes (unpublished observations).* A score of 0 to 3 was considered normal, 4 to 6 intermediate, and 7 to 10 as bacterial vaginosis. Other features of bacterial vaginosis (such as high pH, clue cells, amine odor) were not used because of the potential effect of membrane rupture on them. However, Eschenbach et al.[1] have shown that Gram stain criteria very similar to the ones we used correlated well with other clinical and microbiologic criteria of bacterial vaginosis in an outpatient population. Women who developed puerperal fever were excluded from the analysis because of the confounding influences such as cesarean delivery. Discrete data were compared by χ^2 test; $p < 0.05$ was considered significant.

*The vaginal Gram stain score was determined as follows:

Morphotype	*Points*	*Morphotype*	*Points*
4+ Lactobacilli	0	1 + *G. vaginalis*	1
3+ Lactobacilli	1	2 + *G. vaginalis*	2
2+ Lactobacilli	2	3 + *G. vaginalis*	3
1+ Lactobacilli	3	4 + *G. vaginalis*	4
		1 + 2+ *Mobiluncus* species	1
0 Lactobacilli	4	3 to 4+ *Mobiluncus* species	2

Results

During the first part of the study, there were 408 cases representing approximately 1% of all deliveries and an estimated 40% of all cases of intraamniotic infection. In the remaining cases of intraamniotic infection, no amniotic fluid could be aspirated for study. The overall microbiologic results and neonatal outcomes, have previously been reported in part.[11] As in previous studies, these pregnancies were characterized by long intervals of membrane rupture (23.4 ± 19.7 hours, mean ± SD) and of internal monitoring (12.3 ± 6.8 hours, mean ± SD). Most patients were at term (39.5 ± 3.2 weeks, mean gestational age), and 174 (43%) had cesarean delivery. The mean temperature at diagnosis was 38.3° ± 0.4° C.

There were several significant associations found among organisms. As shown in Table I, strong associations were seen among *M. hominis*, *G. vaginalis*, and some anaerobes. Group B streptococci were associated with *Bacteroides bivius*. When group B streptococci were present in the amniotic fluid, *B. bivius* was present in 42% (25 of 60), whereas in fluid without group B streptococci *B. bivius* was present in 27% (95 of 348, $p = 0.02$). Stronger associations were observed among *Escherichia coli*, enterococci, and other aerobic gram-negative rods. When *E. coli* was present, enterococci were found in 21% (7 of 33) and other aerobic gram-negative rods in 24% (8 of 33). In fluid without *E. coli*, enterococci were present in only 4% (15 of 375), and other aerobic gram-negative rods were present in only 3% (13 of 373, $p < 0.001$ for both). We found no other significant association of any organism in amniotic fluid.

There were 26 (6.4%) mothers with bacteremia, but none had *G. vaginalis* organisms isolated. Only two (0.5%) had anaerobic bacteremia, both with Bacteroides species. Other maternal blood isolates were group B streptococci (11 cases), *E. coli* (7), *Staphylococcus aureus* (2), *Listeria monocytogenes* (2), and one each enterococci, viridans streptococci, *Streptococcus pneumoniae*, and *Pseudomonas aeruginosa*. There were 28 isolates (two patients had polymicrobial bacteremias). Isolates in cases of neonatal bacteremias have been previously reported.

When clinical features for intraamniotic infection associated with any index organism were compared with all other intraamniotic infection cases, there were few pertinent and significant differences. Specifically, clin-

Table I. Associataion of bacterial vaginosis-relatec organisms in amniotic fluid of cases of intraamniotic infection

	Organism in amniotic fluid					
	*M. hominis**			*B. bivius*		
Organism in amniotic fluid	*Yes (n = 125)*	*No (n = 234)*	*p*	*Yes (n = 120)*	*No (n = 288)*	*p*
G. vaginalis	44 (35%)	54 (23%)	0.01	40 (33%)	59 (20%)	0.01
M. hominis	—	—		56 (47%)	66 (24%)	<0.001
B. bivius	56 (45%)	53 (23%)	<0.001	—	—	
Peptostreptococci	20 (16%)	14 (6%)	0.002	14 (12%)	24 (8%)	NS

*Total number of cultures for *M. hominis* is 359 because the remainder were not done or were contaminated.

Table II. Characteristics of women with versus women without bacterial vaginosis and at high risk for intraamniotic infection

Characteristic	*Bacterial vaginosis (n = 32)*	*No bacterial vaginosis (n = 93)*	*p*
Age (yr)*	22.7 ± 6.1	22.6 ± 5.8	
Parity*	0.53 ± 1.4	0.70 ± 1.2	
Gestational age (wk)*	39.6 ± 2.8	39.8 ± 2.2	
Labor duration (hr)*	17.6 ± 10.8	13.4 ± 6.2	0.043
Rupture of membranes (hr)*	21.8 ± 18.4	18.4 ± 14.5	
Vaginal examination (No.)*	7.2 ± 2.0	7.3 ± 2.1	
Internal monitoring (hr)*	10.3 ± 5.2	10.8 ± 5.9	
Intrapartum oxytocin use (%)	88	93	
Cesarean delivery (%)	35	31	

*Data are shown as mean ± 1 SD.

ical features such as maximum temperature in labor, labor duration, and duration of internal monitoring were not significantly different for anaerobe-, *M. hominis*–, or *G. vaginalis*–associated intraamniotic infection.

B. bivius–associated intraamniotic infection was accompanied by an increased number of maternal febrile days (1.8 ± 1.3 vs. 1.4 ± 1.3 days, $p = 0.02$), even though it was not associated with any other variable predisposing to more febrile days such as increased rate of cesarean section. In comparison, cases of *E. coli*–associated intraamniotic infection were accompanied by a significantly increased number of maternal febrile days after delivery (2.4 ± 1.8 vs. 1.5 ± 1.4 days, $p < 0.01$), but group B streptococci-associated intraamniotic infection was not (1.8 ± 1.7 vs. 1.5 ± 1.4 ± 1.3 days, $p = 0.2$).

In the second part of the study, 155 women were enrolled between October 1986 and March 1988. Overall 63 had intraamniotic infection and 62 remained asymptomatic. Thirty who developed puerperal fever were excluded from analysis because of major confounding influences of additional factors such as cesarean section. Bacterial vaginosis was present in 32 patients (26%), determined by Gram stain criteria. Characteristics of women with and without bacterial vaginosis are shown in Table II. The groups were not significantly different in any measure except labor duration.

Women with bacterial vaginosis developed intraamniotic infection in 69% (22 of 32) of cases, whereas those without bacterial vaginosis had intraamniotic infection less commonly, 46% (43 of 93, $p = 0.03$). Women with bacterial vaginosis were more likely to have *G. vaginalis* and *M. hominis* in the amniotic fluid and were less likely to have group B streptococci and enterococci in the amniotic fluid (Table III). Anaerobes were not isolated significantly more often in amniotic fluid of women with bacterial vaginosis.

Comment

In this study we made three observations associating bacterial vaginosis with intraamniotic infection. First, organisms previously reported to be associated with bacterial vaginosis (i.e., anaerobes, *G. vaginalis*, and *M. hominis*) were also found in association with each other in the amniotic fluid of women with intraamniotic infection.[1-4] Second, bacterial vaginosis was significantly associated with intraamniotic infection in a group of women at risk. Third, in this high-risk group, women with bacterial vaginosis were more likely to have *G. vaginalis* ($p < 0.01$) and *M. hominis* ($p = 0.04$) in the amniotic fluid.

The association of *G. vaginalis*, some anaerobes, and *M. hominis* in amniotic fluid of women with intraamniotic infection suggests that the organisms arise from bacterial vaginosis rather than independently from the vagina. We also observed associations of *E. coli*, other aerobic gram-negative rods, and enterococci—all organisms commonly isolated in the lower gastrointestinal tract. Group B streptococci, a major neonatal pathogen,

Table III. Organisms isolated in amniotic fluid by vaginal Gram stain score

	Organism(s) in amniotic fluid				
Vaginal score	*G. vaginalis* (%)	*M. hominis* (%)	*Anaerobes* (%)	*Group B streptococci* (%)	*Enterococci* (%)
0-3 (n = 43)	26	12	14	31	20
4-6 (n = 50)	60	23	23	15	8
7-10 (n = 32)	85	35	26	9	3
p	<0.01	0.04	NS	0.03	0.03

Score: 0 to 3, normal; 4 to 6, intermediate; 7 to 10, bacterial vaginosis.

was associated with *B. bivius*, but the association was weak. Because multiple associations were evaluated, there is danger of an α-type error, yet our major associations had values of $p < 0.05$. Thus from patterns of organisms in amniotic fluid of intraamniotic infection cases bacteria did not appear to enter the amniotic cavity by chance or by independent action. Instead, the bacterial vaginosis group often enter in concert as do the enteric organisms (*E. coli, enterococci,* other aerobic gram-negative rods); group B streptococci act more or less independently.

After we observed the interaction of bacterial vaginosis organisms in amniotic fluid, we then proceeded to determine whether bacterial vaginosis itself (as diagnosed by Gram stain) was associated with intraamniotic infection. Use of Gram stain to diagnose bacterial vaginosis was first suggested by Spiegel et al.[12] in 1983. A diagnosis of bacterial vaginosis was based on mixed flora consisting of gram-positive, gram-negative, or gram-variable bacteria with decreased or absent *Lactobacillus* morphotypes. Gram stains that showed a predominance of *Lactobacillus* morphotypes with or without *G. vaginalis* morphotype were interpreted as normal. Compared with composite clinical criteria (homogeneous discharge, pH ≥4.7, amine odor, and presence of clue cells), Eschenbach et al.[1] found the independent diagnosis of bacterial vaginosis by Gram stain had a sensitivity of 97%, a specificity of 79%, and a positive predictive value of 69%. Gram stain criteria for bacterial vaginosis correlated better than semiquantitative cultures for *G. vaginalis* with composite clinical criteria.

Recently, workers have compared the Gram stain score to clinical findings and culture results in 3985 pregnant women at 23 to 26 weeks' gestation. High scores (≥7) correlated with all typical clinical findings of bacterial vaginosis, increasing isolation from the vagina of anaerobes, genital mycoplasmas and *G. vaginalis*, and decreasing vaginal isolation of lactobacilli (unpublished observations).

We applied the Gram stain score to women in labor with membrane rupture. Others have previously applied Gram stain criteria to patients in labor or with ruptured membranes.[7,8,13] We reasoned that Gram stain criteria would be less influenced by membrane rupture and the conduct of labor (bloody show, vaginal examinations, use of lubricants) than would be description of secretions, pH, odor, or vaginal cultures. Since Gram stain criteria were sensitive measures of bacterial vaginosis in other groups of women, we anticipated that these criteria would also be sensitive during labor.

In the high-risk group, women with bacterial vaginosis were similar to those without bacterial vaginosis in regard to age, parity, gestational age, and five intrapartum characteristics (rupture of membranes, vaginal examination, internal monitoring, oxytocin use, and cesarean delivery). Because mean labor duration was longer in the group with bacterial vaginosis, it may be argued that the longer duration of labor rather than bacterial vaginosis per se explains the excess rate of intraamniotic infection in the bacterial vaginosis group. However, we believe that bacterial vaginosis is the more likely factor for three reasons. First, in a logistic regression of analysis of risk factors in intraamniotic infection on this obstetric service during the same time as the current data were collected, we found that parity, duration of membrane rupture, and duration of internal monitoring were independent risk factors (labor duration was not).[14] These three factors were all similar in the groups with and without bacterial vaginosis. Second, since we were examining nine variables it is possible that this one difference (with $p = 0.043$) occurred by chance. Third, because onset of true labor is difficult to pinpoint, this measure is less objective than any other.

Increasing isolation rates of both *G. vaginalis* and *M. hominis* in the amniotic fluid with increasing Gram stain score support bacterial vaginosis as the source of these organisms, yet we did not find that anaerobes, which play a central role in bacterial vaginosis, were increased in the amniotic fluid of women with bacterial vaginosis. Considering the absolute rate of isolating anaerobes was higher in the group with high scores than

in those with low scores (26% vs. 14%), we suspect that there is a real but undetected difference. Interestingly, group B streptococci and enterococci, organisms unrelated to the pathophysiology of bacterial vaginosis, were found with decreasing frequency in the amniotic fluid of women with bacterial vaginosis. Although a mechanism for this relationship is not certain, there is an implication that the "bacterial vaginosis organisms" compete with group B streptococci, enterococci, and perhaps others for ascent into the amniotic cavity. Unfortunately, our data set did not include detailed vaginal cultures and cannot address this relationship more completely.

Our selection of a very high-risk group, with a prevalence of intraamniotic infection of 50%, increased our likelihood of demonstrating a significant association, yet our findings add support to other reports that show an association between bacterial vaginosis and infection. Gravett et al.[6] also found that bacterial vaginosis was associated with subsequent development of "amniotic fluid infection" (9% in women with bacterial vaginosis vs. 4% in those without; $p < 0.05$; odds ratio 2.1 [1.2 to 3.9, 95% confidence interval]). More recently, Hillier et al.,[13] in a multiple regression analysis controlling for confounding variables, found that the intrapartum diagnosis of bacterial vaginosis was associated with recovery of organisms between the chorion and amnion in both term and preterm pregnancies. We speculate that among women with recognized clinical risk factors for intraamniotic infection such as prolonged rupture of the membranes and duration of prolonged internal monitoring,[14] the detection of bacterial vaginosis may identify a group at still higher risk. Identification of such a high-risk group may lead to the development of an intervention strategy such as administration of systemic or local prophylactic antibiotics in labor.

REFERENCES

1. Eschenbach DA, Hillier S, Critchlow C, Stevens C, DeRouen T, Holmes KK. Diagnosis and clinical manifestations of bacterial vaginosis. AM J OBSTET GYNECOL 1988;158:819-28.
2. Speigel CA, Amsel R, Eschenbach DA, Schoenknecht F, Holmes KK. Anaerobic bacteria in non-specific vaginitis. N Engl J Med 1980;303:601.
3. Blackwell AL, Fox AR, Phillips I, Barlow D. Anaerobic vaginosis (nonspecific vaginitis): clinical, microbiologic and therapeutic findings. Lancert 1982;2:1379-82.
4. Paavonen J, Miettinen A, Stevens CE, Chen KC, Holmes KK. *Mycoplasma hominis* in nonspecific vaginitis. Sex Transm Dis 1983;10(S):271-5.
5. Gardner HL, Dukes CD. *Haemophilus vaginalis* vaginitis. AM J OBSTET GYNECOL 1955;69:962-76.
6. Gravett MG, Nelson HP, DeRouen T, Critchlow C, Eschenbach DA, Holmes KK. Independent associations of bacterial vaginosis and *Chlamydia trachomatis* infection with adverse pregnancy outcome. JAMA 1986;256:1899-1903.
7. Gravett MG, Hummel D, Eschenbach DA, Holmes KK. Preterm labor associated with subclinical amniotic fluid infection and with bacterial vaginosis. Obstet Gynecol 1986;67:229-37.
8. Martius J, Krohn MA, Hillier SL, Stamm WE, Holmes KK, Eschenbach DA. Relationships of vaginal *Lactobacillus* species, cervical *Chlamydia trachomatis*, and bacterial vaginosis to preterm birth. Obstet Gynecol 1988;71:89-95.
9. Gibbs RS, Blanco JD, St. Clair PJ, Castaneda YC. Quantitative bacteriology of amniotic fluid from women with clinical intraamniotic infection at term. J Infect Dis 1982;145:18-25.
10. Kenny GE. Mycoplasmas. In: Lennette EH, ed. Manual of clinical microbiology. 4th ed. Washington, DC: American Society for Microbiology, 1985:407-11.
11. Sperling RS, Newton ER, Gibbs RS. Intraamniotic infection in low-birth-weight infants. J Infect Dis 1988; 157:113-7.
12. Spiegel CA, Amsel R, Holmes KK. Diagnosis of bacterial vaginosis by direct gram stain of vaginal fluid. J Clin Microbiol 1983;18:170-7.
13. Hillier SL, Martius J, Krohn M, Kiviat N, Holmes KK, Eschenbach DA. A case-control study of chorioamniotic infection and histologic chorioamnionitis in prematurity. N Engl J Med 1988;319:972-8.
14. Newton ER, Prihoda TJ, Gibbs RS. Logistic regression analysis of risk factor for intraamniotic infection. Obstet Gynecol 1989;73:571-5.

The incidence of positive amniotic fluid cultures in patients in preterm labor with intact membranes

M. Amanda Skoll, MD, Michael L. Moretti, MD, and Baha M. Sibai, MD
Memphis, Tennessee

The possible relationship between intrauterine infection and preterm labor has received considerable attention in recent years. The purpose of this study was twofold: first, to determine the frequency of asymptomatic infection in patients who came to the hospital in preterm labor, and second, to determine the significance of a positive amniotic fluid culture in relation to latency period and likelihood of preterm delivery. Patients who came to the hospital in preterm labor with intact membranes between 20 and 35 weeks' gestation underwent transabdominal amniocentesis. Amniotic fluid was sent for Gram stain and culture. Patients received tocolytic therapy as clinically indicated. Of 127 patients cultured, seven (5.5%) had positive amniotic fluid cultures. These patients had a significantly decreased latency period from amniocentesis to delivery (4.4 days versus 28.6 days), and a significantly increased chance of being delivered of preterm infants (100% versus 52.5%), as compared with patients with negative cultures. The rate of positive Gram stain was 7 of 125 positive. However, there was no correlation between positive Gram stain and positive culture results. Similarly, positive Gram stain results were not associated with any difference in the latency period or rate of preterm delivery. (Am J Obstet Gynecol 1989;161:813-6.)

Key words: Preterm labor, amniocentesis, infection

Despite the development of prevention programs, pharmacologic therapies and other interventions, the preterm delivery rate remains disappointingly stable at approximately 7% of births. Prematurity is responsible for a disproportionately large fraction of perinatal and neonatal morbidity and mortality. Although the mechansims that lead to the onset of preterm labor are not clearly understood, much interest has been devoted in recent years to the possibility of an infectious cause.

Several investigators have studied the role of infection in preterm labor by performing microbiologic studies on amniotic fluid obtained by amniocentesis. Published rates of positive amniotic fluid cultures of patients who are in preterm labor vary from 0 to 46.8%.[1-10] Unfortunately, many of these studies are hampered by small numbers of patients. In addition, it is difficult to know whether results from a particular population can be extrapolated to other populations.

The purpose of this study was to investigate the incidence of positive amniotic fluid cultures in patients who were in preterm labor when they came to the E. H. Crump Women's Hospital in Memphis, Tennessee.

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of Tennessee, Memphis.
Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.
Reprint requests: M. Amanda Skoll, MD, Department of Obstetrics and Gynecology, University of Tennessee, Memphis, 853 Jefferson Ave., Room E102, Memphis, TN 38103.
6/6/13892

Material and methods

The E.H. Crump Women's Hospital is a tertiary care perinatal center affiliated with the University of Tennessee, Memphis. It serves a primarily indigent population with approximately 80% black and 20% white patients. The study was carried out from January 1, 1986, to July 31, 1987, after approval by the Institutional Review Board of the University of Tennessee.

Patients were included in the study if they came to the hospital with evidence of uterine activity or cervical dilatation or effacement between 20 and 35 weeks' gestation. Gestational age was determined by early ultrasonography if available; otherwise, an estimate was made based on last menstrual period combined with ultrasonography on admission. Patients were excluded if they had ruptured membranes before admission, cessation of contractions with an intravenous fluid bolus and sedation, advanced labor (≥5 cm dilatation), clinical evidence of chorioamnionitis, or refused amniocentesis. Qualifying patients were commenced on tocolytic therapy as per the hospital's current protocol. The first-choice tocolytic agent was ritodrine hydrochloride, administered by intravenous infusion pump. In patients with contraindications to betamimetic therapy, magnesium sulfate was started, also by intravenous infusion.

Patients underwent ultrasound-guided transabdominal amniocentesis. Fluid was sent for pulmonary maturity studies including lecithin to sphingomyelin (L/S) ratio and foam stability index. At the same time, fluid was collected for microbiologic study. After im-

Table I. Organisms isolated in culture

Organism	*No. patients with positive culture**
Fusobacterium sp.	2
Group D streptococci	2
Group B streptococci	1
Bacteroides sp.	1
Bacillus cereus	1
Ureaplasma urealyticum	1
Gardnerella vaginalis	1

*Some patients had positive cultures for more than one organism.

Table II. Clinical characteristics

	Positive culture n = 7	*Negative culture n = 120*
Gravidity	2.0 ± 0.9	2.4 ± 1.6
Parity	1.2 ± 0.8	1.1 ± 1.3
Gestational age on admission (wk)	31.7 ± 1.8	31.6 ± 2.7
Cervical dilatation on admission (cm)	2.1 ± 1.4	1.5 ± 1.2
Time from amniocentesis to delivery (days)	4.4 ± 5.1*	28.6 ± 40.3*

Values are expressed as mean ± 1 SD.
*$p < 0.001$ by two-tailed Student t test.

mediate transfer to the microbiology laboratory, the specimen for Gram stain was centrifuged, treated to standard Gram stain, and read by experienced technicians. Amniotic fluid was plated aerobically on blood agar, chocolate agar, Thayer-Martin agar, McConkey agar, and thioglycollate broth. Amniotic fluid was plated anaerobically on Schaedler's, calcium nutrient agar, *Pisum sativum* agglutinin, and chopped-meat broth. Specific cultures for *Mycoplasma* species were not performed. Results were reported semiquantitatively. The results of microbiology studies were not made available for clinical management.

Patients who responded to tocolytic therapy with cessation of contractions were maintained on intravenous infusion of either ritodrine or magnesium sulfate for approximately 12 hours. They were then weaned onto oral betamimetic therapy and were subsequently discharged from the hsopital in 2 to 3 days if contractions did not recur. Indications for delivery included failed tocolysis, clinical suspicion of chorioamnionitis (based on the presence of fever >100° F, uterine tenderness, or leukocytosis with no other evidence of an infective site), or fetal distress. In addition, in those patients in whom rupture of the membranes occurred subsequent to admission to hospital, the presence of a mature lung profile constituted an indication for delivery. Labor was not induced in patients in whom tocolysis failed. The only patients in whom induction was performed preterm were those with a clinical diagnosis of chorioamnionitis or those with ruptured membranes plus a mature L/S ratio.

Neonatal records were reviewed for evidence of neonatal sepsis as documented by positive blood, urine, or cerebral spinal fluid cultures.

Statistical analysis was performed with Student's t test for unpaired samples and χ^2 where appropriate.

Results

Of the 127 amniotic fluid samples obtained, seven (5.5%) were found to be positive. The organisms are detailed in Table I.

Comparisons between the groups with positive cultures and negative cultures (Table II) do not reveal significant differences with regard to gravidity, parity, gestational age at presentation, or cervical dilatation on admission. The group with positive cultures included four patients who were 3 or 4 cm dilated, and another three who were <2 cm dilated on admission, but demonstrated cervical change despite hydration and sedation. There was a significant difference ($p < 0.001$) in the latent period from amniocentesis to delivery between the two groups based on culture results. Of interest is one patient who was culture positive for rare Group B streptococci after a negative Gram stain. She responded quickly to tocolytic agents and subsequently was delivered of an infant 12 days later and never received antibiotics. Her infant did not have septic complications.

Two of the patients in the group with positive cultures received antibiotics before delivery. The first was a twin gestation at 32 weeks who received erythromycin for *Chlamydia* diagnosed on cervical culture. She ruptured membranes in the hospital and was delivered of infants 10 days after amniocentesis. The other patient was treated with ampicillin and gentamicin for a clinical diangosis of chorioamnionitis based on fever and uterine tenderness. She also ruptured membranes during attempted tocolysis, and was delivered of an infant within 24 hours of admission. None of the remaining five patients with positive cultures had spontaneous rupture of the membranes during admission.

Within the group with negative cultures, 14 of 120 patients received antibiotics, including nine patients treated for urinary tract infections (seven with intravenous ampicillin, one with oral nitrofurantoin, and one with intravenous cefazolin). One patient received intravenous ampicillin and gentamicin as prophylaxis because of mitral valve prolapse, and four patients received the same medications because of clinical chorioamnionitis.

A total of 83 patients (65.4%) were delivered of preterm infants. This includes all seven of the patients with

positive cultures (8.4% of whom were delivered of preterm infants). Table III shows the rates of preterm delivery based on culture results.

There was no correlation between results of the Gram stain and subsequent culture. Of the seven positive culture results, only two (28.6%) had positive Gram stains. In addition, there were five false-positive Gram stains. When we analyzed the incidence of preterm delivery by dividing the patients on the basis of Gram stain results, there was no difference in the rate of preterm delivery with positive Gram stain. In addition, the presence of white blood cells in the amniotic fluid did not influence the rate of preterm delivery (64.7% with white blood cells, versus 67% without).

Of the eight offspring of mothers with positive cultures (including one set of twins), only one had clinical sepsis, with blood cultures positive for *Pseudomonas aeruginosa*. A total of 25 neonates received parenteral antibiotics (ampicillin and gentamicin in all cases). Four of these were from the group with positive amniotic fluid cultures, and 21 from the group with negative cultures. Indications for treatment included maternal diagnosis of chorioamnionitis (four cases), positive blood cultures (one case, as above), and prophylaxis (20 cases).

Table III. Correlation between culture results and preterm delivery

	Positive culture n = 7	*Negative culture n = 120*
Patients delivered of infants <37 weeks (%)	7 (100)*	76 (63.3)*
Patients delivered of infants ≥37 weeks (%)	0 (0)	44 (36.7)

*$p < 0.02$ by χ^2 analysis.

Comment

Our data, like those of other groups, suggest a role for an infectious cause in some patients in preterm labor. Although a rate of positive cultures of 5.5% falls toward the lower end of the reported range, there are several possible explanations. First, no specific cultures were performed for *Mycoplasma* species. Although one of the patients was found to be culture positive for *Ureaplasma urealyticum*, this was fortuitous and the culture techniques were not designed to detect the presence of *Ureaplasma* species. There is some evidence that this may be a significant organism in relation to preterm labor.[11] Second, patients who were seen with cervical dilatation >4 cm were not eligible for amniocentesis because they were not candidates for tocolytic therapy. It may be that the patients who are seen in advanced labor are more likely to harbor otherwise asymptomatic intrauterine infection.

Previous studies at the University of Tennessee, Memphis[12] have shown that up to two thirds of patients who were delivered of infants <1000 gm arrived at the hospital >4 cm dilated; however, such patients would have been excluded from the present study. These findings would explain the discrepancy between our data and that of other groups that have found that patients with positive amniotic fluid cultures tend to present at an earlier gestational age. Hillier et al.[13] have presented evidence from pathologic and microbiologic examination of the placenta that also supports the view that patients who present at earlier gestational ages are significantly more likely to have histologic chorioamnionitis. These are the very patients who would most benefit from prolongation of pregnancy, but are, in fact, least likely to respond to our efforts to achieve this. A few investigations of the use of antibiotics in the treatment of preterm labor may hold promise in this regard.[14, 15]

An attempt to minimize the chance of a false-positive diagnosis of preterm labor was made by excluding all patients who responded to a fluid bolus or sedation with cessation of uterine activity. In view of the well-recognized difficulty in making the diagnosis of preterm labor, it is useful to look at patients who were delivered of preterm infants. Our findings reveal that 8.4% of these patients had positive amniotic fluid cultures.

The lack of correlation between Gram stain and culture is disappointing. False-positive Gram stains may occur when extremely fastidious organisms present in the initial sample fail to grow in culture. To investigate the impact of such so-called "false-negative cultures," we analyzed the occurrence of preterm delivery by Gram stain results. This analysis failed to show any difference in the rate of preterm delivery with positive Gram stain. Our finding is different from that reported by other authors,[10] and at present we have no explanation for this difference. Of note is the fact that the negative predictive value of the Gram stain in our series is still 95.8%, so that a negative Gram stain is still reassuring as to the absence of infection.

False-negative Gram stains may occur when the number of organisms present in the initial Gram stain is very low but the organisms subsequently grow in culture. One method suggested to minimize this problem is to use ultracentrifugation of the specimen before performance of the Gram stain to concentrate the number of organisms. However, Romero et al.[10] failed to show any increase in the sensitivity of the Gram stain of amniotic fluid with this technique.

In terms of the significance of white blood cells found on Gram stain, our results agree with those of other investigators who found no relationship with sepsis or occurrence of preterm delivery.[6, 10]

Overall, we conclude that in a defined population, approximately 5% of patients with preterm labor and intact membranes have positive amniotic fluid cultures. Furthermore, these patients have a significantly shorter time from amniocentesis to delivery, as well as an increased likelihood of preterm delivery as compared with the group of patients with negative cultures. Unfortunately, we have not found the Gram stain to be a sensitive indicator of intrauterine infection, and we cannot suggest active delivery based on a positive Gram stain result. However, a negative Gram stain, with a negative predictive value of >95%, is reassuring.

REFERENCES

1. Bobbit JR, Hayslip CC, Damato JD. Amniotic fluid infection as determined by transabdominal amniocentesis in patients with intact membranes in premature labor. Am J Obstet Gynecol 1981;140:947.
2. Wallace RL, Herrick CN. Amniocentesis in the evaluation of premature labor. Obstet Gynecol 1981;57:483.
3. Hameed C, Tejani N, Verma UL, et al. Silent chorioamnionitis as a cause of preterm labor refractory to tocolytic therapy. Obstet Gynecol 1984;149:726.
4. Wahbeh CJ, Hill GB, Eden RD, et al. Intra-amniotic bacterial colonization in premature labor. Am J Obstet Gynecol 1984;148:739.
5. Weible DR, Randall HW. Evaluation of amniotic fluid in preterm labor with intact membranes. J Reprod Med 1985;30:777.
6. Leigh J, Garite TJ. Amniocentesis and the management of premature labor. Obstet Gynecol 1986;67:500.
7. Gravett MG, Hummel D, Eschenbach DA, et al. Preterm labor associated with subclinical amniotic fluid infection and with bacterial vaginosis. Obstet Gynecol 1986;67:229.
8. Iams JD, Clapp DH, Contos DA, et al. Does extra-amniotic infection cause preterm labor? Obstet Gynecol 1987; 70:365.
9. Duff P, Kopelman JN. Subclinical intra-amniotic infection in asymptomatic patients with refractory preterm labor. Obstet Gynecol 1987;69:756.
10. Romero R, Emamian M, Quintero R, et al. The value and limitations of the Gram stain examination in the diagnosis of intra-amniotic infection. Am J Obstet Gynecol 1988; 159:114.
11. Knudson RB, Driscoll SG, Monson RR, et al. Association of *Ureaplasma urealyticum* in the placenta with perinatal morbidity and mortality. N Engl J Med 1984;310:941.
12. Amon E, Anderson GD, Sibai BM, et al. Factors responsible for preterm delivery of the immature infant (<1000 gm). Am J Obstet Gynecol 1987;156:1143.
13. Hillier SL, Martius J, Krohn M, et al. A case-control study of chorioamniotic infection and histologic chorioamnionitis in prematurity. N Engl J Med 1988;319:972.
14. McGregor JA, French JL, Reller LB, et al. Adjunctive erythromycin treatment for idiopathic preterm labor: results of a randomized double-blinded, placebo-controlled trial. Am J Obstet Gynecol 1986;154:98.
15. Morales WJ, Angel JL, OBrien WF, et al. A randomized study of antibiotic therapy in idiopathic preterm labor. Obstet Gynecol 1988;72:829.

Correction

In the article by Humphreys-Beher and Blackwell, "Identification of a deoxyribonucleic acid allelic variant for β1-4 galactosyltransferase expression associated with male sperm binding/penetration infertility" (Am J Obstet Gynecol 1989;160: 1160-5), it was reported that the complementary deoxyribonucleic acid clone used corresponded with a putative human β1-4 galactosyltransferase. Analysis of the complete nucleotide sequence and expression of this clone has revealed that it is not the catalytic enzyme. Further studies by Lopez and Shur (Biochem Biophys Res Commun 1988;156:1223-9) and our research suggest it indirectly influences enzyme activity.

Infection and labor

V. Prevalence, microbiology, and clinical significance of intraamniotic infection in women with preterm labor and intact membranes

Roberto Romero, MD,* Marina Sirtori, MD, Enrique Oyarzun, MD, Cecilia Avila, MD, Moshe Mazor, MD, Robert Callahan, BS, Virginia Sabo, MSN, Apostolos P. Athanassiadis, MD, and John C. Hobbins, MD

New Haven, Connecticut

Amniotic fluid was retrieved by amniocentesis from 264 patients with preterm labor and intact membranes admitted to Yale–New Haven Hospital from Jan. 1, 1985, to July 31, 1988. The prevalence of a positive amniotic fluid culture was 9.1% (24/264). A total of 111 patients (42%) delivered preterm neonates, and 24 (21.6%) of those had positive amniotic fluid cultures. The diagnostic indexes of the Gram stain of amniotic fluid in the prediction of a positive amniotic fluid culture were as follows: sensitivity, 79.1%; specificity, 99.6%; positive predictive value, 95%; and negative predictive value, 98%. Endotoxin was detected with the limulus amebocyte lysate assay in 4.9% (13/264) of patients with preterm labor. All patients with endotoxin in the amniotic fluid delivered preterm neonates. The three most frequently isolated organisms were *Ureaplasma urealyticum* ($n = 6$), *Fusobacterium* species ($n = 5$), and *Mycoplasma hominis* ($n = 4$). Clinical chorioamnionitis was present in only 12.5% of the patients with positive amniotic fluid cultures. Women with positive amniotic fluid cultures had lower gestational ages and more advanced cervical dilatation on admission than women with negative cultures. Preterm infants born to mothers with positive amniotic fluid cultures had a higher incidence of respiratory distress syndrome and infectious complications than preterm neonates born after negative amniotic fluid cultures. These data underscore the frequency and importance of intraamniotic infections in women with preterm labor. (AM J OBSTET GYNECOL 1989;161:817-24.)

Key words: Preterm labor, prematurity, intraamniotic infection, lipopolysaccharide, limulus amebocyte lysate, Gram stain, amniotic fluid

Intrauterine infection has been recognized as a major etiologic factor for preterm delivery.[1] Bobitt and Ledger[2] were the first to present microbiologic data suggesting that unrecognized amnionitis is causally related to preterm labor and delivery. Subsequently, several investigators have used amniocentesis to assess the microbiologic state of the amniotic cavity in women with preterm labor and intact membranes. The prevalence of positive amniotic fluid cultures has varied from 0%[3] to 25.8%.[3-16] These discrepant results have been attributed to differences in population characteristics and microbiologic techniques.

The study reported herein was conducted on women with preterm labor and intact membranes. The objectives were: (1) to determine the prevalence of intraamniotic infection; (2) to establish the detection rate of lipopolysaccharide in amniotic fluid; (3) to determine the diagnostic value of the Gram stain and the limulus amebocyte lysate test in the detection of intraamniotic infection; (4) to describe the quantitative and qualitative microbiology of intraamniotic infection in this patient population; and (5) to ascertain maternal and neonatal outcomes according to amniotic fluid culture results.

Material and methods

Patient population. Patients admitted to Yale–New Haven Hospital with preterm labor and intact membranes from Jan. 1, 1985, to July 31, 1988, underwent amniocenteses for evaluation of the microbiologic status of the amniotic fluid. Preterm labor was defined as the presence of regular uterine contractions

From the Department of Obstetrics and Gynecology, Yale University School of Medicine.

Supported by grants from the Walter Scott Foundation for Medical Research and the Network of Maternal-Fetal Medicine Units from the National Institute of Child Health and Human Development.

Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.

Reprint requests: Roberto Romero, MD, Department of Obstetrics and Gynecology, Yale University School of Medicine, 333 Cedar St., P. O. Box 3333, New Haven, CT 06510.

**Recipient of a Physician Scientist Award from the National Institute of Child Health and Human Development.*

6/6/13996

Table I. Results of the Gram stain and limulus amebocyte lysate of amniotic fluid, used singly or in combination, in the diagnosis of a positive amniotic fluid culture

	Amniotic fluid culture		
	Positive	*Negative*	*Total*
Gram stain			
Positive	19	1	20
Negative	5	239	244
Limulus amebocyte lysate			
Positive	12	1	13
Negative	12	239	251
Limulus amebocyte lysate and Gram stain combined			
Positive	21	2	23
Negative	3	238	241

with a frequency of at least two every 10 minutes. Nitrazine, pooling, and ferning tests were performed in every patient during a sterile speculum examination to exclude patients with leakage of amniotic fluid into the vagina. Ritodrine, terbutaline, or magnesium sulfate was administered intravenously for tocolysis. Failure of tocolysis was diagnosed when cervical dilatation progressed beyond 5 cm or delivery occurred.

Retrieval of amniotic fluid. Amniotic fluid was retrieved by transabdominal amniocentesis in all cases. Amniocentesis was performed under ultrasound guidance.

Microbiologic culture technique. Amniotic fluid was transported to the laboratory in a capped plastic syringe immediately after collection. These conditions precluded air contact with the specimen. Plating occurred within 30 minutes of collection. Amniotic fluid was cultured for aerobic and anaerobic bacteria, as well as for *Mycoplasma* species.[16] An intraamniotic infection was defined as the presence of a positive amniotic fluid culture.[1]

Endotoxin bioassay. All amniotic fluid samples were assayed with the limulus amebocyte lysate gel clot test for detection of endotoxin. The assay was performed by a method previously described in detail.[5] Briefly, the assay consists of adding 200 μl of freshly obtained, unspun amniotic fluid to a single test vial of 200 μl of high-sensitivity lyophilized limulus amebocyte lysate (sensitivity, 0.06 endotoxin units/ml) (Associates of Cape Cod, Woods Hole, Mass.). The mixture was incubated for 60 minutes in a 37° C motionless water bath. The positive control consisted of Associates of Cape Cod control standard lipopolysaccharide *Escherichia coli* 0113, and the negative control consisted of pyrogen-free water. The formation of an adherent gel was scored as a positive result. The absence of a clot was considered indicative of a negative result. These tests were performed immediately after collection of the amniotic fluid, and the results were not used for patient management.

Gram stain examination. Gram stain examinations were performed with commercial reagents (crystal violet, saffranin, and Gram's iodine [Difco Laboratories, Detroit, Mich.]) under standard conditions. Stained slides were then examined by trained technologists, and the presence or absence of microorganisms was noted. The results of the Gram stain examinations were shared with the clinicians. Patients with negative Gram stain examinations for bacteria were given tocolysis. Patients with positive Gram stain examinations of amniotic fluid for bacteria were given parenteral antibiotics and allowed to labor. A positive Gram stain for bacteria was an indication for discontinuation of tocolysis.

Criteria for the diagnosis of chorioamnionitis, puerperal endometritis, and neonatal sepsis. Intraamniotic infection was diagnosed by the presence of a positive amniotic fluid culture.[1] Clinical chorioamnionitis was defined according to the criteria proposed by Gibbs et al.[6] Neonatal sepsis was diagnosed by the presence of a positive culture of blood, urine, or cerebrospinal fluid. Possible neonatal sepsis was diagnosed in the absence of a positive culture when two or more of the following criteria were present: white blood cell count of $<5000/mm^3$, polymorphonuclear leukocyte count of $<1800/mm^3$, I:T ratio (ratio of bands to total neutrophils) >0.2, or a positive gastric aspirate for polymorphonuclear leukocytes showing >5 white blood cells/high-power field. These criteria have been previously used in the pediatric and obstetric literature.

Statistical analysis. Diagnostic indexes (sensitivity, specificity, positive, and negative predictive values) for the detection of a positive amniotic fluid culture were calculated for both the Gram stain and limulus amebocyte lysate test. Comparisons between proportions were performed with a χ^2 test or Fisher's exact test. Sensitivity and specificity comparisons between the limulus amebocyte lysate and the Gram stain were performed with a modified t test. A Mann-Whitney test was used to compare cervical dilatation between groups because this variable was not normally distributed.

Results

Amniocenteses were performed on 264 women admitted with the diagnosis of preterm labor. Amniotic fluid was obtained in all cases. The prevalence of intraamniotic infection in women admitted with preterm labor was 9.1% (24/264). A total of 111 (42%) women delivered preterm neonates, and 24 (21.6%) of those had positive amniotic fluid cultures.

Table I illustrates the results of the Gram stain of

Table II. Gram stain and limulus amebocyte lysate test results according to the classification of organisms and culture results

	Gram stain			*Limulus amebocyte lysate*		
	Positive	*Negative*	*Sensitivity (%)*	*Positive*	*Negative*	*Sensitivity (%)*
Pure Gram (+)	3	1	75	2	2	50
Pure Gram (−)	5	1	83.3	4	2	66.7
Mixed infection	9	1	90	5	5	37.5
Candida species	1	0	—	1	0	—
Mycoplasma species*	1	2	33.3	0	3	0
Uninfected	1	239	—	1	239	—

**M. hominis* and *U. urealyticum.*

Table III. Microbiology and diagnostic tests in patients with positive amniotic fluid cultures

Patient No.	*Name of organisms*	*cfu/ml*	*Gram stain*	*Limulus amebocyte lysate*
1350	*U. urealyticum*	$>10^5$	+	−
	Fusobacterium species	5,000		
1382	*U. urealyticum*	>80,000	−	−
1668	*U. urealyticum*	$>10^5$	+	+
	Fusobacterium species	$>10^5$		
1587	*M. hominis*	1+	+	−
	Bacteroides species	$>10^5$		
	Peptococcus species	$>10^5$		
	U. urealyticum	1+		
1588	*M. hominis*	$>10^5$	−	−
	U. urealyticum			
1711	*M. hominis*	$>10^5$	+	−
2014	*M. hominis*	$>10^5$	+	+
	U. urealyticum	$>10^5$		
	G. vaginalis	2+		
1297	*Fusobacterium* species	>25,000	−	+
1308	Viridans streptococci	$>10^5$	+	−
1333	*G. vaginalis*	$>10^5$	+	−
1361	*G. vaginalis*	$>10^5$	+	−
1364	*Fusobacterium* species	1+	+	+
1393	*Bacteroides* species (four varieties)	$>10^5$	+	+
1411	*Streptococcus agalactiae*	$>10^5$	+	+
1445	Mixed anaerobic flora	$>10^5$	+	−
	Mixed aerobic flora	1+		
1616	*Bacteroides* species	$>10^5$	+	+
	Peptococcus species	>1,600		
1641	Mixed aerobic and anaerobic flora	1+	+	−
1851	Mixed anaerobic flora, gram-negative rods of three varieties	>45,000	+	−
1878	*S. aureus*	$>10^5$	−	−
1881	*Fusobacterium* species	$>10^5$	+	+
1941	*Peptostreptococcus*	$>10^5$	+	+
1995	Mixed anaerobic flora	$>10^5$	−	+
2012	Mixed anaerobic flora	>5,000	+	+
2055	*Candida tropicalis*	>8,000	+	+

amniotic fluid and the limulus amebocyte lysate in the detection of a positive amniotic fluid culture. The Gram stain had a sensitivity of 79.1% (19/24), a specificity of 99.6% (239/240), a positive predictive value of 95% (19/20), and a negative predictive value of 98% (239/244). One patient had a positive Gram stain of amniotic fluid but a negative culture. The patient had a cervical dilatation of 6 cm at the time of amniocentesis, did not have any clinical evidence of infection, and delivered spontaneously. She did not have clinical evidence of endometritis, and the newborn had no evidence of sepsis. Of the five patients with false-negative Gram stains, two had intraamniotic infections caused by *Mycoplasma* species (these organisms are not visible

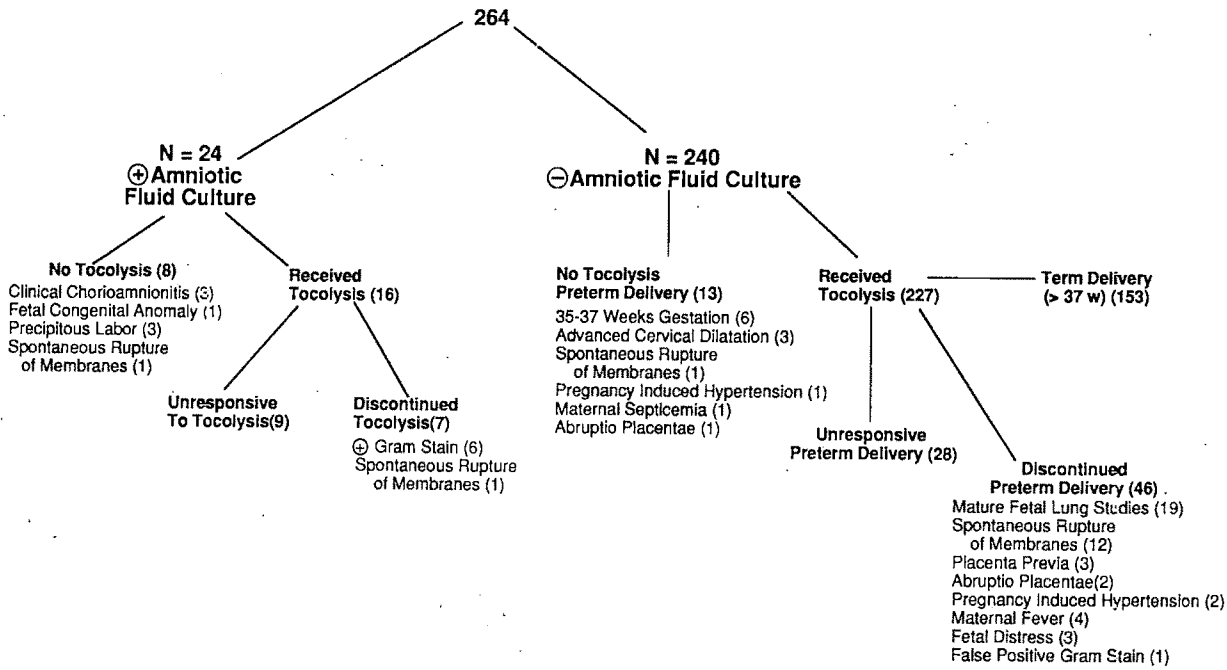


Fig. 1. Amniotic fluid culture and outcome of tocolysis of all patients in this study. *PIH,* Pregnancy-induced hypertension.

in a Gram stain). The remaining three false-negative stains occurred in one patient with *Fusobacterium nucleatum* with 25,000 colony-forming units (cfu) per milliliter, one with mixed flora, and one with *Staphylococcus aureus* ($>10^5$ cfu/ml).

This table also describes the results of the limulus amebocyte lysate assay and culture results. The limulus amebocyte lysate test had a sensitivity of 50% (12/24), a specificity of 99.6% (239/240), a positive predictive value of 92.3% (12/13), and a negative predictive value of 95.2% (239/251). One patient had a positive limulus amebocyte lysate test but a negative culture. This patient underwent a repeat amniocentesis four days later because of premature rupture of membranes. The second culture was positive for *Streptococcus viridans* ($>10^5$ cfu/ml).

Finally, Table I illustrates the results of combining the Gram stain of amniotic fluid with the limulus amebocyte lysate assay. The sensitivity was 87.5% (21/24), specificity was 99% (238/240), positive predictive value was 91% (21/23), and negative predictive value was 98.8% (238/241). The combined use of the Gram stain and the limulus amebocyte lysate did not result in a significant improvement in sensitivity with this sample size ($p > 0.05$).

Table II displays the results of the limulus amebocyte lysate and the Gram stain according to the classification of the microorganisms and culture results. The sensitivity of the Gram stain and limulus amebocyte lysate in detecting positive amniotic fluid cultures in pure infection for gram-positive or gram-negative bacteria was not different.

Table III illustrates the results of quantitative and qualitative microbiology, Gram stain, and limulus amebocyte lysate results in all 24 patients with positive amniotic fluid cultures. The three most frequent isolates were *U. urealyticum* ($n = 6$), *Fusobacterium* species ($n = 5$), and *M. hominis* ($n = 4$). Fifty percent (12/24) had more than one organism isolated from the amniotic fluid. Seventy-one percent (17/24) of all patients with positive amniotic fluid cultures had an inoculum size with more than 10^5 cfu/ml.

Table IV displays maternal clinical characteristics and complications according to amniotic fluid culture results. Women with positive amniotic fluid cultures had lower gestational ages and more advanced cervical dilatation on admission than did women with negative amniotic fluid cultures. All patients with abruptio placentae and toxemia of pregnancy had negative amniotic fluid cultures.

Fig. 1 shows the administration and response to tocolysis according to the results of the amniotic fluid culture. Successful tocolysis was more common in women with negative amniotic fluid cultures than in women with positive amniotic fluid cultures (84.5% [153/181] versus 0% [0/9]; $p < 0.001$, Fisher's exact test).

Table V shows the neonatal outcome according to culture results. Neonates born to mothers with intra-amniotic infections had lower gestational ages and birth weights than those born to mothers with negative amniotic fluid cultures. The incidence of respiratory distress syndrome, neonatal infectious morbidity, and low Apgar scores was greater in newborn infants of mothers

Table IV. Maternal demographic data and complications according to amniotic fluid culture in the population of infants delivered preterm

	(+) Amniotic fluid culture	*(−) Amniotic fluid culture*	*p value*
No. of patients	24	87	
Maternal age (mean in yr)	28 ± 6	25 ± 6	NS
Primipara	7 (29.2%)	20 (23%)	
Gestational age at admission (wk)	29 ± 4.1	32 ± 3.8	<0.01
Cervical dilatation (median in cm)	3 (range, 0-8)	2 (range, 0-8)	0.02
Interval between amniocentesis and delivery (median in hr)	6 (range, 2-264)	48	<0.0001
Clinical chorioamnionitis	3/24 (12.5%)	1/87 (1.2%)	<0.03
Toxemia of pregnancy	0	6	NS
Abruptio placentae	0	7	NS
Endometritis after cesarean section	0/3	4/27	NS
Endometritis after vaginal delivery	2/21	0/60	NS

Table V. Neonatal outcome according to culture results in the population of infants delivered preterm

	(+) Amniotic fluid culture (%)	*(−) Amniotic fluid culture (%)*	*p value*
No. of neonates	24	87	
Gestational age at birth (mean in wk)	29.4 ± 5.0 (SD)	32.6 ± 3.7 (SD)	0.0004
Birth weight (mean in gm)	1471 ± 761 (SD)	2188 ± 734 (SD)	0.0001
Neonatal deaths	2/21 (9.5)	9/71 (12.7)	NS
Respiratory distress syndrome	13 (54.2)	20 (22.9)	0.009
Infectious morbidity (sepsis,* possible sepsis, pneumonia, and meningitis)	6 (25.0)	3 (3.4)	0.008
Small for gestational age	0	1	
Intraventricular hemorrhage	5 (20.1)	7 (8.0)	NS
Congenital anomalies	2 (8.3)	5 (5.8)	NS
Apgar <7, 1 min	15 (62.5)	26 (29.9)	0.006
Apgar <7, 5 min	12 (50)	14 (16.1)	0.002

*One case.

with positive amniotic fluid cultures. A positive blood culture was documented in one neonate born to a mother with an intraamniotic infection. The amniotic fluid grew *Gardnerella vaginalis, M. hominis,* and *U. urealyticum,* and the neonatal blood culture was positive for *G. vaginalis.* On the other hand, one neonate born to a mother with a negative amniotic fluid culture had a positive blood culture for *Candida albicans.* The interval between amniocentesis and delivery was 72 hours, and results of histologic examination of the placenta were negative for chorioamnionitis. Two congenital anomalies were identified in the group of fetuses with positive amniotic fluid cultures (ascites and a bowel obstruction) and five in the group with negative amniotic fluid cultures (four cases of multiple congenital anomalies and one case of triploidy associated with a partial mole).

Comment

The prevalence of positive amniotic fluid cultures in the present study was 9.1%. Previous reports that used amniocentesis to determine the microbiologic state of the amniotic cavity in patients with preterm labor and intact membranes have documented a positive culture mean rate of 13.6% (46/339), with a range from 0% to 25.8%.[7] The disparity among different series is probably attributable to a combination of factors, including the definition of preterm labor, microbiologic techniques (i.e., cultures for *Mycoplasma* species), and the threshold required to perform an amniocentesis in patients in preterm labor among the different centers.

To circumvent the problems associated with differences in the diagnosis of preterm labor among the reports, we have analyzed the data by looking at patients with preterm labor leading to preterm delivery. In this case, the prevalence of positive amniotic fluid cultures is 21.6%. Of interest is that these findings are compatible with those of pathologic studies of the placenta. Guzick and Winn[15] found a 20.9% prevalence of histologic chorioamnionitis in patients delivering preterm infants without premature rupture of membranes. This information implies that one of every five preterm births occurring in women with premature labor and intact membranes is associated with intraamniotic infection. These data have major implications for the understanding and management of preterm labor.

The natural history of intraamniotic infection has not been well established. Several authors have suggested

Table VI. Prevalence of intraamniotic infection and microbiology in patients with preterm labor and intact membranes

Author	No. of patients	Gestational age (wk)	Indication for amniocentesis	Positive AF cultures		Microbiology study for Mycoplasma species
				N	%	
Bobitt et al.[7]	31	<35	Diagnosis of preterm labor after period of observation not stated	8	25.8	No
Wallace and Herrick[8]	25	26-34	Preterm labor after variable period of observation	1	4	No
Wahbeh et al.[9]	33	<35	Preterm labor	7	21.2	No
Hameed et al.[10]	37	24-34	Preterm labor suitable for tocolysis	4	10.8	No
Weible and Landall[11]	35	24-34	Preterm labor	1	2.9	No
Leigh and Garite[12]	59	<37	Preterm labor	7	11.8	No
Gravett et al.[13]	54	<35	Preterm labor	13	24	Yes
Duff and Kopelman[14]	24	20-35	Preterm labor	1	4.2	No
Romero et al.[16]	41	<36	Preterm labor	4	9.7	Yes
TOTAL	339			46	13.6	

AF, Amniotic fluid.

that women with positive amniotic fluid cultures and preterm labor are more likely to be refractory to tocolysis, to rupture membranes spontaneously, and to develop clinical chorioamnionitis than women with negative amniotic fluid cultures (reviewed in reference 1). Our data support these claims, since women with positive amniotic fluid culture were more likely to be refractory to tocolysis and develop clinical chorioamnionitis. A limitation of our study is that the result of the Gram stain examination of amniotic fluid was used in patient management; therefore the natural history of intraamniotic infection was altered. However, the net effect of this intervention was to remove patients with a larger inoculum size. Therefore our data indicate that refractoriness to tocolysis occurs even in patients with small microbial colony counts in the amniotic fluid. No empiric data suggest that women with a larger inoculum size behave differently.

The sensitivity of the Gram stain of amniotic fluid for the detection of intraamniotic infection was considerably better than our previous report (79.1% versus 44.8%). However, that study was conducted predominantly in patients with ruptured membranes. The improvement in the sensitivity noted in this study is probably attributable to greater experience with the test and the greater proportion of patients with an inoculum size of greater than 10^5 cfu/ml. Three of the five false-negative Gram stains occurred in patients with intraamniotic infections with *Mycoplasma* species. *Mycoplasma* species are not visible with the Gram stain. It is of note that one patient with an intraamniotic infection with *M. hominis* had a positive Gram stain for bacteria. This can be interpreted as either a false-positive stain or failure of the culture to grow fastidious microorganisms. The correlation between quantitative microbiology and Gram stain results has been previously discussed.[16] In amniotic fluid, as in other body fluids, an inoculum size $>10^5$ cfu/ml is associated with a positive Gram stain in 80% of the cases.[14] In this report, 15 of 17 patients with more than 10^5 cfu/ml had positive Gram stains of amniotic fluid (88%). The only false-positive Gram stain occurred in a patient who was dilated 6 cm and had a spontaneous delivery.

The diagnostic indexes of the limulus amebocyte lysate test were similar to those previously reported by our laboratory.[8] All patients with endotoxin in the amniotic fluid as detected by the limulus amebocyte lysate went on to deliver spontaneously. Although most of these patients had positive Gram stains of amniotic fluid and therefore did not receive tocolysis, one patient with a negative Gram stain and positive limulus amebocyte lysate test was refractory to tocolysis. The culture of amniotic fluid was positive for *Fusobacterium* species.

We have previously reported that the combined use of the limulus amebocyte lysate and the Gram stain increases the sensitivity of each test for the detection

Organisms isolated
Candida (n = 2); *Pseudomonas* (n = 1), *Bacteroides* (n = 2); *Coccobacillus* (n = 1); *Veillonella* (n = 1); enterococci group D (n = 1)
Bacteroides and *Fusobacterium* species (n = 1)
Fusobacterium (n = 3); *Bacteroides* (n = 2); *Lactobacillus* (n = 1); *Propionibacterium* (n = 3); diphtheroids (n = 1); *Streptococcus* nonhemolytic (n = 1)
E. coli (n = 1); *S. epidermidis* (n = 1); *S. mitis* (n = 1)
Fusobacterium and *Bacteroides* species
Fusobacterium (n = 3); *Bacteroides* (n = 1); *Candida* (n = 1); *Streptococcus viridans* (n = 1); *L. monocytogenes* (n = 1)
Fusobacterium (n = 2); *U. urealyticum* (n = 6); *Mycoplasma* (n = 6); *Candida* (n = 1); *G. vaginalis* (n = 3); *Bacteroides* (n = 1)
Corynebacterium species (n = 1)
Streptococcus viridans (n = 1); *Enterococcus* (n = 1); *Fusobacterium* (n = 1); mixed anaerobic (n = 1); *Streptococcus* β-hemolytic

of intraamniotic infection.[8] In the current study, the addition of the limulus amebocyte lysate increased the sensitivity of the Gram stain from 79.1% to 87.5%. However, this increment is not statistically significant. We attribute this apparent discrepancy with our previous report to the significant improvement in the sensitivity of the Gram stain (44.8% in the previous study).[16]

An interesting finding in our current study is that several patients had a positive limulus amebocyte lysate assay of amniotic fluid in the absence of gram-negative bacteria (Table II). We had documented similar findings in a study of women with preterm premature rupture of membranes.[17] These observations could be explained by (1) failure of fastidious gram-negative organisms to grow in culture, (2) presence of endotoxin in amniotic fluid from nonviable gram-negative bacteria, or (3) presence of other microbial products in amniotic fluid that stimulate the gelation of the limulus amebocyte lysate. Indeed, peptidoglycans from gram-positive bacteria, mannan from yeast, and lipoglycans from *Ureaplasma* species can yield a positive limulus amebocyte lysate test. However, the procedure used to purify these materials cannot exclude endotoxin contamination. It has been estimated that 1000 to 400,000 times higher concentrations of peptidoglycans than endotoxin are required to produce a positive limulus amebocyte lysate test. Contamination of these microbial preparations with as little as 0.00025% of endotoxin could account for the reported findings. Such low degrees of contamination cannot be ruled out in any material and may be found in "purified material."

A major finding of the current study is that the three most frequently isolated microorganisms from amniotic fluid are *Fusobacterium* species, *M. hominis*, and *U. urealyticum*. Previous studies have also hinted at the importance of *Fusobacterium* species in this setting (Table VI). The high prevalence of *M. hominis* and *U. urealyticum* was an unexpected observation; however, these findings are consistent with those found after culturing the membranes of patients with histologic chorioamnionitis.[18] This observation has clinical importance. First, microbiologic studies of the amniotic cavity that do not include cultures for *Mycoplasma* species may underestimate the rate of intraamniotic infection. Second, treatment of intraamniotic infection may require the addition of agents that are active against these organisms. Third, many of the suspected but not confirmed cases of neonatal sepsis may be caused by *Mycoplasma* organisms. Indeed, recent data indicate that these organisms have been isolated from the cerebrospinal fluid and pulmonary secretions of neonates.[19, 20]

Our results indicate that clinical chorioamnionitis is an insensitive indicator of intraamniotic infection. Indeed, only 12.5% of the women with positive amniotic fluid cultures met the clinical definition of chorioamnionitis; therefore reliance on standard clinical signs of infection results in an underestimation of the association between intraamniotic infection and preterm labor.

Another finding of this study is that neonates born to women with positive amniotic fluid cultures had lower gestational ages and a greater incidence of neonatal complications (respiratory distress syndrome and infectious morbidity) than preterm neonates of women with negative amniotic fluid cultures. The greater incidence of respiratory distress syndrome is probably related to the significantly lower gestational age at birth. Similar findings have been reported by other investigators.[7, 9, 12, 13]

Advanced cervical dilatation on admission has been traditionally considered a poor prognostic indicator of response to tocolysis. Our study indicates that women with preterm labor and intraamniotic infection had greater cervical dilatation than women with negative amniotic fluid cultures. Therefore it is possible that the unresponsiveness to tocolysis in women with advanced cervical dilatation is at least partially attributable to the presence of an intraamniotic infection.

One third of preterm neonates are born to women with preterm labor and intact membranes, a second third to patients with preterm PROM, and the remaining third to women with maternal or fetal indications for premature delivery. Our data suggest that in the

setting of preterm labor with intact membranes 22% of deliveries occurred to mothers with a positive amniotic fluid culture, while in the setting of preterm PROM 55% of patients will have a positive amniotic fluid culture at the time of the onset of parturition.[21] On the basis of these figures, we estimate that at least one of every four preterm deliveries occurs to a woman with an intraamniotic infection. We conclude that an intrauterine infection, which is often subclinical in nature, is a major etiologic factor for preterm delivery.

REFERENCES

1. Romero R, Mazor M. Infection and preterm labor. Clin Obstet Gynecol 1988;31:553-84.
2. Bobitt JR, Ledger WJ. Amniotic fluid analysis: its role in maternal and neonatal infection. Obstet Gynecol 1978; 51:56-62.
3. Iams JD, Clapp DH, Contos DA, et al. Does extra-amniotic infection cause preterm labor? Gas-Liquid chromatography studies of amniotic fluid in amnionitis, preterm labor, and normal controls. Obstet Gynecol 1987;70:365-8.
4. Miller JM, Pupkin MJ, Hill GB. Bacterial colonization of amniotic fluid from intact fetal membranes. Am J Obstet Gynecol 1980;136:795-804.
5. Romero R, Kadar N, Hobbins JC, Duff GW. Infection and labor: the detection of endotoxin in amniotic fluid. Am J Obstet Gynecol 1987;157:815-9.
6. Gibbs RS, Blanco JD, St Clair PJ, Castaneda YS. Quantitative bacteriology of amniotic fluid from women with clinical intraamniotic infection at term. J Infect Dis 1982;145:1-8.
7. Bobitt JR, Hayslip CC, Damato JD. Amniotic fluid infection as determined by transabdominal amniocentesis in patients with intact membranes in premature labor. Am J Obstet Gynecol 1981;140:947-52.
8. Wallace RL, Herrick CN. Amniocentesis in the evaluation of premature labor. Obstet Gynecol 1981;57:483-6.
9. Wahbeh CJ, Hill GB, Eden RD, et al. Intra-amniotic bacterial colonization in premature labor. Am J Obstet Gynecol 1984;148:739-43.
10. Hameed C, Tejani N, Verma UL, et al. Silent chorioamnionitis as a cause of preterm labor refractory to tocolytic therapy. Am J Obstet Gynecol 1984;149:726-30.
11. Weible DR, Randall HW. Evaluation of amniotic fluid in preterm labor with intact membranes. J Reprod Med 1985;30:777-80.
12. Leigh J, Garite TJ. Amniocentesis and the management of premature labor. Obstet Gynecol 1986;67:500-6.
13. Gravett MG, Hummel D, Eschenbach DA, et al. Preterm labor associated with subclinical amniotic fluid infection and with bacterial vaginosis. Obstet Gynecol 1986;67:229-37.
14. Duff P, Kopelman JN. Subclinical intraamniotic infection in asymptomatic patients with refractory preterm labor. Obstet Gynecol 1987;69:756-9.
15. Guzick DS, Winn K. The association of chorioamnionitis with preterm delivery. Obstet Gynecol 1985;65:11-6.
16. Romero R, Emamian M, Quintero R, et al. The value and limitations of the Gram stain examination in the diagnosis of intraamniotic infection. Am J Obstet Gynecol 1988;159:114-9.
17. Romero R, Roslansky P, Oyarzun E, et al. Labor and infection. II. Bacterial endotoxin in amniotic fluid and its relationship to the onset of preterm labor. Am J Obstet Gynecol 1988;158:1044-9.
18. Hillier SL, Martius J, Krohn M, Kiviat N, Holmes KK, Eschenbach DA. A case-control study of chorioamnionic infection and histologic chorioamnionitis in prematurity. N Engl J Med 1988;319:972-8.
19. Waites KB, Rudd PT, Crouse DT, et al. Chronic *Ureaplasma urealyticum* and *Mycoplasma hominis* infections of central nervous system in preterm infants. Lancet 1988;1:17-21.
20. Cassell GH, Waites KB, Crouse DT, et al. Association of *Ureaplasma urealyticum* infection of the lower respiratory tract with chronic lung disease and death in very-low-birth–weight infants. Lancet 1988;2:240-4.

Diagnosis of birth asphyxia on the basis of fetal pH, Apgar score, and newborn cerebral dysfunction

Larry C. Gilstrap III, MD, Kenneth J. Leveno, MD, Jody Burris, RN, M. Lynne Williams, RN, and Bertis B. Little, PhD

Dallas, Texas

Imprecise diagnosis of birth asphyxia coupled with uncertainties about causal factors for neurologic abnormalities in the newborn have greatly fueled the current litigation crisis in obstetrics. Our goal was to more precisely define birth asphyxia based on fetal condition as measured by umbilical artery blood pH, Apgar scores, and neurologic condition of newborns. We selected for study 2738 patients with singleton pregnancies with cephalic presentations who were delivered of infants at term to avoid complications such as prematurity, which may affect infant outcome independent of birth condition. The basis for study of these particular patients were defined criteria for high risk and an indicated arterial cord pH value. A total of five infants demonstrated cerebral dysfunction as evidenced by seizures during the neonatal period. Infection was linked to seizures in three of these infants; one infant had neonatal asphyxia and only one infant's clinical course could be attributed solely to birth events (uterine rupture). Stratification of umbilical artery blood pH values, Apgar scores, and combinations of these dependent variables in relation to newborn clinical outcomes revealed that infants must be severely depressed at delivery before birth asphyxia can be reliably diagnosed. Such depression includes Apgar scores ≤3 at 1 and 5 minutes plus umbilical artery pH values <7.00. (Am J Obstet Gynecol 1989;161:825-30.)

Key words: Birth asphyxia, acidemia, Apgar score

Perinatal and *asphyxia* are overused terms in current obstetric-legal parlance. Typically these terms are used to implicate "birth asphyxia" as the sole cause of neurologically damaged infants. Imprecise definition of these terms coupled with uncertainties about causal factors for neurologic abnormalities in the newborn have greatly aided the current litigation crisis in obstetrics.

The term *perinatal* may be used to denote many different time periods during gestation. For example, according to *Webster's Dictionary,*[1] perinatal refers to "the period around the time of birth." However, this term is defined in *Dorland's Medical Dictionary*[2] as "beginning with completion of the twentieth to twenty-eighth week of gestation and ending 7 to 28 days after birth." The term *asphyxia* is also variously defined in these dictionaries. In *Webster's Dictionary,*[1] asphyxia is defined as "a lack of oxygen or excess of carbon dioxide that is usu. caused by interruption of breathing and that causes unconsciousness." According to *Dorland's Medical Dictionary,*[2] asphyxia is a "lack of oxygen in respired air, resulting in impending or actual cessation of apparent life." Other commonly used terms include *hypoxia,* defined as low content of oxygen, or *ischemia* to signify reduced perfusion.[3] Such variable and therefore imprecise definitions, when applied by laymen to neurologically damaged newborns, are usually accepted to signify that the infant's outcome was a result of oxygen deprivation during the birth process or "birth asphyxia."

The most common cause of hypoxia in the fetus is hypoperfusion, or ischemia.[3] Many investigators now emphasize that very recent hypoxia-ischemia can only be implicated as a cause of brain damage when central nervous system signs and symptoms are present in the immediate newborn period.[3-5] Cerebral dysfunction or encephalopathy in the newborn may be defined as a syndrome in which tone and primitive reflexes are altered from the norm and there is evidence of cerebral irritation manifested by seizure activity.[3, 6] Long-term neurologic outcome is related to the severity and duration of these signs in the newborn. For example, in a study by Robertson and Finer[5] seizures in the immediate newborn period were a marker for abnormal neurologic sequelae.

Ideas about the obstetric antecedents of cerebral palsy are dangerously simplistic[3, 7] and we sought to more precisely define "birth asphyxia" on the basis of both fetal condition at birth and clinical performance of the newborn in the nursery. Our goal was to correlate immediate neurologic outcomes of term infants with

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of Texas Southwestern Medical Center.

Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1989.

Reprint requests: Larry C. Gilstrap, III, MD, Department of Obstetrics and Gynecology, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75235-9032.

6/6/13711

Table I. Maternal demographic in 2738 term pregnancies with cephalic presentation and live births

Characteristic	*No.*	*%*
Age (yr)		
≤19	759	28
29-29	1597	58
30-39	368	13
≥40	14	0.5
Race		
Hispanic	1092	40
Black	866	32
White	741	27
Other	39	1
Nulliparous patients	1203	44
Prenatal care	2412	88

Table II. Results of umbilical artery blood pH determinations in 2738 singleton live births delivered at term

Umbilical artery blood pH	*Live births*	
	No.	*%*
≥7.25	1833	67
7.20-7.24	520	19
7.15-7.19	217	8
7.10-7.14	90	3.2
7.00-7.09	60	2.2
<7.00	18	0.6

fetal acid-base status as measured in umbilical artery blood and newborn condition as reflected by Apgar score. Singleton term infants with cephalic presentations were selected for study to avoid complications such as breech presentation, which may affect newborn condition. Similarly, preterm births were excluded inasmuch as complications of prematurity such as respiratory disease and intracranial hemorrhage may affect infant outcome independent of birth events.

Material and methods

Between August 1, 1987, and February 28, 1988, blood was obtained from the umbilical artery for pH measurement in 2738 complicated pregnancies delivered at Parkland Memorial Hospital. These study participants were chosen from a total of 8678 patients who were delivered of infants at our institution during this time period. The following complications were used to identify pregnancies for umbilical blood pH determination: cesarean section, forceps delivery, meconium in the amnionic fluid, oxytocin stimulation of labor, and abnormal fetal heart rate (FHR). Umbilical artery specimens were drawn from doubly clamped cord segments into 3 ml plastic syringes flushed with 1000 U/ml of heparin solution and the specimens were transferred in ice to the hospital laboratory for analysis. Results of these analyses were prospectively computerized and linked to a similarly computerized obstetric data base.

Term singleton pregnancies, defined to include birth weights ≥2500 gm, morphologically normal infants, and cephalic presentations, were subsequently selected for analysis. Pediatric house officers attended all deliveries and assigned Apgar scores. Low Apgar scores were defined in this study as ≤6 at 1 and 5 minutes because this score represented the criteria commonly used to define mild asphyxia and as ≤3 at 1 and 5 minutes because this score repsented the criteria used to define severe asphyxia.[8] Newborn records were systematically reviewed by research nurses with expertise in neonatal special care, and clinical indices of immediate newborn clinical performance were prospectively computerized. Special attention was given to neurologic signs and symptoms and their time of onset. The diagnosis of seizures and hypotonia was based on assessments by the attending pediatricians. Only hypotonia that persisted 24 to 48 hours after birth was considered significant. Follow-up the infants after discharge was not available.

Tests for statistical significance were performed by means of χ^2 contingency tables or Fisher's probability (Center for Disease Control Statistical Analysis Package written by J. D. Erickson). Probability values ≤0.05 were considered significant.

Results

The maternal demographic characteristics of 2738 pregnancies that resulted in live births and were included in this investigation are summarized in Table I. These demographic features were similar to those of the general obstetric population at Parkland Hospital. Pregnancy complications in the study group reflected criteria for umbilical artery sampling and included prolonged rupture of membranes (2%), chorioamnionitis (5%), hypertension (16%), use of oxytocin (25%), cesarean section (44%), meconium in the amniotic fluid (45%), and electronic FHR monitoring (70%).

The three most common indications for cesarean section were repeat operation (42%), dystocia (25%), and fetal distress (18%). The majority (85%) of participants who were delivered of infants vaginally had spontaneous births and the remainder were assisted by forceps.

The results of umbilical artery blood gas determinations are summarized in Table II. The majority (86%) of infants had pH values ≥7.20 and 18 (0.6%) had PH values <7.00.

The relation between low 1-minute Apgar scores (≤6 and ≤3) and umbilical artery blood pH is depicted in Fig. 1. Only 143 (8%) of the 1833 newborns with an umbilical artery pH ≥7.25 had 1-minute Apgar scores

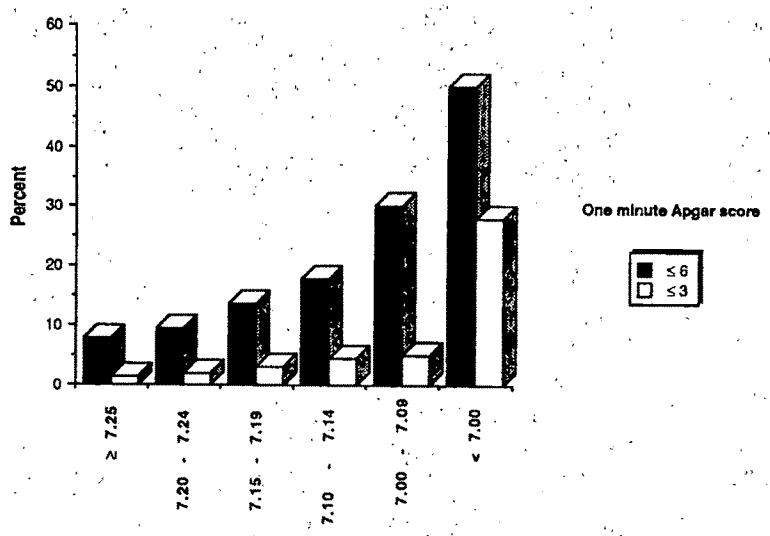


Fig. 1. Relation between low 1-minute Apgar scores and umbilical artery blood pH.

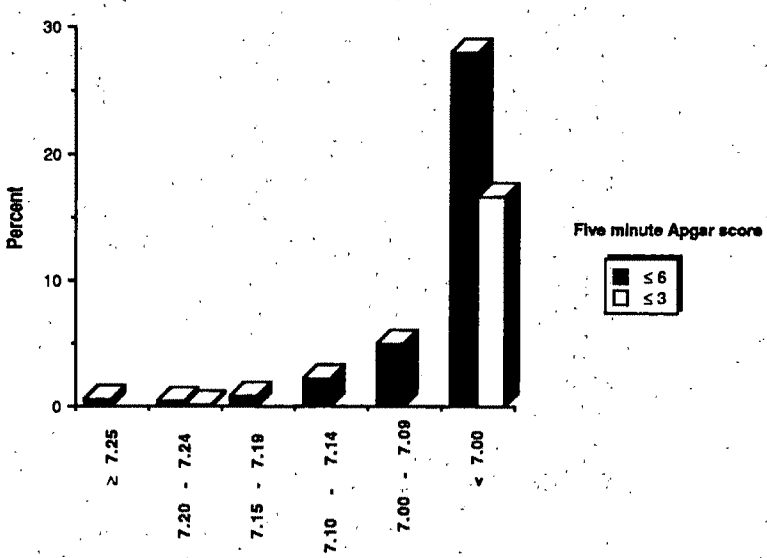


Fig. 2. Relation between low 5-minute Apgar scores and umbilical artery pH.

≤6. In contrast, 50% ($n = 9$) of those with a pH <7.00 had such Apgar scores at 1 minute ($p < 0.05$). There was a similar relation for 1-minute Apgar scores ≤3 (24 of 1833 or 1.3% versus 5 of 18 or 28%; $p < 0.05$). Shown in Fig. 2 is the distribution of these Apgar scores at 5 minutes according to pH. Apgar scores ≤6 at 5 minutes occurred in 10 of 1833 (0.5%) infants with pH ≥7.25 compared with 5 of 18 (28%) with a pH <7.00 ($p < 0.05$). The results for Apgar scores <3 at 5 minutes were 0 of 1833 with pH ≥7.25 versus 3 of 18 (17%) with pH <7.00 ($p < 0.05$). Apgar scores ≤3 at 10 minutes after birth were assigned to two infants and both had an umbilical artery blood pH <7.00.

Neonatal complications in relation to umbilical artery blood pH are summarized in Table III. With the exception of meconium aspiration syndrome, neonatal complications increased as the umbilical artery blood pH decreased and those with a pH <7.00 ($n = 18$) had singificantly more morbidity than did those in any other group. One third of these 18 infants required intubation, and the incidence of respiratory disease and hypotonia was 28% and 17%, respectively.

A total of five infants had seizures and these are summarized in Table IV. Four of these infants had seizures within the first 24 hours after birth and one convulsed on day 10. Three of the four neonates with early seizures had an umbilical artery blood pH <7.15 and two of these had a pH <7.00. Both of these infants

Table III. Selected neonatal complications in relation to umbilical artery blood pH values

	Umbilical artery blood pH value											
	>7.25 (n = 1833)		*7.20-7.24 (n = 520)*		*7.15-7.19 (n = 217)*		*7.10-7.14 (n = 90)*		*7.00-7.09 (n = 60)*		*<7.00 (n = 18)*	
Neonatal complications	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
Intubation in delivery room	13	0.7	6	1	2	1	4	4†	2	3	6	33‡
Respiratory disease*	45	3	16	3	13	6§	6	7‖	5	8¶	5	28#
Meconium aspiration syndrome	4	0.2	1	0.2	1	0.5	2	2	1	2	0	
Hypotonia	35	2	10	2	2	0.9	4	4	4	7**	3	17††

*Ambienit oxygen administered by either halo or mechanical ventilation.

χ^2 Statistical comparison to pH group ≥7.25:

†Odds ratio 6.5, $p < 0.007$

‡Odds ratio 70.0, $p < 0.0001$

§Odds ratio 2.5, $p < 0.006$

‖Odds ratio 2.8, $p < 0.03$

¶Odds raio 3.6, $p < 0.02$

#Odds ratio 15.3, $p < 0.0001$

**Odds ratio 3.7, $p < 0.03$

††Odds ratio 10.3, $p < 0.005$

had metabolic acidemia, defined as a bicarbonate level ≥17.3 mEq/l and a P_{CO_2} ≤65 mm Hg.[9] All four of the infants with early seizures had 1-minute Apgar scores ≤3 and 5-minute scores ≤6. As shown in Table IV, two of the infants with early seizure activity had neonatal complications (group B streptococcal sepsis and syphilis) associated with such neurologic dysfunction.

Inasmuch as Apgar scores and acid-base status may be dependent variables in the determination of the newborn condition, we analyzed combinations of these indices and the results are summarized in Table V. None of the 13 neonates with a pH <7.00 and a 1-minute Apgar score >3 had either seizures or hypotonia. There were 12 of these 13 neonates who had either respiratory or a mixed pattern of acidemia.[9] Conversely, among those with pH values ≥7.00 and an Apgar score ≤3, seven (14%) had hypotonia and two (4%) had a seizure. One infant had an umbilical artery blood pH of 7.11 and early onset group B streptococcal sepsis. Importantly, when infants had both low Apgar scores and pH values the frequency of serious neonatal morbidity significantly increased ($p < 0.05$). Specifically, all required resuscitation at birth and approximately one half demonstrated neurologic dysfunction.

Comment

Both the American College of Obstetricians and Gynecologists[10] and the American Academy of Pediatrics[11] have challenged use of the Apgar score alone to define birth asphyxia. As both of these organizations have emphasized, Apgar scores are unsatisfactory for the prediction of neurologic outcomes of newborns. It is important to recognize that there are several other factors that can account for low Apgar scores besides "asphyxia" such as gestational age, maternal medications, type of anesthetic administered, and the person who assigned the score.[9] In an attempt to more objectively define immediate newborn condition, several investigators have recommended use of umbilical blood acid-base determinations.[9, 12-14] However, reliance solely on newborn acid-base status to define birth asphyxia has been thwarted by the unrealistically high pH values used to define pathologic acidosis. For example, a commonly used definition of newborn acidosis or "acidemia" is umbilical blood pH <7.20, but the majority of such newborns wil be vigorous at birth and will not have obvious neonatal sequelae.[9, 15] This was borne out in our investigation; 385 infants (14%) were acidotic by this definition and yet the majority were vigorous at birth and without neurologic sequelae.

It also has been reported that there is a poor correlation between Apgar scores and umbilical blood pH values. Sykes and associates[12] defined pathologic acidosis as pH <7.11 and reported that 81% of infants with low 5-minute Apgar scores (<7) were not acidotic at birth; conversely, 73% of those who were acidotic had normal Apgar scores. Regardless, the Apgar scoring system continues to be the most commonly used method to define birth asphyxia. for example, according to *The International Classification of Disease,*[8] mild asphyxia is defined as a 1-minute Apgar score ≤6 and severe asphyxia is defined as a 1-minute Apgar score ≤3. If these definitions were used in the present study,

Table IV. Characteristics and neonatal outcome of five infants with seizures

Infant	*Day of seizure*	*Umbilical artery blood pH*	*Apgar scores**	*Complications*	*Outcome*
1	10	7.31	9, 9	rH isoimmunization, meningitis	Discharged day 18
2	1	7.11	3, 5, 7	Meconium aspiration, group B streptococcal sepsis	Died day 2
3	1	6.59	2, 3, 4	Congenital syphilis	Discharged day 11
4	1	7.20	3, 6, 8	Meconium aspiration, pneumothorax, pneumomediastinum	Died day 22
5	1	6.59	1, 3, 3	Uterine rupture	Discharged day 30; cerebral palsy

*Scores at 1 minute, 5 minutes, and 10 minutes.

Table V. Neonatal morbidity in relation to combination of low 1-minute Apgar scores and low umbilical artery blood pH values

	pH <7.00; Apgar score >3 (n = 13)			*pH ≥7.00; Apgar score ≤3 (n = 49)*			*pH <7.00 Apgar score ≤3 (n = 5)*	
Newborn complications	*No.*	*%*	*Comparison**	*No.*	*%*	*Comparison**	*No.*	*%*
Intubation	1	8	NS	8	16	0.0004	5	100
Admission to special care nursery	1	8	NS	15	31	NS	2	40
Respiratory disease	2	15	NS	13	27	NS	3	60
Hypotonia	0		NS	7	14	0.03	3	60
Seizures	0		NS	2	4	0.03	2	40

*Statistical comparison of adjacent groups shown in table.

267 or approximately 10% of the newborns would have been diagnosed to have mild asphyxia and 55 (2%) to have severe asphyxia. Yet virtually all of these infants were normal in the immediate newborn period.

Because neither the Apgar score nor acid-base status alone correlated well with neonatal morbidity, we analyzed the effects of combined low Apgar scores and low pH values. Of the newborn who had an Apgar score ≤3 and an umbilical artery blood pH <7.00, all resuscitation at birth and approximately one half had neurologic abnormalities that included hypotonia or seizures. No infant with an Apgar score >3 and a pH <7.00 had seizures. One infant with a 1-minute Apgar score of 3 and a pH of 7.11 had seizure activity in the first 24 hours of life. This infant had early onset group B streptococcal sepsis, which likely was the cause of hypoperfusion leading to brain damage and seizures. The combination of an Apgar score ≤3 and pH <7.00 was a sensitive predictor of all cases of serious neonatal morbidity.

Cerebral dysfunction as evidence by seizures in the newborn period may result from metabolic abnormalities (e.g., hypoglycemia), infection, malformations, trauma, or true birth asphyxia.[3] There were five (1.8 per 1000) infants in the present study who had significant neurologic sequelae (i.e., seizures) during the neonatal period. These five infants demonstrate the plurality of causes for newborn cerebral dysfunction. One newborn had a seizure on day 10 that was due to meningitis. Infection (group B streptococcal sepsis and congenital syphilis) was implicated in two other infants. The fourth infant had an umbilical artery blood pH of 7.20 at delivery and pulmonary insufficiency developed as a result of meconium aspiration. The pH of this infant decreased acutely to 6.91 soon after arrival in the nursery and the infant subsequently convulsed. This time sequence suggests neonatal asphyxia as opposed to birth asphyxia. The fifth infant had an umbilical artery blood pH of 6.59, 1- and 5-minute Apgar scores of 1 and 3, respectively, and was delivered by cesarean section because of fetal distress due to uterine rupture. This is the only infant in the entire study population who had seizures and no other associated causative factors for this brain disorder. Cerebral palsy was diagnosed in this infant before discharge.

It is evident from this investigation of 2738 term pregnancies with intrapartum complications that new-

born cerebral dysfunction manifested as seizures and attributable to true birth asphyxia is extremely rare. The study results now reported suggest the infant must be severely depressed at birth to implicate birth asphyxia as the sole cause of newborn seizures. Such depression includes (1) extremely low Apgar score ≤3, (2) umbilical artery blood pH <7.00, and (3) need for resuscitation at birth. Towbin[4] has also concluded that infants who sustain central nervous system damage are severely depressed at birth. He stated, "It is implausible to believe that cerebral palsy developing in an infant can be attributed to massive acute cerebral damage incurred intranatally in an infant who was in good clinical condition soon after delivery." Other also have observed that seizures within the first day of life may be a sign of newborn brain dysfunction as a result of asphyxia.[3, 16-18] This observation that newborn brain disorders can only be attributed to birth asphyxia when the infant is extremely ill at birth is also supported by animal studies. Such studies indicate that animals must have almost lethal hypoxia before encephalopathy and neurologic damage develop.[5, 19] We conclude that birth asphyxia that leads to newborn seizures is very rare and that such neurologic dysfunction may result from a plurality of causes. Caution must be exercised before attributing birth asphyxia as the sole explanation for seizures in the immediate newborn period. An infant who is asphyxiated at birth must be severely depressed to include Apgar scores ≤3, fetal pH <7.00, require resuscitation, and have seizures in the first day after birth. The impact of lesser degrees of asphyxia as measured by Apgar score and acid-base status at birth remains unknown. A limitation in the design of this study is that there is now follow-up beyond the newborn period and it is possible that other infants in the study group would later show signs of cerebral dysfunction. Thus long term follow-up studies are necessary to completely elucidate the role of birth events in the genesis of infant brain disorders. Until such information becomes available, we believe it is inappropriate to diagnose birth asphyxia unless the infant is both severely depressed and acidotic at birth.

REFERENCES

1. Webster's ninth new collegiate dictionary. Springfield, Massachusetts: Merriam-Webster, Inc., 1985.
2. Dorland's illustrated medical dictionary, 26th ed. Philadelphia: WB Saunders, 1985;129, 990.
3. Freeman JM, Nelson KB. Intrapartum asphyxia and cerebral palsy. Pediatrics 1988;82:240-9.
4. Towbin A. Obstetric malpractice litigation: the pathologists view. AM J OBSTET GYNECOL 1986;155:927-35.
5. Robertson C, Finer N. Term infants with hypoxic-ischemic encephalopathy: outcome at 3.5 years. Dev Med Child Neurol 1985;27:473-84.
6. O'Brien MJ, Ash JM, Gilday DL. Radionuclide brain-scanning in perinatal hypoxial ischemia. Dev Med Child Neurol 1979;21:161-5.
7. Hey E. Fetal hypoxia and subsequent handicap: the problem of establishing a causal link. In: Chamberlain GVP, Orr CJB, Sharp F, eds. Litigation and obstetrics and gynecology. London: Royal College of Obstetricians and Gynaecologists, 1985:223-42.
8. The international classification of diseases, 9th revision, clinical modifications, 2nd ed. Washington, D.C.: 1980; DHHS publication no. (PHS)80-1260.
9. Gilstrap LC, Hauth JC, Hankins GDV, Beck AW. Second stage fetal heart rate abnormalities and type of neonatal acidemia. Obstet Gynecol 1987;70:193-5.
10. American College of Obstetricians and Gynecologists Committee on Obstetrics (maternal-fetal medicine). Use and misuse of the Apgar score (ACOG committee on the fetus and the newborn). Washington, D.C.: Nov 1986.
11. American Academy of Pediatrics. Use and abuse of the Apgar score. Pediatrics 1986;7:1148-9.
12. Sykes GS, Johnson E, Ashworth F. Do Apgar scores indicate asphyxia? Lancet 1982;1:494-6.
13. Silverman F, Suidan J, Wasserman J, et al. The Apgar score: is it enough? Obstet Gynecol 1985;66:331-6.
14. Dijxhoorn MJ, Visser GHA, Fidler VJ, et al. Apgar score, meconium, and acidaemia at birth in relation to neonatal neurological morbidity in term infants. Br J Obstet Gynaecol 1986;93:217-22.
15. Suidan JS, Young BK. Acidosis in the vigorous newborn. Obstet Gynecol 1985;65:361-4.
16. Nelson KB, Ellenberg JH. Neonatal signs as predictors of cerebral palsy. Pediatrics 1979;64:225-32.
17. Mellits ED, Holden KR, Freeman JM. Neonatal seizures. II. A multivariate analysis of factors associated with outcome. Pediatrics 1982;70:177-84.
18. Nelson KB, Ellenberg JH. Antecedents of cerebral palsy: multivariate analysis of risk. N Engl J Med 1986; 315:81-6.
19. Meyers RE. Experimental models of perinatal brain damage: relevance to human pathology. In: Gluck L, ed. Intrauterine asphyxia and the developing fetal brain. Chicago: Yearbook Medical Publishers, Inc., 1977:37-97.

First-trimester midgut herniation: A high-frequency transvaginal sonographic study

Ilan E. Timor-Tritsch, MD, Wendy B. Warren, MD, David B. Peisner, MD, and Exenia Pirrone, LPN

New York, New York

The application of high-frequency transvaginal sonography offers new opportunities in scanning for malformations during the first trimester of pregnancy. Scanning of the anterior abdominal wall and the umbilical cord insertion at this gestational age is feasible. A cross-sectional study of 61 embryos-fetuses from 7 to 12 weeks of gestation was performed to observe the physiologic midgut hernia. This herniation was detected in 64% of the cases at 8 weeks, in 100% during weeks 9 and 10, and in 25% at 11 weeks' gestation. None of the fetuses studied at 12 weeks had a midgut herniation. Vaginal sonography of the well-dated fetus at 12 weeks can confirm the final development of the anterior abdominal wall. (Am J Obstet Gynecol 1989;161:831-3.)

Key words: Embryo, fetus, ultrasonography, transvaginal ultrasonography, ventral wall hernia, physiologic midgut hernia

High-frequency transvaginal transducer probes produce a high-resolution image of the first-trimester embryo.[1] Indications for vaginal scans in the first trimester include the detection of structural abnormalities. Familiarity with the potential of this technique is necessary to discover various anatomic defects. Relatively common birth defects of the ventral wall are omphalocele and gastroschisis, which occur at a rate of 1 in 4000 to 1 in 6000 live births. The normal process of the physiologic midgut herniation is well known. Timely diagnosis of a fetal ventral wall defect at the end of the first trimester would benefit the obstetric management of the patient and the surgical management of the fetus or neonate.

We focused our study on the complete disppearance of the physiologic midgut herniation to determine any deviation from the norm in patients who were at high risk for a congenital abdominal wall defect.

Material and methods

Sixty-one first-trimester pregnancies ranging from 7 to 12 weeks (inclusive) were scanned to observe the appearance or disappearance of the midgut herniation into the umbilical cord. Every patient was studied only once. A 6.5 MHz mechanical sector transducer probe (ESI-1000 Elscint, Inc., Haifa, Israel) was used.

From the Department of Obstetrics and Gynecology, and the Division of Ultrasound, Columbia University College of Physicians and Surgeons.

Presented at the Ninth Annual Meeting of the Society of Perinatal Obstetricians, New Orleans, Louisiana, February 2-4, 1988.

Reprint requests: Ilan E. Timor, MD, Department of Obstetrics and Gynecology, Columbia Presbyterian Medical Center, 622 West 168th St., Room 1212, New York, NY 10032.

6/6/14091

Gestational ages are given in menstrual weeks and days calculated from the first day of a certain and regular last menstrual period or the known date of conception. Crown-rump length was measured for each patient. The mean values for the gestational age in weeks and the patient distribution are given in Table I. We examined views of a transverse plane at the level of the abdominal insertion of the umbilical cord and a midsagittal section of the embryo to demonstrate the same anatomic area (Figs. 1 and 2). If the cross-sectional measurement at the immediate abdominal insertion of the cord was at least 1.5 times larger than the size of the cord, the presence of the midgut herniation was assumed.

Regardless of its position in the cord or in the abdominal cavity, before and after 12 weeks, respectively, the midgut could be easily located by its hyperechoic properties.

Results

The gestational ages by mean measurement of the crown-rump length correlated well (± 1 to 2 days); the mean gestational age was calculated by the clinical dating for each gestational week.

During the seventh week, the exact location of the abdominal insertion of the cord could not be detected. Therefore the presence or the absence of a midgut herniation could not be seen.

The thickening of the cord was clearly seen in 7 of the 11 (64%) fetuses studied throughout the eighth week.

All fetuses studied during the ninth and tenth weeks (11 and 7 cases, respectively) demonstrated a significant thickening of the cord at the level of the abdominal

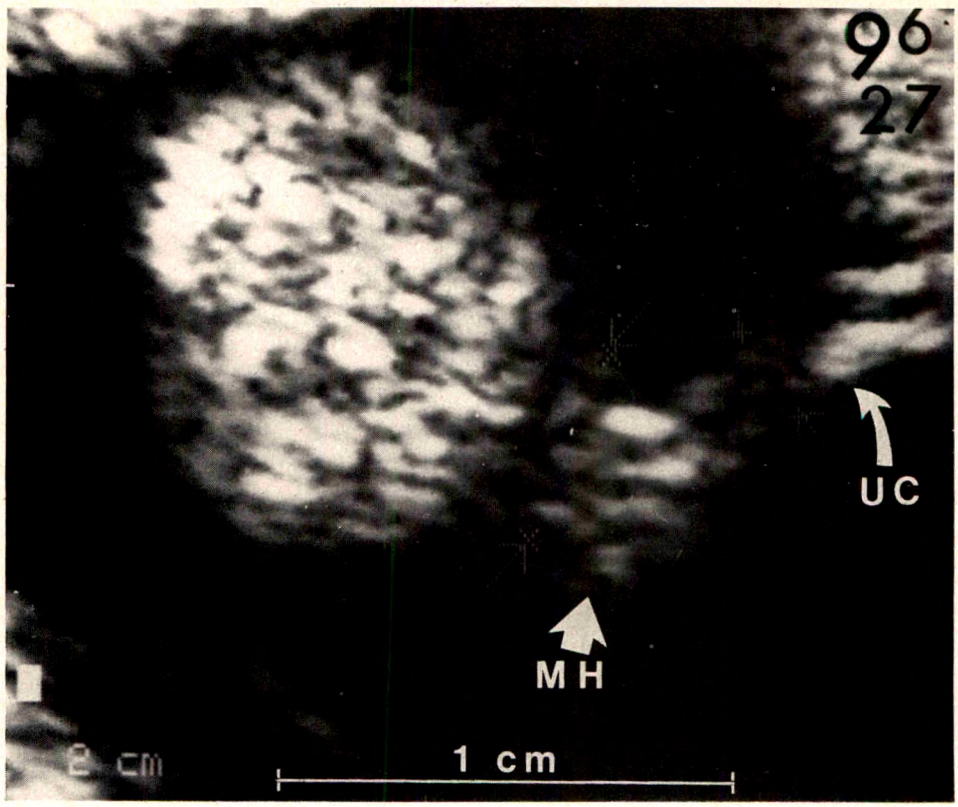


Fig. 1. Cross-section of 9-week 6-day fetus with crown-rump length of 27 mm at level of abdominal insertion of umbilical cord. Note that umbilical cord *(UC)* measures 2 mm and that diameter at its thickened abdominal insertion representing herniation of midgut *(MH)* is 4 mm.

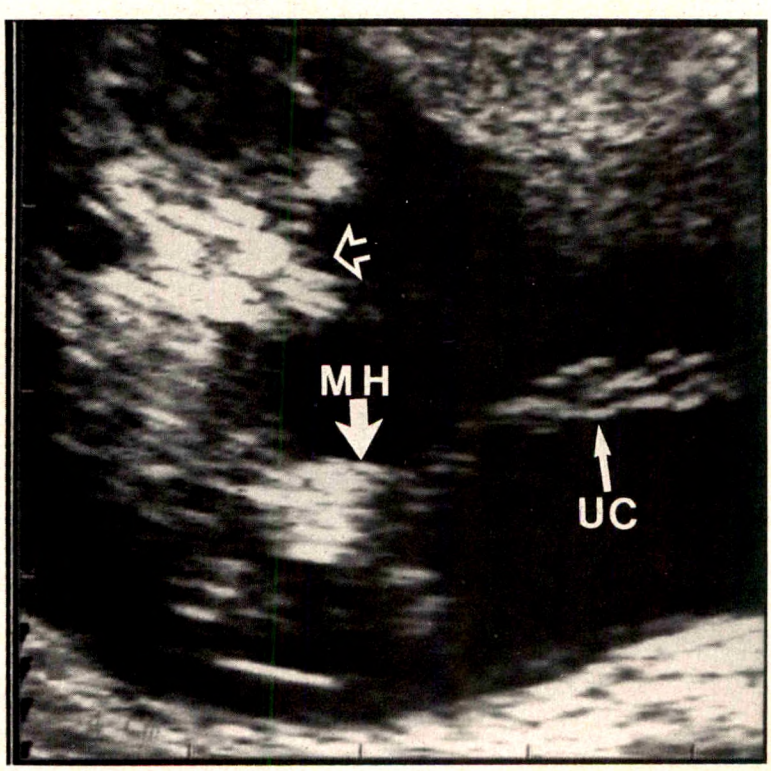


Fig. 2. Midsagittal section demonstrates difference in caliber between umbilical cord *(UC)* and hyperechogenic midgut herniation. Obvious high echogenicity of herniated midgut *(MH)* equals that generated by facial structures of face and upper limb *(open arrow)*.

Table I. Distribution of patient population

Gestation (wk)	*N*	*Mean gestational age by clinical dating*	*Mean crown-rump length (mm)*	*Gestational age by mean crown-rump length**	*Ventral hernia seen*
7	18	7 + 3	11.8	7 + 4	0
8	11	8 + 3	16.8	8 + 2	7 (64%)
9	11	9 + 3	26.0	9 + 3	11 (100%)
10	7	10 + 2	33.6	10 + 2	7 (100%)
11	8	11 + 2	48.0	11 + 5	2 (50%)
12	6	12 + 3	53.0	12 + 1	0
TOTAL	61				

*Gestational age expressed in weeks + days. Crown-rump length as determined by Robinson and Fleming.[4].

insertion. During this period it was relatively easy to image the finding.

Only four of the eight fetuses (25%) studied at 11 weeks demonstrated the midgut herniation. The remainder of the six fetuses were considered to have normal cord insertions.

The cord thickening containing the hyperechogenic content could not be imaged in any of the six fetuses scanned at 12 weeks.

Comment

Throughout its early embryologic development, the gut undergoes a counterclockwise rotation during which it is "forced" to assume an extracorporeal position within and along the base of the umbilical cord. This physiologic midgut herniation is probably caused by the relative lack of space created by the fast-growing liver during menstrual weeks 7 to 11.

The successful detection and significance of the physiologic midgut herniation by transabdominal ultrasonography has been reported.[2, 3] However, we project that the use of the high-frequency transvaginal ultrasonographic scanning method, when applied in the first and early second trimesters, will replace the ultrasonographic malformation workup currently done by transabdominal ultrasonography. The projected increase in the use of transvaginal scanning prompted us to evaluate its diagnostic abilities in imaging the physiologic midgut hernia.

The distinct hyperechogenicity of the bowel facilitated the identification of this well-defined structure in the cord before 11 to 12 weeks or within the abdominal wall during and after the twelfth week. The ventral herniation was not seen by vaginal ultrasonography in fetuses after 12 completed weeks of gestation. The particularly exaggerated curve of the fetal body before 8 weeks makes it difficult to identify the insertion of the umbilical cord; therefore the midgut herniation was not detected before 8 weeks.

The results of this study suggest that the final development of the fetal anterior abdominal wall, either normal or abnormal, can be seen by vaginal ultrasonography by 12 completed weeks, assuming that the gestation is well dated. Early identification of a ventral wall defect would allow time for further genetic evaluation of the fetus (i.e., chorionic villus sampling).

We conclude that vaginal ultrasonography by means of a higher-frequency probe, enables a reliable and relatively easy detection of the migrating midgut and its retraction into the fetal abdominal cavity before 12 weeks. This information is clinically important and useful.

REFERENCES

1. Timor-Tritsch IE, Farine D, Rosen MG. A close look at early embryonic development with the high-frequency transvaginal transducer. AM J OBSTET GYNECOL 1988; 59:676-81.
2. Schmidt W, Yarkoni S, Crelin ES, Hobbins JC. Sonographic visualization of physiologic anterior abdominal wall hernia in the first trimester. Obstet Gynecol 1987;69:911-5.
3. Green JJ, Hobbins JC. Abdominal ultrasound examination of the first-trimester fetus. AM J OBSTET GYNECOL 1988;159:165-75.

CORRESPONDENCE

Calcium intake and pregnancy-induced hypertension

To the Editors:

Belizan, Villar, and Repke in their article (The relationship between calcium intake and pregnancy-induced hypertension: up-to-date evidence. AM J OBSTET GYNECOL 1988;158:898-902) detailed the relationship of calcium intake and the incidence of pregnancy-induced hypertension (PIH). They noted that supplementation of 1.0 gm of elemental calcium was associated with the reduction of diastolic blood pressure in reproductive women. They also cited a study from Equador in which calcium supplementation was associated with a lower (6.5%) incidence of PIH, as compared with the placebo group (28.2%).

In addition to their studies, they sited several studies in which the calcium intake was associated with a lower incidence of PIH even in the poor (often-sted as high-risk group for PIH). No mention was made to the relevance and function of magnesium, a critical counterpart of calcium. I would like to know whether the magnesium levels in their patients were noted either in their observations or in those to which they referred.

I am surprised to learn that if indeed "it has been widely documented that there is an inverse relationship between calcium intake and blood pressure" that "to date, there are no available reports conclusively demonstrating this association" and that "it could be premature to recommend calcium supplementation to all pregnant women with the prevention of pregnancy-induced hypertension as the only objective."

On the basis of my understanding of the role of magnesium in the physiology of pregnancy, I have used supplementation of magnesium[1, 2] in all pregnant women and have observed beneficial effects on PIH (incidence and severity) and the effects on low birth weight rates[3] (premature and intrauterine growth retardation).

Stefan Semchyshyn, MD

22 Old Short Hills Road
Livingston, NJ 07039

REFERENCES

1. Altura BM, Altura BT, Carella A. Magnesium deficiency-induced spasms of umbilical vessels: relation to preeclampsia, hypertension, growth retardation. Science 1983;221: 376-8.
2. Conradt A, Weidinger H, Algayer H. The decrease of the incidence of preeclampsia in pregnancies treated with betamimetics and additional magnesium. Geburtshilfe Frauenheilkd 1984;44:118-23.
3. Semchyshyn S. Preventing prematurity, part II: managing early labor. Female Patient 1986;11:20-32.

Reply

To the Editors:

We thank Dr. Semchyshyn for his interest in our article and the opportunity to discuss some aspects of the relationship between magnesium load and prevention of pregnancy-induced hypertension (PIH).

As we mentioned in our article, magnesium reduces the transport of ionized calcium into the cell and then decreases the degree of tension of vascular smooth muscle cells. We hypothesized that the same effect is produced by a rise in calcium intake and could therefore explain why calcium and magnesium load could have beneficial effects on PIH.

We do not have experience with magnesium supplementation to prevent PIH but Dr. Semchyshyn quoted a retrospective study of Conradt et al.[1] that showed a reduction of the frequency of fetal growth retardation and PIH with magnesium supplementation. In a double-blinded randomized clinical trial of 568 pregnant women (278 with 15 μmol of magnesium aspartate-hydrochloride and 290 with a placebo) from the 16 weeks' gestation onward, Spälting and Spälting[2] showed no reduction in PIH in the supplemented group. However, they showed a reduction on the incidence of low birth weight infants (≤2500 gm) and a significant reduction to one half on the incidence of preterm deliveries (<37 weeks' gestation).

These observations in conjunction with the adjunt mechanisms of these two minerals on the intracellular calcium levels, encourage the performance of research on this subject with supplementation studies with a factorial design with independent and combined calcium and magnesium.

With regard to magnesium levels in our studies, no significant changes were evident in magnesium serum levels in calcium-deprived rats,[3] in calcium-supplemented young men and women,[4] and in pregnant women[5] in comparison with their control groups.

Finally, we would like to point out a pitfall in Dr. Semchyshyn's interpretation of the test. Although we said "it has been widely documented that there is an inverse relationship between calcium intake and blood pressure" when we stated that "to date, there are no available reports conclusively demonstrating this association," we referred to the association between calcium supplementation during pregnancy and reduction of PIH. Consequently "it could be premature to recommend calcium supplementation to all pregnant women with the prevention of pregnancy-induced hypertension as the only objective."

José M. Belizán, MD
Centro Rosarino de Estudios Perinatales
Br. Oroño 500
(2000) Rosario, Argentina

José Villar, MD
Prevention Research
National Institute of Child Health and Human Development
National Institutes of Health
Executive Plaza North
Bethesda, MD 20892

REFERENCES

1. Conradt A, Weidinger H, Algayer H. The decrease of incidence of preeclampsia in pregnancies treated with beta-

mimetics and additional magnesium. Geburtshilfe Frauenheilkd 1984;44:118-23.
2. Spälting L, Spälting G. Magnesium supplementation in pregnancy. Br J Obstet Gynaecol 1988;95:120-5.
3. Belizán J, Pineda O, Menendez A, Villar J. Rise of blood pressure in calcium-deprived pregnant rats. Obstet Gynecol 1981;141:163-9.
4. Belizán J, Villar J, Pineda O, Gonzalez A, Sainz E, Garrera G, Sibrian R. Reduction of blood pressure with calcium supplementation in young adults. JAMA 1983;249:1161-5.
5. Belizán J, Villar J, Zalazar A, Rojas L, Chan D, Bryce G. Preliminary evidence of calcium supplementation on blood pressure in normal pregnant women. AM J OBSTET GYNECOL 1983;146:175-80.

Analytical and clinical sensitivity and specificity in pregnancy testing

To the Editors:

Emanicipator et al. (Emanicipator K, Cadoff EM, Burke MD. Analytical versus clinical sensitivity and specificity in pregnancy testing. AM J OBSTET GYNECOL 1988;158:613-6) stated that whereas the clinical value of the newer immunoenzymetric monoclonal assays for human chorionic gonadotropin (hCG) are sufficient for early pregnancy testing, analytical sensitivity is still inferior to serum radioimmunoassay. There can be no doubt that these assays are well established as reliable and convenient methods of detection of urinary hCG in a variety of clinical states. We have shown that there is an approximately unitary relationship between blood and urine values of hCG in patients with first trimester pregnancy, whether the gestational sac is intrauterine[1] or in the fallopian tube.[2] This permits the use of qualitative urine hCG measurement to determine prgnancy status, and we have shown in several studies that the Tandem Icon pregnancy test (Hybritech Inc., San Diego, Calif.) will detect urine hCG in every case of ectopic pregnancy with a positive predictive index of 100% and a negative index of 99.6%[2-4] (serum radioimmunoassay results, 96.7% and 99.6%, respectively). Therefore, Emanicipator et al. confirm our proposal that the immunoenzymetric assays for hCG have a high clinical sensitivity and specificity for pregnancy.

However, they overlook the fact that when these newer assays are applied to urine the assays may have superior analytic qualities in addition to their clinical value. We have demonstrated that the Tandem Icon test can be made sufficiently sensitive to 1 IU/L (first international reference preparation) by a simple modification of filtration of 8 ml of urine.[5] The assay can be performed at the bedside by nonlaboratory personnel in 5 minutes[6] and has no significant crossreaction with undefined urinary interfering substances. All these parameters are superior to the conventional radioimmunoassays in which both serum and urine samples may give background "noise." In addition, newer densitometric equipment permits the quantitation of hCG levels with the Tandem Icon test, whether the test fluid is urine or (preferably) serum.

There is little doubt that serum radioimmunoassay has been replaced by qualitative immunoenzymetric hCG assays in the detection of pregnancy status. Where radioimmunoassay still has a role is in the determination of quantitative levels for discriminatory zones or rate of increase of hCG to assist in the determination of the site of the gestation.

R. J. Norman, MD
Department of Obstetrics and Gynecology
University of Adelaide
Queen Elizabeth Hospital
Woodville, South Australia 5011

REFERENCES

1. Norman RJ, Menabawey M, Lowings C, Chard T. Relationship between blood and urine concentrations of intact human chorionic gonadotropin and its free subunits in early pregnancy. Obstet Gynecol 1987;69:590-3.
2. Norman RJ, Buck RH, Rom L, Joubert SM. Blood or urine measurement of human chorionic gonadotropin for detection of ectopic pregnancy? Obstet Gynecol 1988;71:315-8.
3. Buck RH, Norman RJ, Reddi K, Moodley J, Joubert SM. Various methods for determining urinary choriogonadotropin evaluated for the early diagnosis of ectopic pregnancy. Clin Chem 1986;32:879-82.
4. Buck RH, Gihwala N, Rom L, Joubert SM, Norman RJ. Detection of urinary human chorionic gonadotropin by rapid immunoconcentration method is the first-line test for suspected ectopic pregnancy. Fertil Steril 1987;48:761-5.
5. Buck RH, Norman RJ, Joubert SM. A bedside test for human chorionic gonadotropin as sensitive as serum radioimmunoassays. Clin Chem 1986;32:1596.
6. Buck RH, Pather N, Moodley J, Joubert SM, Norman RJ. Bedside application of an ultrasensitive urine test for hCG in patients with gynaecological emergencies. Ann Clin Biochem 1987;24:268-72.

Reply

To the Editors:

Our study[1] showed that results of the Tandem Icon human chorionic gonadotropin (hCG) urine pregnancy test, when performed according to the manufacturer's instructions, were positive in the overwhelming majority of cases in which there was detectable hCG in the serum by radioimmunoassay (RIA). Of nine patients with adequate clinical follow-up, negative results of a Tandem Icon hCG urine test, and detectable hCG in the serum by RIA, only one proved to be pregnant. By increasing the analytical sensitivity of the urine pregnancy test, a small increase in the clinical sensitivity would be achieved, but only at the expense of an equivalent decrease in clinical specificity. The net clinical efficiency would be slightly reduced. In general, there seems to be no advantage to increasing the analytical sensitivity of the urine pregnancy test.

Arguably, in certain circumstances it may be desirable to maximize clinical sensitivity at the expense of clinical specificity. Buck et al.[2] propose that two such circumstances are a woman awaiting roentgenography in the second half of her menstrual cycle and a woman with suspected ectopic pregnancy. With proper shielding of the uterus, roentgenography of the skull, chest, cervical spine, or extremities roentgenography increase the risk of birth defects by only 0.00001% to 0.0001%. Therefore necessary roentgenography should never be withheld. Elective roentgenography should be scheduled

during the first 10 days of the menstrual cycle.[3] Urine pregnancy tests with increased sensitivity may have a role in the evaluation of ectopic pregnancy; in our experience (unpublished data) and that of others,[4] undetectable serum hCG virtually excludes the diagnosis. On the other hand, as we have stated previously, experience has shown that it is safe to delay operation in patients with ectopic pregnancy and very low serum hCG levels, and that doing so probably increases preoperative diagnostic accuracy.[5]

We did not mean to imply that enzyme-linked monoclonal immunoassays in general have inferior analytical sensitivity compared with RIA. We stated that the analytical sensitivity of urine pregnancy tests (commercially available at the time) were inferior to that of the RIA. Recently, a quantitative serum enzyme-linked monoclonal immunoassay for hCG with an analytical sensitivity of 0.34 IU/L (first international reference preparation) was described.[6] Clearly, this assay rivals any RIA.

Finally, it should be noted that shortly after the conclusion of our study, Hybritech, Inc. (San Diego, Calif.) introduced the Tandem Icon II hCG urine test. This assay is more analytically sensitive than the older version. Fortunately, an internal standard allows the user to determine whether detectable urinary hCG levels are > or <50 IU/L (first international reference preparation). Therefore the test has three possible results: "negative," "borderline" (<50 IU/L), and "positive" (>50 IU/L). The physician may act on borderline results as deemed appropriate for the clinical situation.

Kenneth Emancipator, MD

National Institutes of Health
Building 10, Room 2C407
Bethesda, MD 20892.

REFERENCES

1. Emancipator K, Cadoff EM, Burke MD. Analytical versus clinical sensitivity and specificity in pregnancy testing. AM J OBSTET GYNECOL 1988;158:613-6.
2. Buck RH, Norman RJ, Joubert SM. A bedside test for human chorionic gonadotropin as sensitive as serum radioimmunoassays. Clin Chem 1986;32:1596.
3. Hockberger RS. Obstetric emergencies. In: Tintinalli JE, Rothstein RJ, Krome RL, eds. Emergency medicine. New York: McGraw Hill, 1985:515-24.
4. Romero R, Kadar N, Copel JA, Jeanty P, DeCherney AH, Hobbins JC. The effect of different human chorionic gonadotropin assay sensitivity on screening for ectopic pregnancy. AM J OBSTET GYNECOL 1985;153:72-4.
5. Romero R, Kadar N. Reply [Letter]. AM J OBSTET GYNECOL 1986;155:682-4.
6. Browne PJ, Mitchell DC. A multi-center evaluation of a new immunoenzymometric assay for human choriogonadotropin. Clin Biochem 1988;21:151-7.

Neonatal seizures and cesarean section

To the Editors:

Spellacy and colleagues (Spellacy WN, Peterson PQ, Winegar A, Quilligan EJ. Neonatal seizures after cesarean delivery: higher risk with labor. AM J OBSTET GYNECOL 1987;157:377-9) have shown that for infants with birth weights >2500 gm, primary cesarean section with labor is associated with a significantly higher incidence of neonatal seizures than is repeat elective cesarean section without labor. They suggest that labor, of itself, may cause a threefold increase in the incidence of seizures in these larger infants. Paradoxically, infants with birth weights <2500 gm did not appear to be adversely affected by labor, despite their inherent susceptibility to the adverse effects of intrapartum hypoxia. By looking only at patients delivered by cesarean section, the authors have biased their analysis. To attribute blame to labor they should have compared outcomes of all patients who labored with outcomes of those delivered by cesarean section. However, if Spellacy and colleagues are correct, the explanation must lie in the characteristics of labor in the group with larger infants. Further analysis of the data base may help to clarify matters. It is likely that the majority of cases of primary cesarean section involved primigravid women, whereas all the repeat cesarean sections were, by definition, in multiparous women. It is probable that in Illinois, as elsewhere, the incidence of seizures is higher in first-born infants.[1] The data from Illinois also suggest that the incidence of cesarean section in labor was significantly higher in primigravid women, similar to experience in our institution.[2]

If my interpretation of the data is correct, the real question revolves around what characteristics of *first* labors in Illinois predispose patients to a high incidence of both neonatal seizures and cesarean section where birth weight is >2500 gm. Previous studies suggest that the incidence of seizures may be related to the duration of labor.[1, 3] It would therefore be interesting to know the average duration of labor in primigravid women delivered of infants in Illinois. Perhaps a high incidence of prolonged or abnormal labor (dystocia) has resulted in a high incidence of both seizures and cesarean section. In primigravid patients at our institution the average duration of spontaneous labor is 7.0 hours (11.0 hrs if labor is induced), the incidence of cesarean section in spontaneous labor is 10%, and the incidence of seizures is 1.9 per 1000. Our low incidence of cesarean section is attributed to active management of labor.[4] An explanation of the lower incidence of seizures is not immediately apparent, although information with regard to duration of labor in primigravid women in Illinois might help us reach an answer. The fact that labor did not appear to have an effect on the smaller infants supports the proposition that length of labor has a significant influence on seizure incidence, inasmuch as labor is generally shorter with low birth weight. A solution to the apparently high incidence of seizures reported by Spellacy and colleagues may lie in a different approach to management of labor, such as active management, rather than the implied solution of more liberal use of cesarean section.

Finally, I fear that the figures reported by Spellacy and colleagues may encourage more liberal use of cesarean section in the management of dystocia in pri-

migravid women, a practice largely responsible for the current cesarean epidemic.[2,4]

REFERENCES

1. Minchom P, Niswander K, Chalmers I, et al. Antecedents and outcome of very early neonatal seizures in infants born at or after term. Br J Obstet Gynaecol 1987;94:431-9.
2. Boylan P, Frankowski R. Dystocia, parity, and the cesarean problem. AM J OBSTET GYNECOL 1986;155:455-6.
3. MacDonald D, Grant A, Sheridan-Pereira M, et al. The Dublin randomized controlled trial of intrapartum fetal heart rate monitoring. AM J OBSTET GYNECOL 1985; 152:524-39.
4. O'Driscoll K, Foley M, MacDonald D. Active management of labor as an alternative to cesarean section for dystocia. Obstet Gynecol 1984;63:485-90.

Peter Boylan, MD
Associate Professor
University College Dublin
Department of Obstetrics and Gynecology
National Maternity Hospital
Holles Street
Dublin 2, Ireland

Reply

To the Editors:

I appreciate the thoughtful letter from Dr. Boylan concerning our article on neonatal seizures and cesarean delivery. As expected, there were more primigravidous patients in the primary cesarean section group, but we could not show more seizures in first-born children when controlled for birth weight.

Whereas the duration of labor may be an important variable, even more critical may be the development of fetal hypoxia during that labor. The article did not suggest that long labors should be treated by cesarean section but rather that infant brain insults can occur during labor, as opposed to them all occurring before labor, and that this critical time in the infant's life must be carefully monitored and managed by the obstetrician.

William N. Spellacy, MD
Department of Obstetrics and Gynecology
College of Medicine
University of South Florida
4 Columbia Dr.
Tampa, FL 33606

Is central venous pressure monitoring "contraindicated" in patients with severe preeclampsia?

To the Editors:

We read with interest the article by Clark and Cotton in your March issue (Clark SL, Cotton DB. Clinical indications for pulmonary artery catheterization in the patient with severe preeclampsia. AM J OBSTET GYNECOL 1988;158:453-8). We found it to be a well-written review of the literature on the hemodynamic findings associated with severe preeclampsia. However, we do not believe that the literature to date supports all their conclusions. We are not convinced that simple central venous pressure monitoring is *contraindicated* in these patients. Nor have we seen any literature to support their contention that such patients can be managed better clinically than with the additional information provided by central venous pressure monitoring. Finally, we believe the proposal to place a pulmonary artery catheter each time the use of nitroprusside "is contemplated" would unjustifiably increase the use of pulmonary artery catheters.

We agree that one must not seek to translate a central venous pressure value into a value of pulmonary capillary wedge pressure as this has been repeatedly shown to be inaccurate.[1-3] But we hope that the clinician does not treat any of his patients (preeclamptic or not) solely on the basis of a central venous pressure reading or any other single number. It is important to note that the literature does show some relationships of the central venous pressure level to the pulmonary capillary wedge pressure values in patients with severe preeclampsia. It can be noted in the Cotton et al.[1] study in the same March issue, and in previous studies,[2-3] that when the central venous pressure level is <4 mm Hg the pulmonary capillary wedge pressure will be <18 mm Hg. The study by Cotton et al.[1] demonstrated that 51% of their patients will be seen with a central venous pressure value <3 mm Hg; thus it follows that the central venous pressure level alone will produce reliable information in the majority of patients. Only when the central venous pressure begin to rise above this value (typically to values of 7 or 8 mm Hg) is there a risk that the patient will have an elevated pulmonary capillary wedge pressure level with a normal central venous pressure level. This information can be used to assist therapeutic decisions without subjecting the patient to the unique risks of floating a pulmonary artery catheter (particularly in patients with potential bleeding disorders).[4] In fact, the central venous pressure level may be a "more accurate" indicator of low intravascular volume than is the wedge pressure.[1] This is often the information that is necessary when attempts are made to replenish a patient's intravascular volume before initiation of an epidural or general anesthetic.[5] It is important to realize the limitations of central venous pressure monitoring; but if used with caution in the proper circumstances it can provide clinically useful information with minimal risk to the patient.[6] One can also use the initial central venous pressure value and its subsequent response to a gentle fluid challenge to help determine the need to go on to pulmonary artery catheter monitoring.[4]

It is stated by Clark and Cotton that a patient with severe preeclampsia should be managed either clinically or with the aid of a pulmonary artery catheter. Multiple studies have demonstrated the inaccuracy of attempts to predict clinically the volume status in critically ill patients.[7-8] There is no data to suggest otherwise in patients with severe preeclampsia. Nor is there any study that supports the contention that physicians can determine a patient's volume status more accurately by

clinical examination alone than they can by clinical examination and central venous pressure monitoring.

Finally, Clark and Cotton dictate that when nitroprusside "is contemplated" a pulmonary artery catheter should be placed. This statement would encompass all severe and many moderate preeclamptic patients about to receive a general anesthetic agent inasmuch as nitroprusside (or another potent vasodilator) is usually on standby. This practice would greatly increase the number of pulmonary artery catheters placed in obstetrics suites across the United States. In light of the extra cost and potential risks, this recommendation should be supported by comparative outcome studies before it is labeled as "mandatory."

In conclusion, although the central venous pressure value cannot be directly translated into a number that always matches the pulmonary capillary wedge pressure value, it is certainly not a useless piece of information. We believe it is unwarranted to claim that this long-used indicator of intravascular volume is contraindicated in the patient with severe preeclampsia.

David G. Woodward, MD
Mark E. Romanoff, MD

Clinical Instructors of Anesthesiology
Department of Anesthesia
Wilford Hall USAF Medical Center
Lackland Air Force Base
San Antonio, TX 78236

REFERENCES

1. Cotton DB, Wesley L, Huhta JC, Dorman KF. Hemodynamic profile of severe pregnancy-induced hypertension. Am J Obstet Gynecol 1988;158;523-9.
2. Newsome LR, Bramwell RS, Curling PE. Severe preeclampsia: hemodynamic effects of lumbar epidural anesthesia. Anesth Analg 1986;65:31-6.
3. Cotton DB, Gonik B, Dorman KF, Harrist R. Cardiovascular alterations in severe pregnancy-induced hypertension: relationship of central venous pressure to pulmonary capillary wedge pressure. Am J Obstet Gynecol 1985;1551:762-4.
4. Gutsche BB, Cheek TG. Anesthetic considerations in preeclampsia-eclampsia. In: Shnider SM, Levinson G, eds. Anesthesia for obstetrics. Baltimore: Williams and Wilkins, 1987:225-42.
5. James FM III. Pregnancy-induced hypertension. In: James FM, Wheeler AS, Dewan DM, eds. Obstetric anesthesia: the complicated patient. Philadelphia: F. A. Davis, 1988:411-37.
6. Gutsche B. The experts opine: is epidural block for labor and delivery and for cesarean section a safe form of analgesia in severe preeclampsia? Surv Anesth 1986;30:304-11.
7. Connors AF, McCaffree DR, Gray BA. Evaluation of right-heart catheterization in the critically ill patient without acute myocardial infarction. N Engl J Med 1983;308: 263-7.
8. Eisenberg PR, Jaffe AS, Schuster DP. Clinical evaluation compared to pulmonary artery catheterization in the hemodynamic assessment of critically ill patients. Crit Care Med 1984;12:549-53.

Reply

To the Editors:

We would like to thank Drs. Woodward and Romanoff for their interest in our article. These authors focus on the utility of central venous pressure monitoring for the determination of intravascular volume in patients with severe preeclampsia. This is puzzling inasmuch as it is well known that patients with severe preeclampsia are volume contracted; the clinician does not need any type of invasive monitoring to be assured of this principle.

The information we seek from pulmonary artery catheterization goes far beyond simple assessment of intravascular volume. Assessment of myocardial function and systemic vascular resistance is far more important in complicated preeclamptic patients than is the assessment of intravascular volume, which is already known to be decreased. When volume considerations are important in any individual patient, it is meaningless to know that central venous pressure levels correlate with wedge pressure levels in "most" (70% to 80%) patients.

Further, we fail to understand the authors' statement with regard to "unique risks" of a pulmonary artery catheter in patients with bleeding disorders. In such patients obtaining central venous access is the problem. The additional risks of floating the pulmonary artery catheter through the central venous sheath have nothing to do with bleeding disorders. Large studies that deal with both obstetric and nonobstetric patients have shown clearly that most complications of pulmonary artery catheterization involve obtaining central venous access and would be the same whether simple central venous pressure assessment or the more comprehensive information derived from pulmonary artery catheterization is obtained.[1-2]

Finally, the authors are concerned that our recommendations "would encompass all severe and many moderate preeclamptic patients about to receive a general anesthetic agent." With very few exceptions, epidural anesthesia is the anesthetic method of choice for patients with severe preeclampsia. Thus, preeclamptic patients who are about to receive a general anesthetic agent should be few and far between in most modern obstetric centers. For such patients, agents that are less potentially dangerous including β-blockers and nitroglycerine are effective in the reduction of blood pressure fluctuations associated with induction of general anesthesia. The use of such agents would not require invasive monitoring.

We remain emphatic in our belief that the overwhelming majority of patients with severe preeclampsia may be expertly managed without resorting to invasive monitoring of any type. However, for those few complicated patients in whom invasive monitoring is contemplated, we see no benefit in being content with central venous pressure assessment of intravascular volume. Simple assessment of central venous pressure levels has essentially disappeared from modern critical

care medicine. As obstetricians become familiar with pulmonary artery catheterization, assessment of central venous pressure levels in obstetrics should likewise become obsolete.

Steven L. Clark, MD
Utah Valley Regional Medical Center
1034 N. 500 W.
Provo, Utah 84604

David B. Cotton, MD
Department of Obstetrics and Gynecology
Baylor University College of Medicine
1 Baylor Plaza
Houston, TX 77030

REFERENCES

1. Clark SL, Horenstein JM, Phelan JP, et al. Experience with the pulmonary artery catheter in obstetrics and gynecology. AM J OBSTET GYNECOL 1985;152:374-8.
2. Sise MJ, Hollingsworth P, Brimm JE, et al. Complications of the flow-directed pulmonary artery catheter. Crit Care Med 1981;9:315.

Recurrence of functional ovarian cysts after laparoscopic fenestration

To the Editors:

After preoperative vaginal examination and echography, we performed operative laparoscopic fenestrations in young women with ovarian cysts. The mean diameter of the cysts was 6.0 cm (range, 3.7 to 11.3 cm). Only follicular and corpus luteum cysts were studied. As in endometriomas, teratomas, and paraovarian cysts, we made a total laparoscopic extraction of the cyst wall.

In a series of 104 patients with a mean age of 30.4 years, the ovarian cysts were fenestrated[1] by the same endoscopic surgeon during 1985 to 1987. We saw no postoperative complications such as bleeding or pelvic inflammatory disease. The histopathology of the window revealed 58% follicular cysts and 42% corpus luteum cysts; no malignancy was found.

When we looked at the influence of combined oral contraceptives taken preoperatively we found that 29% of the patients took oral contraceptives at the time the functional cyst developed. A total of 91 women were followed up. The mean follow-up time was 20 months. With the use of vaginal examination and echography, we found a recurrence rate of 8% (7/91). It was, however, impossible to distinguish between a new disease or a recurrent cyst. Again, no malignancy was found.

When pelvic adhesions were seen at the laparoscopy, a higher recurrence rate of 11%, compared with 6% (Table I) was observed. At-random postoperative hormonal therapy with progestogenic agents or combined oral contraceptives showed no protective effect (Table II). Follicular cysts had a recurrence rate of 2%; corpus luteum cysts recurred in 14% (Table III).

We can conclude from our study there was no protective effect of postoperative hormonal therapy. We demonstrated a higher recurrence rate for corpus luteum cysts than that for follicular cysts. In the presence of pelvic adhesions, new cysts tend to occur. Our data confirm the safety and efficacy of the operative laparoscopic fenestration of functional ovarian cysts in young women and point out the factors that lead to a higher recurrence rate.

Table I. Influence of pelvic adhesions (n = 91)

	No adhesions	*Adhesions*
No recurrence	59	25
Recurrence	4	3
Recurrence rate	4/63 (6%)	3/28 (11%)

Table II. Influence of postoperative hormonal therapy (n = 91)

	No hormonal therapy	*Progestogens-combined oral contraceptives*
No recurrence	63	21
Recurrence	3	4
Recurrence rate	3/66 (5%)	4/25 (16%)

Table III. Influence of histopathology (7 patients had both types) (n = 91)

	Follicular cyst	*Corpus luteum cyst*
No recurrence	53	38
Recurrence	1	6
Recurrence rate	1/54 (2%)	6/44 (14%)

Rudy L. De Wilde, MD
Department of Obstetrics and Gynecology
University of Muenster
Bethesda Clinic
Hainstrasse 35, D-5600
Wuppertal 1, West Germany

REFERENCE

1. Semm K. Atlas of gynecologic laparoscopy and hysteroscopy. Philadelphia: Saunders, 1977:246-9.

Criticism of publication of pharmaceutical-supported research results draws response

To the Editors:

A thought-provoking letter by Dr. Robert Goodlin was published in the March 1989 issue of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY (Goodlin RC. Pharmaceutical-supported supplements. 1989; 160:764). Inasmuch as I was cited in the letter as the editor of a criticized pharmaceutical-supported sym-

posium, I would like to comment on Dr. Goodlin's views.

I have a different view of antibiotic research than does Dr. Goodlin. I agree with him that there are probably too many antibiotics, but I disagree with the logic that "It is not necessarily progress to develop and test new antibiotics, particularly when present drugs and regimens do so well." This was the same type of statement that I heard as a medical student in the 1950s when I was taught that prophylactic antibiotics were of no value and the treatment of choice for postpartum endomyometritis was penicillin G and streptomycin. Infections have been a dynamic process in which changes in the bacterial pathogens and antibiotic susceptibilities have modified our therapeutic approaches. Much of our knowledge about the importance of such things as anaerobic bacteria and *Chlamydia trachomatis* in pelvic infections have come from studies supported by the pharmaceutical industry. For the future health of our specialty, I hope that support continues.

I disagree with Dr. Goodlin's view that results in the symposium in question have been modified to present only favorable findings. As the editor for this symposium, I know the clinical studies were adequately controlled with results accurately portrayed. Initial reports of antibiotics are usually enthusiastic, but that is the rule, not the exception for any new treatment regimen. Not every study is controlled in the symposium, but even Dr. Goodlin has recommended new therapeutic interventions from studies without concomitant controls (Goodlin RC. Surgical treatment of patients with hour glass–shaped or ruptured membranes prior to the 25th week of gestation. Surg Gynecol Obstet 1987;165:410.).

Like Dr. Goodlin, I am concerned about the lack of journal space. I believe that symposia should be published as supplements separate from the main journal. This is a recent decision by the JOURNAL and it is an improvement over the inclusion of the supplement within regular journal pages. However, I believe the weakness of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY has always been the publication of meeting papers. Despite peer review, I do not think the articles from the meetings of the American Gynecological and Obstetrical Society, Central Association of Obstetricians and Gynecologists, South Atlantic Association of Obstetricians and Gynecologists, and Pacific Coast Obstetrical and Gynecological Society are as good as the other published articles. This is a two-tier system that causes pressure on JOURNAL space for articles outside of those meetings.

William J. Ledger, MD

The New York Hospital
Cornell University Medical Center
525 E. 68th St.
New York, NY 10021

Reply

Dr. Ledger properly brings out the point that infections are a dynamic process in which changes in bacterial pathogens and antibiotic susceptibilities have modified therapeutic approaches. Research and development continue to bring out new approaches to therapy and education in obstetrics and gynecology, medicine, and surgery. Dr. Ledger correctly noted that the pharmaceutical industry has provided support for many of these advancements. With this we agree.

The AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY does publish reports of observations and randomized, blinded, controlled trials in the various sections of the JOURNAL, including symposia, if they are believed to have value for our readers.

Dr. Ledger expressed concern about lack of JOURNAL space and mentioned that symposia should be published as freestanding supplements, not within regular JOURNAL pages. The inference is that a symposium published within a regular issue of the JOURNAL with consecutively numbered pages reduces the number of pages for the publication of manuscripts that are individually submitted to the JOURNAL. Such is not the case. Whether the supplement is freestanding or within the JOURNAL issue, no space (pages) is taken or will be taken from that allotted for regularly submitted manuscripts.

The publication of society papers as a means of disseminating information to a wide spectrum of obstetricians and gynecologists was the objective of the predecessor of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY, namely, the *American Journal of Obstetrics and Diseases of Women and Children,* which was founded and began publication in 1868. The first and subsequent issues contained society articles. In 1876 they published papers of the first meeting of the American Gynecological Society and in 1888 the papers of the first meeting of the American Association of Obstetricians and Gynecologists. Articles from both societies have been published yearly since then (now, with the union of the two, as the American Gynecological and Obstetrical Society). When the C.V. Mosby Company purchased the *American Journal of Obstetrics and Diseases of Women and Children* in 1920, it maintained this objective and, as new societies were formed, selected the ones for which papers would be published. In addition to the ones mentioned by Dr. Ledger the Society for Gynecologic Investigation and the Society of Perinatal Obstetricians have been added. Societies, their annual meetings, and publication of their papers have played and are playing an important role in the development and progress of obstetrics and gynecology. Each has a purpose. We are pleased to play a role, both historically and presently, by publishing articles from their annual meetings.

The Editors

Books received

Annual Report on the Results of Treatment in Gynecological Cancer—FIGO-1988. Twentieth Volume. 192 pages, illustrated. Edited by Folke Pettersson. Sweden, 1988, published under the patronage of the International Federation of Gynecology and Obstetrics.

Appleton & Lange's Review of Obstetrics and Gynecology. Fourth Edition. Louis A. Vontver and Thomas M. Julian. 369 pages. East Norwalk, Connecticut, 1988, Appleton & Lange. $19.95 (soft cover).

Atlas of Fetal Skeletal Radiology. Asher Ornoy, Zvi Borochowitz, Ralph Lachman, and David L. Rimoin. 148 pages, illustrated. Chicago, 1988, Year Book Medical Publishers. No price listed.

The Biological Clock: Balancing Marriage, Motherhood and Career. Molly McKaughan. 343 pages. New York, 1989, Penguin Books. $7.95 (soft cover).

The Biology of Surfactant. B. A. Hills. 408 pages, illustrated. New York, 1988, Cambridge University Press. $89.50.

Candida and Candidosis: A Review and Bibliography. Second Edition. F. C. Odds. 470 pages. London, 1988, Bailliere & Tindall. No price listed.

The Columbia University College of Physicians and Surgeons Complete Guide to Pregnancy. W. Duane Todd, Donald F. Tapley, Genell J. Subak-Sharpe, and Diane M. Goetz. 288 pages, illustrated. $24.95.

Douglas-Stromme Operative Obstetrics. Fifth Edition. Frederick P. Zuspan and Edward J. Quilligan. 727 pages, illustrated. East Norwalk, Connecticut, 1988, Appleton & Lange. $89.95.

The Endocrine Control of the Fetus: Physiologic and Pathophysiologic Aspects. W. Kunzel and A. Jensen. 427 pages, illustrated. New York, 1988, Springer-Verlag. $125.40.

Family Planning Handbook for Doctors. Edited for the International Planned Parenthood Federation by Ronald L. Kleinman. 361 pages. London, 1988, International Planned Parenthood Federation. $18.00.

Female Contraception: Update and Trends. B. Runnebaum, T. Rabe, and L. Kiesel. 450 pages, illustrated. New York, 1988, Springer-Verlag, $79.80.

Fitness and Pregnancy: A Proven Strategy. Garnet Fougere Mailey. 117 pages, illustrated. Dubuque, Iowa, 1988, Kendall Hunt Publishing Company. $17.95 (soft cover).

Helping Women Keep Well. Richard H. Blum and W. LeRoy Heinrichs. 462 pages. New York, 1988, Irvington Publishers, Inc. $49.95.

Hysterectomy: Before & After. Winnifred B. Cutler. 451 pages. New York, 1988, Harper & Row. $19.95.

Maternal-Fetal Medicine: Principles and Practice. Second Edition. Robert K. Creasy and Robert Resnik. 1238 pages, illustrated. Philadelphia, 1989, W. B. Saunders Company. $95.00.

Modern Instrumental Delivery. John Patrick O'Grady. 272 pages, illustrated. Baltimore, 1988, Williams & Wilkins. $34.95.

New Trends in Urinary Tract Infections: The Single-Dose Therapy. International Symposium on New Trends in Urinary Tract Infections, Rome, January, 1987. 358 pages, illustrated. H. C. Neu and J. D. Williams. Basel, Switzterland, 1988, S. Karger. SFr. 246/US $164.

A Piece of My Mind: A Collection of Essays from the *Journal of the American Medical Association.* Edited by Dr. Bruce B. Dan and Roxanne K. Young. 256 pages. Los Angeles, 1988, Feeling Fine Programs, Inc. $18.95.

A Practical Guide to Labour Management. Donald Gibb. 152 pages, illustrated. Chicago, 1988, Year Book Medical Publishers. No price listed.

Prenatal Care: Reaching Mothers, Reaching Infants. Sarah S. Brown. 254 pages. Washington, D.C., 1988, National Academy Press. $17.95 (soft cover).

Rape: The Evidential Examination and Management of the Adult Female Victim. William M. Green. 175 pages. Lexington, Massachusetts, 1988, Lexington Books. No price listed.

Reproduction, Obstetrics and Gynaecology. Edited by M. G. Elder. 333 pages, illustrated. Oxford, 1988, Heinemann Professional Publishing. Distributed by Butterworths, Stoneham, Massachusetts. $59.95.

Sexology. W. Eicher, G. Kockott, H. J. Vogt, V. Herms, and R. Wille. 405 pages, illustrated. New York, 1988, Springer-Verlag. $101.50.

1988 Year Book of Perinatal/Neonatal Medicine. Marshall H. Klaus and Avroy A. Fanaroff. 344 pages, illustrated. Chicago, 1988, Year Book Medical Publishers. No price listed.

ITEMS

Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60.00 U.S. per insertion and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, The C.V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318.

International Perinatal Doppler Society (I.P.D.S.), Paris, France, September 13-15, 1989. For more information contact: Dr. Ph. Arbeille, Secretary of the I.P.D.S., Unite INSERM 316, C.H.U. Bretonneau, 37044 Tours, France.

Update in Gynecologic Oncology: An International Perspective, October 16 and 17, 1989, Worcester, Massachusetts. Sponsored by the University of Massachusetts Medical School, Department of Obstetrics and Gynecology. For further information contact: Office of Continuing Education, UMMS, 55 Lake Avenue North, Worcester, MA 01655. Tel.: (508) 856-3041.

Problem Solving in Gynecologic Endocrinology and Infertility, December 8-9, 1989, The Knickerbocker Hotel, Chicago, Illinois. For information contact: The University of Chicago, Center for Continuing Medical Education, 5841 Maryland, Box 139, Chicago, IL 60637. Tel.: (312) 702-1056.

Third Annual Symposium and Workshop on Operative Laparoscopy, Pelviscopic Surgery and Laser Endoscopy—"Advances and Controversies," St. Louis, Missouri, October 20-21, 1989. Sponsored by St. Luke's Hospital, Department of OB/GYN, in conjunction with St. Luke's Hospital Laser Institute. For further information contact: Patty Fogertey, Program Coordinator, Department of Education, St. Luke's Hospital Institute for Health Education, 222 Woodsmill Road, Suite 350 North, St. Louis, MO 63017. Tel.: (314) 851-6006.

2nd Annual Review Course in Obstetrics and Gynaecology, May 1-5, 1990, King Edward Hotel, Toronto, Ontario, Canada. AMA Category 1 credit hours. For further information contact: Continuing Education, Faculty of Medicine, University of Toronto, Medical Sciences Building, Toronto, Ontario, Canada M5S 1A8. Tel.: (416) 978-2718.

45th Annual Postgraduate Assembly, sponsored by OB-GYN Assembly of Southern California, February 15-18, 1990, Beverly Hilton Hotel, Beverly Hills, California. For information contact: Director of Medical Education, OB-GYN Assembly of So. California, 5820 Wilshire Blvd., #500, Los Angeles, CA 90036. Tel.: (213) 937-5514.

Clinical Problems in Gynecologic Surgery, January 27–February 1, 1990, Frenchman's Reef Hotel Conference Center, St. Thomas, Virgin Islands. Sponsored by The Johns Hopkins University School of Medicine, in conjunction with Emory University School of Medicine and Brown University School of Medicine. For information contact: Program Coordinator, Office of Continuing Education, The Johns Hopkins University School of Medicine, 720 Rutland Ave., Baltimore, MD 21205-2195. Tel.: (301) 955-6046.

7th World Congress of Cervical Pathology and Colposcopy, Rome, Italy, May 13-17, 1990. For information contact: Studio EGA Professional Congress Organizers, 7th World Congress of Cervical Pathology and Colposcopy, Viale Tiziano, 19, 00196 Roma, Italy. Tel.: 06/3221806. Telex: 614357. Fax: 06/3222006.

Maternal-Fetal Medicine

The Department of Obstetrics and Gynecology at the University of Tennessee Medical Center at Knoxville, Tennessee is currently accepting applications for an additional Maternal-Fetal Medicine Specialist.

This position requires Board certification or eligibility in Maternal-Fetal Medicine. Responsibilities include resident and medical student education, participation in Regionalization Program and clinical or basic research. Specialized areas of interest such as critical care, ultrasonography and Doppler studies, and prenatal diagnosis are encouraged. A private practice opportunity is available.

Academic rank is commensurate with qualifications and experience.

Interested persons should send curriculum vitae to:

Michael R. Caudle, M.D.
Associate Professor and Gynecology
Department of Obstetrics and Gynecology
For more information call (615) 544-9306

THE UNIVERSITY OF TENNESSEE
Medical Center at Knoxville
1924 ALCOA HIGHWAY
KNOXVILLE, TENNESSEE 37920
EEO/An Affirmative Action/Title IX/Section 504 Employer

GYNECOLOGIST

Busy GYN Clinic in beautiful downtown Salt Lake City offers its patients a full range of reproductive health services—here's what we can offer you:

—Competitive salary
—Outstanding benefit package
—Financial incentive leading to full partnership
—Near 3 Universities
—Close to mountains; great skiing, camping, hiking

Send C.V. to:

Sally Burgess
Utah Women's Health Center
515 South 400 East
Salt Lake City, Utah 84111
(801) 531-9192

from Churchill Livingstone...
Books for the best in gynecologic patient care!

PRINCIPLES AND PRACTICE OF CLINICAL GYNECOLOGY, Second Edition

Edited by **Nathan G. Kase,** MD,
Allan B. Weingold, MD, and **David M. Gershenson,** MD
With 36 contributors

The revised, up-to-date **new edition** — with many new expert contributors and first-time chapters on geriatric patients . . . genital tract lesions . . . risk management; plus crucial new information on toxic shock syndrome and AIDS. The clinical approach stresses understanding of the basic sciences as key to proper diagnosis and treatment.

November 1989. 1,056 pages plus index. 754 illustrations, 280 tables. $89.95 *tentative*

OBSTETRICS

Edited by **Sir Alec Turnbull,** CBE MD FRCOG, and
Geoffrey Chamberlain, MD FRCS FRCOG
With 85 contributors

A comprehensive account of all aspects of current obstetric knowledge and practice, presented by a distinguished team of experts. Includes major sections on fetal monitoring, abnormal pregnancy, neonatal care, contraception and medicolegal issues.

October 1989. 1,201 pages. 350 illustrations. $150.00

MANUAL OF GYNECOLOGIC ONCOLOGY

By **Enrique Hernandez,** MD FACOG FACS, and
Neil B. Rosenshein, MD FACOG

Provides the fundamentals of effective care for gynecologic cancer patients. The book features disease- **and** procedure-related coverage, *plus* discussions of nutritional support, blood component therapy, and the Hickman catheter — topics not often found in gynecology texts.

1989. 184 pages. 24 illustrations, 37 tables. Flexible binding. $24.95

ORDER TOLL FREE 1-800-548-2299
24 Hours a Day/7 Days a Week • For Customer Inquiries Please Call (800) 553-5426

Clip & Mail

CHURCHILL LIVINGSTONE FULFILLMENT CENTER
5 S 250 Frontenac Road, Naperville, IL 60563-1711

Yes! Please send me:

_____ copy/ies of **Kase et al:** PRINCIPLES AND PRACTICE OF CLINICAL GYNECOLOGY, 2E (0443086362) @ $89.95 *tentative*
_____ copy/ies of **Turnbull/Chamberlain:** OBSTETRICS (0443035393) @ $150.00
_____ copy/ies of **Hernandez/Rosenshein:** MANUAL OF GYNECOLOGIC ONCOLOGY (0443086427) @ $24.95

Name _____
(Please print.)
Signature _____
Address _____
(Publisher will not deliver to a P.O. Box. Street address required.)
City _____ State _____ Zip _____

Save postage and handling with prepayment!
Charge it: ☐ Visa ☐ MasterCard ☐ American Express
Account Number _____ Exp. Date _____
☐ Check enclosed for $_____ Add sales tax if you reside in NY (8.25%) or IL (7.25%).
☐ Bill me on 30-day approval plus postage and handling.

Non-USA residents (other than Canada), please mail orders to: Churchill Livingstone/Robert Stevenson House/1-3 Baxter's Place/ Leith Walk/Edinburgh EH1 3AF, Scotland. **In Canada,** send orders to: Copp Clark Pitman Ltd., 2775 Matheson Blvd. East, Mississauga, Ontario L4W 4P7.
Prices subject to change without notice and valid only in the U.S.A. 30-day approval offer valid only in the U.S.A. OBG 090

GYNECOLOGIC ONCOLOGIST

State University of New York at Stony Brook

The Department of Obstetrics and Gynecology at the State University of New York at Stony Brook is currently recruiting for a Gynecologic Oncologist. This is a full-time faculty position requiring board certification or eligibility in gynecologic oncology. The responsibilities include undergraduate and graduate medical teaching, patient care, and clinical or laboratory research.

Academic rank and salary are commensurate with experience and qualifications.

Curriculum vitae should be sent to:

William J. Mann, M.D.
Director of Gyn Oncology
Department of OB/GYN
SUNY at Stony Brook-HSC
Stony Brook, New York 11794-8091

An Equal Opportunity/Affirmative Action Employer

Immediate Opening
ASSISTANT OR ASSOCIATE PROFESSOR
MATERNAL-FETAL MEDICINE

The Department of Obstetrics & Gynecology, MacDonald Hospital for Women, University Hospitals of Cleveland, and Department of Reproductive Biology, Case Western Reserve University, is actively recruiting two full-time Perinatologists for its Division of Maternal-Fetal Medicine. The department of OB-GYN delivers approximately 3,000 infants per year, with a large number of high risk referrals.

Excellent opportunity to be involved in teaching, ultrasound and prenatal diagnosis including CVS, PUBS, and Fetal Echocardiography. Clinical and basic research are encouraged. There is a Board approved fellowship program. Board eligibility or certification in Maternal-Fetal Medicine is required. Interested candidates should send curriculum vitae to:

J. C. Veille, M.D.
Director, Maternal-Fetal Medicine
Department of Obstetrics & Gynecology
MacDonald Hospital for Women
University Hospitals of Cleveland
2074 Abington Road
Cleveland, OH 44106
Phone: 216-844-3338

The University is an equal opportunity/affirmative action employer.

Sheboygan, Wisconsin — Seek one or two BC/E OB/GYNs to begin practice immediately. $120,000 salary guarantee plus $10,000 signing bonus. Referrals will be supported by 40 member independent practice group including Pediatrics, Family Practice, Surgery, and Internal Medicine. Beautiful community located on Lake Michigan near major cities offers wide range of recreational opportunities.

Send C.V. to:

William Trager, M.D.
P.O. Box 1339
Sheboygan, WI 53082
(414) 457-4372

MATERNAL-FETAL MEDICINE
(Board eligible or Certified)

600 Bed University-affilited Level III Hospital with 3,600 deliveries/year, 24-hour in-house Anesthesiology and Neonatology/large NICU, active maternal transfer service, perinatal outreach programs, and fully-approved OB/GYN residency program has immediate opening for a full-time position in Perinatology. Duties to include residency teaching, maternal transfer care, and private practice incentive. Competitive guaranteed salary plus incentive. Excellent public school system, housing, and Lake St. Clair in very close proximity.

CALL COLLECT: 313-343-3495
MINU KASHEF, M.D.
ST. JOHN HOSPITAL
DEPT. OB/GYN
DETROIT, MI 48236

SINAI HOSPITAL OF BALTIMORE
ASSOCIATE DIRECTOR — OB/GYN
Baltimore, Maryland

Dynamic, active OB/GYN service. All modern facilities available on site; vaginal sonography, pelviscopy, Doppler velocimetry, genetic counselor, active dedicated research lab, etc. 4,000 deliveries. 16 residents. Independent residency. Position requires extensive clinical interaction with residents, in conjunction with large faculty of full time and private attendings. Applicant should have several years of clinical experience and/or subspecialty training.

Please send resume to:

Dr. Phillip J. Goldstein
Obstetrician & Gynecologist-in-Chief
Sinai Hospital of Baltimore
Belvedere Avenue at Greenspring
Baltimore, Maryland 21215

DEPARTMENT CHAIR
OBSTETRICS AND GYNECOLOGY

The University of South Alabama invites applications and/or nominations for the position of Professor and Chair of the Department of Obstetrics and Gynecology. The College of Medicine admitted its first class of medical students in 1973. It is a relatively small, state assisted, medical school with approximately 55 basic science faculty and 140 full-time clinical faculty members. Residency programs are well established in all major specialties, with a total of approximately 175 resident positions. This position provides an outstanding opportunity to build an excellent, progressive department in this geographic region. Candidates should have demonstrated academic administration and clinical experience. Proven leadership skills are required. Candidates must be committed to excellence in patient care, teaching, and research.

Please send nominations and/or applications to:

Joseph H. Coggin, Jr., Ph.D.
Chair, Search Committee
Department of Microbiology, 2118 MSB
University of South Alabama
Mobile, AL 36688

Applications should include curriculum vitae and the names and addresses of those who will provide letters of reference.

The University of South Alabama is an Affirmative Action/Equal Opportunity employer.

OB/GYN
Boston, MA

We are a community health center in Boston currently seeking a temporary full-time, or permanent part-time OB/GYN. Strong medical network includes CNM and NP support.

We offer a competitive salary and generous fringe benefits including malpractice insurance.

Please send CV, or contact: Dr. Albert K. Yee, Medical Director, South Cove Community Health Center, 885 Washington Street, Boston, MA 02111, (617) 482-7555.

SOUTH COVE COMMUNITY HEALTH CENTER

健康

Generalist in OB-GYN

We have an opportunity for a generalist in obstetrics and gynecology to join the full time faculty of the University of Rochester Department of Obstetrics and Gynecology at Rochester General Hospital. Rochester General is a 540 bed hospital, located in upstate New York. We serve women from the inner city and suburban areas with a successful mix of privately insured and medicaid patients. Our recently opened Birth Center is the first phase of our master plan to modernize our labor and delivery and surgery facilities. The successful candidate will have a commitment to clinical excellence and patient teaching. The teaching of residents and medical students requires a solid knowledge of general obstetrics and gynecology. Excellent communication and interpersonal skills are essential.

The University of Rochester offers an excellent salary and benefit package commensurate with academic credentials and clinical experience. Board certified or eligible individuals are requested to submit your curriculum vitae in confidence to:

Phyllis C. Leppert, CNM, MD, PhD
Chief of Obstetrics & Gynecology
Rochester General Hospital
1425 Portland Avenue
Rochester, New York 14621

The University of Rochester is an equal opportunity employer.

Medical Staff Fellowships

Genetics and Endocrinology
National Institutes of Health

The National Institute of Child Health and Human Development of the National Institutes of Health is offering a limited number of three-year Medical Staff Fellowship positions to candidates of the highest caliber. Fellowships begin July 1, 1990 and 1991. Positions are available for physicians who have completed at least two years of training in Internal Medicine, Pediatrics, or Obstetrics and Gynecology. Other medical specialty credentials will be considered on a case by case basis. Candidates must be United States citizens or permanent residents; graduates of foreign medical schools must be certified by the ECFMG.

The training programs consist either of one clinical year and two years of laboratory-based research (Human Genetics, Adult Endocrinology, Pediatric Endocrinology, Gynecologic Endocrinology). The endocrinology fellowships are integrated with the NIH Interinstitute Endocrinology Fellowship Program, and the human genetics fellowships are integrated with the NIH Interinstitute Medical Genetics Program. The gynecologic-endocrinology fellowship is integrated with programs at the Walter Reed Hospital and the National Naval Medical Center.

Research experience will be obtained in the Laboratory or Branch of the Fellow's choice from among the Institute's three clinical research Branches and nine basic science Laboratories.

The major emphasis of the NICHD Intramural Research Program is on basic and clinically applied research in the broad area of developmental biology. Areas presently being examined include developmental endocrinology, reproductive biology, cellular differentiation, human genetics and inborn metabolic errors, molecular endocrinology; developmental and molecular immunology, developmental neurobiology, molecular genetics (bacteria, virus, yeast, drosophila, and animal cell), pharmacogenetics, growth factors, neurochemistry and neuroimmunology, theoretical and physical biology, cell biology, (especially receptors and organelles), and comparative ethology.

For physicians interested in pursuing a career in academic medicine and research, these Fellowships offer an opportunity to train with scientists and physicians of international stature. Excellent facilities are available on the 306-acre NIH campus in Bethesda, Maryland, including a 480-bed research and teaching hospital, more than 1,000 laboratories, and the National Library of Medicine. Medical Staff Fellows receive an initial salary of $37,000 with increments of $2,000 per year and may be appointed to the Civil Service or the Public Health Service. Moving and travel allowances and Federal health care benefits are available.

Applications may be obtained from:

The NIH Medical Staff Fellowship Program
Ms. Vicki Malick
National Institutes of Health
Building 10, Room 1C292
9000 Rockville Pike
Bethesda, Maryland 20892
(301) 496-2427

Further information may be obtained from:

Arthur S. Levine, M.D.
Scientific Director, National Institute of Child Health and Human Development
National Institutes of Health
Building 31, Room 2A50
9000 Rockville Pike
Bethesda, Maryland 20892
(301) 496-2133

Deadline for applications is November 10, 1989.

NIH IS AN EQUAL OPPORTUNITY EMPLOYER.

Michael Reese Health Plan

The **Michael Reese Health Plan,** a large well-established HMO serving its members in 20 Health Centers throughout the Chicago Metro area, is seeking additional Board Certified/Eligible physicians in the following specialty:

Obstetrics Gynecology

Physicians with the Health Plan enjoy a full practice with modern facilities and equipment as well as opportunities for teaching.

A highly-competitive salary and excellent benefits complement a rewarding practice and lifestyle.

Interested applicants should address inquiries to:

Scott P. Smith, M.D.
MICHAEL REESE HEALTH PLAN
2545 Dr. Martin Luther King Dr.,
Chicago 60616
or call (312) 808-4551

an equal opportunity employer m/f/h

REPRODUCTIVE ENDOCRINOLOGIST

The Allentwon Hospital—Lehigh Valley Hospital Center, an 800-bed tertiary care teaching hospital in eastern Pennsylvania, is seeking a board certified reproductive endocrinologist (may also be active candidate for certification). The hospital will help the successful candidate to set up a pivate practice and will look to the physician to provide leadership and medical education in reproductive endocrinology and infertility. Total income will be guaranteed for a period of time.

Ours is a burgeoning area. We are located in a beautiful section of Pennsylvania, one and one half hours from New York City and one hour from Philadelphia.

Please submit your curriculum vitae in complete confidence to: Sze-ya Yeh, M.D., Chairman, Department of Obstetrics & Gynecology, C/O HealthSearch, 50 College Drive, Allentown, PA 18104, 215-778-7993. We are an equal opportunity employer m/f/h/v.

Join a Leader
We're The Ohio Permanente Medical Group, Inc.

and we are looking for board certified/board eligible **Obstetrician/Gynecologists** to help us keep up with rapid growth of the Kaiser Permanente Program in Northeastern Ohio. OPMG is the multispecialty group practice that provides health care services to the more than 185,000 Kaiser members in the Cleveland-Akron area.

Our wealth of experience of over 40 plus years (25 in Ohio) makes Kaiser Permanente a mature, solid, leader in the managed care sector of the health care industry. The rewards of practice with us are substantial—excellent salary and benefit packages, company-paid retirement plan, full malpractice coverage, a stimulating, collegial environment in which to practice quality medicine, and more.

Kaiser Permanente's Ohio Region is located in the heart of the dynamic, resurgent industrial Midwest. The area offers the best of big city sophistication and culture in an affordable, accessible living area.

Please send your resume to: Ronald G. Potts, M.D., Medical Director, Ohio Permanente Medical Group, Inc., 1300 E. 9th Street, Suite 1100, Cleveland, OH 44114. Or you may call us collect at (216) 623-8780.

KAISER PERMANENTE

OB/GYN

46-physician multi-specialty group located 40 miles north of Sacramento seeks a physician to join our OB/GYN Department. Excellent compensation package the first year. Partnership opportunity thereafter. Contact:

Warren J. Boyer, Jr., M.D.
Marysville Medical Group
800 Third Street
Marysville, CA 95901

MARYSVILLE MEDICAL GROUP

MATERNAL-FETAL MEDICINE
ASHEVILLE, NORTH CAROLINA

The University of North Carolina Department of OB/GYN and the Mountain Area Health Education Center has a full time faculty position open in Asheville, NC.

Assistant/Associate Professor: Individual should be experienced in prenatal diagnosis to include ultrasonography, fetal therapy and counseling with experience in management of high risk pregnancy. Candidates should have demonstrated abilities in clinical practice, teaching and research and have completed fellowship training in Maternal-Fetal Medicine.

The salary and rank are commensurate with experience and training. To apply send CV to:

Tom Bacon, DrPH
Chairman, Search Committee
Mountain Area Health Education Center
501 Biltmore Avenue
Asheville, NC 28801

MAHEC and the University of North Carolina is an Affirmative Action Equal Opportunity Employer.

OBSTETRICS AND GYNECOLOGY
WISCONSIN

We are looking for a Board Certified/Board Eligible obstetrician/gynecologist to join a 7 physician OB/GYN Department in a large multi-specialty group practice located near Milwaukee, Wisconsin, to share coverage at two clinic locations and two hospitals. Competitive salary and fringe benefits.

Forward inquiries and CV to:

Administrator
P.O. Box 427
Menomonee Falls, WI 53051

OB-GYN

The University of Rochester has expanded its residency program to include Rochester General Hospital. Located in northern Rochester in upstate New York near lakes and other recreational areas. Rochester General is a 540 bed hospital. We currently have a position for a board certified or eligible obstetrician-gynecologist with a major interest in state-of-the-art gyn laparoscopy to join the full time faculty. The position requires strong communication and interpersonal skills and excellent teaching ability. It is anticipated that the successful applicant will provide leadership in the development of ambulatory gyn surgery and contribute to the scientific literature.

The University of Rochester offers an excellent salary and benefits package commensurate with academic credentials and clinical experience.

For consideration please submit your curriculum vitae in confidence to:

Phyllis C. Leppert, CNM, MD, PhD
Chief of Obstetrics & Gynecology
Rochester General Hospital
1425 Portland Avenue
Rochester, New York 14621

The University of Rochester is an equal opportunity employer.

POSITIONS OPEN – OBSTETRICS AND GYNECOLOGY
University of Utah – School of Medicine

Division of General Obstetrics and Gynecology

We are seeking an individual at the rank of Instructor or Assistant Professor who is committed to a career in academic medicine. We have a particular need for someone interested in practicing and teaching obstetrics. A variety of opportunities for clinical investigation and career development are available. The applicant must be board certified or an active candidate for such certification.

Division of Maternal-Fetal Medicine

We are seeking someone to join four certified perinatologists and two fellows at the rank of Assistant Professor. The applicant must be interested in research, teaching, and patient care. We are especially interested in someone with interest and special expertise in obstetric ultrasound, and fetal therapy. Applicants must be certified in maternal fetal medicine, or active candidates for certification.

Interested candidates should send curriculum vitae to:

James R. Scott, M.D.
Professor and Chairman
Department of Obstetrics and Gynecology
University of Utah School of Medicine
Salt Lake City, Utah 84132

Applications must be postmarked by September 01, 1989. Applications received after that deadline may be considered if no qualified applications have been received by that time. The University of Utah is an Equal Opportunity Affirmative Action Employer.

FELLOWSHIP
MATERNAL-FETAL MEDICINE
JULY, 1990

The Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, MacDonalc Hospital for Women, University Hospitals of Cleveland, and Case Western Reserve University, has an opening at the instructor level beginning July 1, 1990. Applicants should desire a career in academic medicine and must be Board certified/eligible by the American Board of Obstetrics and Gynecology.

Fellows will be involved in teaching medical students and house officers. The fellowship program emphasizes clinical and basic research in clinical perinatology with a particular interest in ultrasound, Doppler and perinatal diagnosis. The individual will also be responsible for some general duties in obstetrics and gynecology.

Applicants should send a curriculum vitae to:

J.C. Veille, M.D.
Director, Division of Maternal-Fetal Medicine
MacDonald Hospital for Women, University Hospitals of Cleveland
2074 Abington Road
Cleveland, OH 44106
216/844-3338

The University is an equal opportunity/affirmative action employer.

FACULTY POSITION OB/GYN GENERAL SPECIALIST

The Knoxville Unit of The University of Tennessee College of Medicine is currently accepting applications for a General Specialist in the Department of Obstetrics and Gynecology at The University of Tennessee Medical Center at Knoxville.

This position requires board certification or eligibility in Obstetrics and Gynecology. Responsibilities include: resident and medical student education; participation in Regionalization Program; and clinical or basic research.

Specialized areas of interest such as benign GYN surgery and/or reproductive immunology are encouraged.

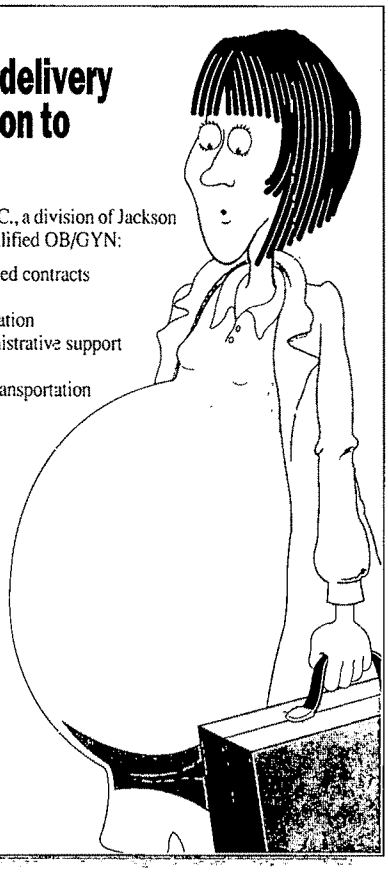
Academic rank is commensurate with both qualifications and experience.

Interested persons should sent curriculum vitae:

Michael R. Caudle, M.D.
Associate Professor and Chairman
Department of Obstetrics and Gynecology

THE UNIVERSITY OF TENNESSEE
Medical Center at Knoxville
1924 ALCOA HIGHWAY
KNOXVILLE, TENNESSEE 37920
EEO/An Affirmative Action/Title IX/Section 504 Employer

Be in on the delivery of the solution to OB staffing.



LOCUM TENENS, INC., a division of Jackson & Coker, offers the qualified OB/GYN:

- 3 to 9 month guaranteed contracts
- competitive daily rate
- licensing and coordination
- knowledgeable administrative support
- round trip airfare
- on-site housing and transportation
- permanent practice opportunities

Call 1-800-544-1987
or write to:
Locum Tenens, Inc.
400 Perimenter
Center Terrace
Suite 760 OGLT9
Atlanta, Georgia 30346

LOCUM TENENS, INC.
A Division of Jackson and Coker

OBSTETRICIAN GYNECOLOGIST

Expanding Western Massachusetts staff model Health Maintenance Organization is seeking a full-time Board Certified/Board Eligible Obstetrician/Gynecologist to join a staff of eight (8) physicians and six (6) midwives.

Our multi-specialty group practice employs seventy (70) full-time physicians and provides care for a growing membership of over 70,000 from modern suburban facilities, in-patient care is provided at 900-bed Tufts affiliated teaching hospital.

Attractive Pioneer Valley location with fine educational, cultural and recreational opportunities. Excellent salary and comprehensive benefit program. Please send curriculum vitae to: Oded Rencus, M.D., Chief of OB/GYN, 305 Bicentennial Highway, Springfield, MA 01118.

MedicalWest
Community Health Plan
A BLUE CROSS & BLUE SHIELD HMO

We are an equal opportunity/affirmative action employer

SPECIAL DELIVERIES. SPECIAL CHALLENGES.

The Southern California Permanente Medical Group invites you to join a unique prepaid health care program. Our multispecialty group practice is a partnership composed of and managed by SCPMG physicians.

Board eligible/certified **PERINATOLOGISTS** are needed for our high-volume obstetric services which handles 3,000-6,000 deliveries per year. Opportunities exist both to join and create high risk services.

There are positions available to practice Perinatology exclusively. Perinatologists who are interested in performing other aspects of obstetrics and gynecology may do so. We also have positions available for Endocrinologists and General Obstetrician-Gynecologists. University affiliation and clinical research are encouraged.

Our compensation is competitive and benefits are outstanding. They include: professional liability, medical and dental coverage, vacation and sick leave, continuing education, life insurance and retirement plans. **After two years full-time employment, physicians are eligible to be considered for partnership.**

For a physician application, please call or write to: William G. McCormick, M.D., Physician Recruitment, Dept. 006, Walnut Center, Pasadena, CA 91188-8013. (714) 829-5365.

Equal Opportunity Employer M/F/H

KAISER PERMANENTE
Good People. Good Medicine.

DIRECTOR

DEPARTMENT OF OBSTETRICS AND GYNECOLOGY

THE MT. SINAI MEDICAL CENTER, CLEVELAND

The Mt. Sinai Medical Center, a 450 bed tertiary care teaching hospital with 12 GME programs, is seeking a Director for its Department of Obstetrics and Gynecology.

The Medical Center is located proximate to the Case Western Reserve University School of Medicine in the University Circle area of Cleveland, the educational, cultural and medical hub of Northeastern Ohio.

The successful candidate will have background and experience in academic, clinical and research activities to provide strong leadership for the full time and voluntary staffs as well as qualifications enabling an appointment to the Faculty of the School of Medicine.

Individuals should indicate their interest with a letter, accompanied by their curriculum vitae, to:

Jeffrey L. Ponsky, M.D.
Director, Department of Surgery, and
Chairman, Search Committee
The Mt. Sinai Medical Center • One Mt. Sinai Drive
Cleveland, Ohio 44106
(216) 421-4058

An affiliate of the Case Western Reserve University School of Medicine

Mt. Sinai is an affirmative action, equal opportunity employer.

JOHNS HOPKINS HEALTH SYSTEM medical group needs additional physicians in OB/GYN for full-time and part-time positions at new and established offices throughout the greater Baltimore area. Physicians must be board certified or active candidates, and eligible for Maryland license and DEA certificate. Interviewing now for next summer; some positions available immediately. Equal Employment Opportunity Employer. Send current curriculum vitae to: Recruiting Coordinator; Office of Medical Affairs; Wyman Park Medical Associates; 3100 Wyman Park Drive; Baltimore, Maryland 21211.

OB/GYN

BE/BC OB/GYN needed to join 14 man multi-specialty practice in Alabama. LARGEST practice in a 45 mile radius. 150 bed hospital located across the street from center. Excellent small town atmosphere. Perfect place to raise children. Local school system above national average. Excellent salary and full benefits. Senior partnership in 2 years.

For more information send your C.V. to Jordan/Fairfax, 6560 N. Scottsdale Rd., Suite J-106, Scottsdale, AZ 85253, Attention: Lori K. Bedford or call (800) 258-9900, (602) 991-8100.

The Doctor Is In...

...private practice in the perfect community

If that's your goal, Humana can help. We have over 80 hospitals in 20 states. Chances are we know of a private practice opportunity in the location of your choice. Consider these communities:

- **Huntsville, AL**
- **Los Angeles Co., CA**
- **Orlando, FL**
- **South Florida**
- **St. Petersburg, FL**
- **Atlanta, GA area**
- **Augusta, GA**
- **Chicago, IL**
- **Indianapolis, IN**
- **Kansas City, KS**
- **Louisville, KY**
- **Somerset, KY**
- **New Orleans, LA**
- **Oakdale, LA**
- **Springhill, LA**
- **Chattanooga, TN**
- **Lebanon, TN**
- **Richmond, VA**
- **Greenbrier Valley, WV**

We have other opportunities not listed here.

For further information, call TOLL-FREE 1-800-626-1590, or send your curriculum vitae to: Manager, Professional Relations, Humana, Inc., Dept. OB-9, 500 West Main Street, Louisville, KY 40201-1438.

Humana®

DIRECTOR OF PERINATOLOGY SERVICES

The Irvine Medical Center, located in Irvine, California, is seeking a full-time, Board Certified Perinatologist to oversee the perinatology services of a 177-bed hospital with a current Medical Staff of 600 physicians. The Irvine Medical Center is currently under construction and is the only hospital in Irvine, a rapidly growing, well established community located in Orange County. The Perinatology Director will have the opportunity to develop the perinatology services for the Medical Center complex.

Please send CV to: Perinatology Search Committee, Irvine Medical Center, 4605 Barranca Parkway, Suite 200, Irvine, CA 92714.

Life.

It's our life's work.

If your mission in life is to practice the ideals of medicine without administrative demands, we invite your inquiry.

Group Health Plan in St. Louis is a staff model HMO that promotes the ideals of private practice while freeing doctors from the financial baggage of the private practitioner.

Group Health Plan isn't for every physician. Just for those few who want to practice medicine without compromise.

For an interview please talk with Tim Walton at (314) 993-5955. Or write Group Health Plan, 11475 Olde Cabin Road, St. Louis, Mo. 63141. An equal opportunity employer.

GROUP HEALTH PLAN

REPRODUCTIVE ENDOCRINOLOGIST

Teaching department with successful Reproductive Endocrinology Division, inclusive of highly successful IVF program, is searching for a well qualified individual as **DIVISION CHIEF** and a **JUNIOR MEMBER** of the division. Candidates for the position of Division Chief should be subspecialty Board certified, while candidates for the junior position may be subspecialty Board eligible. Academic appointments commensurate with experience level.

We are looking for individuals with an interest in research as well as clinical medicine. Please submit your Curriculum Vitae to: N. Gleicher, M.D., Chairman, Department of Obstetrics and Gynecology, Mount Sinai Hospital Medical Center, Room F208, California Avenue at 15th Street, Chicago, Illinois 60608.

OBSTETRICIANS

A progressive, non-profit 300 bed Regional HealthCare Center in Elizabethtown, Kentucky serving a population base of 225,000 is in need of obstetricians.

Located in Hardin County, one of the fastest growing counties in Kentucky, Elizabethtown's actively expanding industrial base, good schools, small town congeniality, and nearby access to the benefits of a major city make it a very desirable location.

Our offer includes:

- Guaranteed income
- Site visit
- Relocation expenses
- Other negotiable benefits

For further information, please contact:
Carol Jones, Physician Liaison
Hardin Memorial Hospital
North Dixie Avenue
Elizabethtown, KY 42701
502-765-1728

Hardin Memorial Hospital
A Regional HealthCare Center



North Dixie Avenue, Elizabethtown, Kentucky

OB/GYN

HITCHCOCK CLINIC

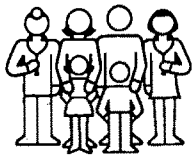
Outstanding opportunity for BC/BE Ob-Gyn physician to join a busy general Ob-Gyn practice in rural Vermont. Association with board certified Ob-Gyn. Employment through Hitchcock Clinic offers guaranteed income, benefits and faculty appointment at Dartmouth Medical School. Randolph, Vermont is centrally located to most of Vermont's major ski areas, but off the tourist track. Convenient to Boston, Montreal. Many practice and lifestyle advantages other practice opportunities cannot provide.

For information contact: Brent Burgee, M.D., Hitchcock Associates of Randolph/Ob-Gyn, 1 Maple Street, Randolph, Vermont 05060; (802) 728-4431.

THE HITCHCOCK CLINIC

An equal opportunity/ affirmative action employer

A Component of the Dartmouth-Hitchcock Medical Center



Central Minnesota
Group Health Plan

OB/GYN

Rapidly growing, innovative primary care clinic has openings for two board eligible/certified OB/GYN physicians. New clinic facility in the fastest growing urban area in Minnesota. St. Cloud is a community of 45,000 located on the upper Mississippi River 70 miles from Mpls./St. Paul. Be close to lakes, ski areas and excellent schools, including three universities. Join a supportive primary care staff and receive competitive salary, outstanding benefits, liberal vacation and educational leave, malpractice coverage, etc. Contact Physician Services, Central Minnesota Group Health Plan, 1245 15th Street North, St. Cloud, MN 56303. 612/253-5220. EOE.

WORK IN PLEASANT SURROUNDINGS

Long established **OB-GYN** practice in Bergen County, New Jersey (10 minutes from New York City) seeks new partner in July 1990. Brand new high-tech office 5 minutes from modern hospital.

Submit CV, special interests and foreign language proficiencies.

FOX, HIRSCH & FERNANDEZ
870 Palisade Ave.
Teaneck, New Jersey 07666

For further information call:
(210) 907-0417



PENNSYLVANIA

Well established OB/GYN. private practice in Eastern Pennyslvania is seeking F/M fourth member. Area offers suburban living with advantages offered by close proximity to most major East Coast cities.

Salary, malpractice insurance, health insurance, fringe benefits. Send CV or call:

College Heights Ob/Gyn
3131 College Heights Blvd.
Allentown, PA 18104
(215) 437-1931
(800) 343-8173

BLUE RIDGE MOUNTAINS VIRGINIA

BC/BE American Trained OB/GYN sought to join a busy two-man practice in the scenic Blue Ridge Mountains of Virginia. Needed July 1, 1990. Area is served by a modern 150 bed hospital with over 500 deliveries a year and very active GYN surgical service, including Laser. Excellent opportunity for the right person interested in this area bordering western North Carolina.

Send inquiries and c.v. to:
GALAX OBSTETRICS &
GYNECOLOGY, INC.
106 Doctors Park
Galax, VA 24333

WANTED!

OBSTETRICIAN/GYNECOLOGIST

For private practice and small group practice opportunities.

— JCAH accredited community hospital in beautiful Sacramento, California seeks BE/BC OB/GYN to fill a number of practice opportunities.

— Extremely attractive assistance and relocation package available including: malpractice coverage, marketing assistance, moving expenses, and a one-time recruitment bonus.

— The Sacramento area is one of America's fastest growing communities. The income potential for OB/GYN physicians is **OUTSTANDING!**

For further information, please write or call:

AMERICAN RIVER HOSPITAL
Physician Services Department,
c/o Larry Furia
3609 Mission Avenue, Suite E,
Carmichael, CA 95608
(916) 486-2261

Central Maine Healthcare Recruitment: is conducting a search for BC/BE OBG specialist interested in practicing in the Western Mountain Region of Maine. The opportunity is flexible offering the physician a choice of practice types and is supported by a 90 bed hospital facility dedicated to continued staff development. The community serves a population of 23,000 and is in an area that boasts of easy access to year round outdoor activities. For more information: phone or send CV and letter to:

E. Bailey,
Director of Physician Recruitment,
76 High St. Ste. 203,
Lewiston, ME 04240,
207-795-2389.

OBSTETRICS & GYNECOLOGY

Obstetrician/Gynecologist needed to join a five physician department at a major regional multispecialty clinic in Billings, Montana. Clinic setting includes all specialties and is adjacent to an outstanding tertiary care referral hospital. Excellent opportunity for a top notch board eligible or board certified Obstetrican/Gynecologist looking for a challenging practice in a geographic area offering unlimited recreational potential. Those interested send CV to:

John W. Heizer, M.D.,
Medical Director
The Billings Clinic
P.O. Box 35100
Billings, Montana 59107-5100

FELLOWSHIP IN PEDIATRIC & ADOLESCENT GYNECOLOGY

The Section of Pediatric & Adolescent Gynecology, Division of Gynecology, Department of Obstetrics and Gynecology at The University of Tennessee, Memphis is offering a one year fellowship in Pediatric and Adolescent Gynecology, beginning July, 1990. The fellowship program follows the educational objective of the North American Society for Pediatric & Adolescent Gynecology. Applicants must have completed a residency in obstetrics and gynecology. Eligibility for medical licensure in Tennessee is required. The fellowship provides a comprehensive experience in Pediatric & Adolescent Gynecology, as well as an opportunity for interaction with other specialties (Reproductive Endocrinology, Pediatric Endocrinology, Pediatric Psychiatry, and Reproductive Genetics). Fellows will be actively involved in clinical research. Applicants should send a curriculum vitae to David Muram, M.D., Chief, Section of Pediatric & Adolescent Gynecology, University of Tennessee, Memphis/ University Physicians Foundation, Inc., 853 Jefferson Avenue, Memphis, Tennessee 38103. The University of Tennessee, Memphis is an equal employment opportunity/affirmative action/Title IX/ Section 504 employer.

INDEX TO ADVERTISERS

American River Hospital
Opportunity Available 57A

Bay Shores Medical Group
Opportunity Available 37A

Berlex Laboratories, Inc.
Tri-Levlen 30A,31A,32A

Billings Clinic
Opportunity Available 57A

Central Maine Healthcare
Opportunity Available 57A

Central Minnesota Group Health Plan
Opportunity Available 56A

College Heights Ob/Gyn
Opportunity Available 56A

Churchill Livingstone
Books 47A

Corometrics Medical Systems, Inc.
Aloka 620 ..... 8A

Fox Hirsch & Fernandez
Opportunity Available 56A

Galax Obstetrics & Gynecology, Inc.
Opportunity Available 56A

Group Health Plan
Opportunity Available 55A

Hardin Memorial Hospital
Opportunity Available 55A

HealthSearch
Opportunity Available 55A

Hitchcock Clinic
Opportunity Available 55A

Humana, Inc.
Opportunity Available 54A

Irvine Medical Center
Opportunity Available 54A

Johns Hopkins Health System
Opportunity Available 54A

Jordan/Fairfax
Opportunity Available 54A

Kaiser Permanente
Opportunity Available 53A

Kaiser Permanente Medical Center
Opportunity Available 50A

Locum Tenens, Inc.
Opportunity Available 53A

Marysville Medical Group
Opportunity Available 51A

Medical West
Opportunity Available 53A

Michael Reese Health Plan
Opportunity Available 50A

Mount Sinai Hospital Medical Center
Opportunity Available 50A

Mount Sinai Medical Center
Opportunity Available 53A

Mountain Area Health Education Center
Opportunity Available 51A

National Institutes of Health
Fellowships 50A

Nellcor Incorporated
N-200 ..... 42A,43A

Ortho Pharmaceutical Corporation
All-Flex 6A
Ortho-Novum 7/7/7 ..... Third Cover, Fourth Cove

Rochester General Hospital
Opportunity Available 49A,52A

Sinai Hospital of Baltimore
Opportunity Available 49A

Searle & Company
Demulen 1/35 ..... 33A,34A,35A,36A

Serono Laboratories, Inc.
Serophene 3A,4A

Springer-Verlag
Book 37A

St. John Hospital
Opportunity Available 49A

South Cove Community Health Center
Opportunity Available 49A

SUNY at Stony Brook-HSC
Opportunity Available 48A

Syntex Laboratories, Inc.
Femstat Prefill 38A,39A,40A
Tri-Norinyl ..... Second Cover,1A,2A

2V Medtech International Corp.
2V STAT 40A

Trager, William M.D.
Opportunity Available 48A

University Hospitals of Cleveland
Fellowship 52A
Opportunity Available 48A

University of South Alabama
Opportunity Available 48A

University of Tennessee Medical Center at Knoxville
Opportunity Available 47A,52A

University of Utah School of Medicine
Opportunity Available 52A

University Physicians Foundation
Opportunity Available 57A

Utah Women's Health Center
Opportunity Available 47A

VHA Physician Placement Services
Opportunities Available 29A

Wyeth-Ayerst Laboratories
Lo/Ovral 44A,45A,46A
Premarin 20A,21A,22A

While every precaution is taken to insure accuracy, we cannot guarantee against the possibility of an occasional change or omission in the preparation of this index.

21- and 28-day regimens

ORTHO-NOVUM® 7/7/7 Tablets

norethindrone/ethinyl estradiol

Each white tablet contains 0.5 mg norethindrone and 0.035 mg ethinyl estradiol. Each light peach tablet contains 0.75 mg norethindrone and 0.035 mg ethinyl estradiol. Each peach tablet contains 1 mg norethindrone and 0.035 mg ethinyl estradiol. Each green tablet in 28-day regimen contains inert ingredients.

IMPORTANT NOTE—This information is a BRIEF SUMMARY of the complete prescribing information provided with the product and therefore should not be used as the basis for prescribing the product. This summary was prepared by deleting from the complete prescribing information certain text, tables and references. The physician should be thoroughly familiar with the complete prescribing information before prescribing the product.

INDICATIONS AND USAGE: PREVENTION OF PREGNANCY.

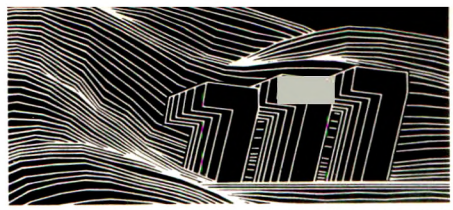
CONTRAINDICATIONS: Oral contraceptives should not be used in women who currently have the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Carcinoma of the endometrium or other known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Cholestatic jaundice of pregnancy or jaundice with prior pill use. 8. Hepatic adenomas or carcinomas. 9. Known or suspected pregnancy.

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia, and gallbladder disease, although the risk of serious morbidity or mortality is very small in healthy women without underlying risk factors. The risk of morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, obesity and diabetes. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks. The information contained in this brief summary is principally based on studies carried out in patients who used oral contraceptives with higher formulations of estrogens and progestogens than those in common use today. The effect of long term use of the oral contraceptives with lower formulations of both estrogens and progestogens remains to be determined. Throughout this brief summary epidemiological studies reported are of two types: retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of a disease, namely a *ratio* of the incidence of a disease among oral contraceptive users to that among nonusers. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide a measure of attributable risk, which is the *difference* in the incidence of disease between oral contraceptive users and nonusers. The attributable risk does provide information about the actual occurrence of a disease in the population. For further information, the reader is referred to a text on epidemiological methods. 1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. a. Myocardial Infarction. An increased risk of myocardial infarction has been associated with oral contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary artery disease such as hypertension, hypercholesterolemia, morbid obesity, and diabetes. The relative risk of heart attack for current oral contraceptive users has been estimated to be two to six. The risk is very low under the age of 30. Smoking in combination with oral contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women in their mid-thirties or older with smoking accounting for the majority of excess cases. Mortality rates associated with circulatory disease have been shown to increase substantially in smokers, especially in those 36 years of age and older among women who use oral contraceptives. Oral contraceptives may compound the effects of well-known risk factors, such as hypertension, diabetes, hyperlipidemias, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and cause glucose intolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see section 9 in WARNINGS). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors. b. Thromboembolism. An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to non-users to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. Cohort studies have shown the relative risk to be somewhat lower, about 3 for new cases and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease associated with oral contraceptives is not related to length of use and disappears after pill use is stopped. A two- to four-fold increase in relative risk of post-operative thromboembolic complications has been reported with the use of oral contraceptives. The relative risk of venous thrombosis in women who have predisposing conditions is twice that of women without such medical conditions. If feasible, oral contraceptives should be discontinued at least four weeks prior to and for two weeks after elective surgery of a type associated with an increase in risk of thromboembolism and during and following prolonged immobilization. Since the immediate postpartum period is also associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than four weeks after delivery in women who elect not to breast feed. c. Cerebrovascular diseases. Oral contraceptives have been shown to increase both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes), although, in general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and non-users, for both types of strokes, and smoking interacted to increase the risk of stroke. In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for nonsmokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk is also greater in older women. d. Dose-related risk of vascular disease from oral contraceptives. A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high density lipoproteins (HDL) has been reported with many progestational agents. A decline in serum high density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the activity of the progestogen used in the contraceptive. The activity and amount of both hormones should be considered in the choice of an oral contraceptive. Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral contraceptive agents should be started on preparations containing 0.035 mg or less of estrogen. e. Persistence of risk of vascular disease. There are two studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40-49 years who had used oral contraceptives for five or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. However, both studies were performed with oral contraceptive formulations containing 50 micrograms or higher of estrogens. 2. ESTIMATES OF MORTALITY FROM CONTRACEPTIVE USE. One study gathered data from a variety of sources which have estimated the mortality rate associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is low and below that associated with childbirth. However, smokers 35 and older and non-smokers 40 and older who use oral contraceptives have a significant increase in mortality higher than those using other methods of birth control. These facts must be weighed in conjunction with failure rates for other methods and the risk associated with subsequent pregnancy. 3. CARCINOMA OF THE REPRODUCTIVE ORGANS. Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. While there are conflicting reports the overall evidence in the literature suggests that use of oral contraceptives is not associated with an increase in the risk of developing breast cancer. The Cancer and Steroid Hormone (CASH) study also showed no latent effect on the risk of breast cancer for at least a decade following long term use. Some studies have shown a slightly increased relative risk of developing breast cancer. Some studies suggest that oral contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of

Revised 11/88



women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors. In spite of many studies of the relationship between oral contraceptive use and breast and cervical cancers, a cause and effect relationship has not been established. 4. HEPATIC NEOPLASIA. Benign hepatic adenomas are associated with oral contraceptive use, although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases/100,000 for users, a risk that increases after four or more years of use especially with oral contraceptives of higher dose. Rupture of benign, hepatic adenomas may cause death through intra-abdominal hemorrhage. Studies from Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (>8 years) oral contraceptive users. However, these cancers are rare in the U.S. and the attributable risk (the excess incidence) of liver cancers in oral contraceptive users approaches less than one per million users. 5. OCULAR LESIONS. There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia; papilledema, or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately. 6. ORAL CONTRACEPTIVE USE BEFORE OR DURING EARLY PREGNANCY: RISK OF BIRTH DEFECTS. Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. The majority of recent studies also do not indicate a teratogenic effect, particularly in so far as cardiac anomalies and limb reduction defects are concerned, when taken inadvertently during early pregnancy. The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion. It is recommended that for any patient who has missed two consecutive periods pregnancy should be ruled out before continuing oral contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral contraceptive use should be discontinued until pregnancy is ruled out. 7. GALLBLADDER DISEASE. Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral contraceptive formulations containing lower hormonal doses of estrogens and progestogens. 8. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. Oral contraceptives have been shown to cause a decrease in glucose tolerance in a significant percentage of users. This effect has been shown to be directly related to estrogen dose. Progestogens increase insulin secretion and create insulin resistance; this effect varying with different progestational agents. However, in the non-diabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects prediabetic and diabetic women in particular should be carefully monitored while taking oral contraceptives. A small proportion of women will have persistent hypertriglyceridemia while on the pill. As discussed earlier (see WARNINGS 1a and 1d), changes in serum triglycerides and lipoprotein levels have been reported in oral contraceptive users. 9. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in women taking oral contraceptives, and this increase is more likely in older oral contraceptive users and with extended duration of use. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing progestational activity. Women with a history of hypertension or hypertension-related diseases, or renal disease, should be encouraged to use another method of contraception. If women elect to use oral contraceptives, they should be monitored closely and if significant elevation of blood pressure occurs, oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives, and there is no difference in the occurrence of hypertension between former and never users. 10. HEADACHE. The onset or exacerbation of migraine or development of headache with a new pattern which is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause. 11. BLEEDING IRREGULARITIES. Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first three months of use. Non-hormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out. Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was preexistent. 12. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. **PRECAUTIONS:** 1. PHYSICAL EXAMINATION AND FOLLOW UP. A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology, and relevant laboratory tests. In case of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care. 2. LIPID DISORDERS. Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult. 3. LIVER FUNCTION. If jaundice develops in any woman receiving such drugs, the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function. 4. FLUID RETENTION. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention. 5. EMOTIONAL DISORDERS. Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 6. CONTACT LENSES. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist. 7. DRUG INTERACTIONS. Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar association, though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines. 8. INTERACTIONS WITH LABORATORY TESTS. Certain endocrine and liver function tests and blood components may be affected by oral contraceptives: a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. b. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radio-immunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. c. Other binding proteins may be elevated in serum. d. Sex-binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged. e. Triglycerides may be increased. f. Glucose tolerance may be decreased. g. Serum folate levels may be depressed by oral contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives. 9. CARCINOGENESIS. See WARNINGS section. 10. PREGNANCY. Pregnancy Category X. See CONTRAINDICATIONS and WARNINGS sections. 11. NURSING MOTHERS. Small amounts of oral contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child. **INFORMATION FOR THE PATIENT. See Patient Package Insert. ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS section). Thrombophlebitis and venous thrombosis with or without embolism. Arterial thromboembolism. Pulmonary embolism. Myocardial infarction. Cerebral hemorrhage. Cerebral thrombosis. Hypertension. Gallbladder disease. Hepatic adenomas or benign liver tumors. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea. Vomiting. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Amenorrhea. Temporary infertility after discontinuation of treatment. Edema. Melasma which may persist. Breast changes: tenderness, enlargement, secretion. Change in weight (increase or decrease). Change in cervical erosion and secretion. Diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted: Pre-menstrual syndrome. Cataracts. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. Acne. Changes in libido. Colitis. **OVERDOSAGE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea and withdrawal bleeding may occur in females.

ORTHO PHARMACEUTICAL CORPORATION
Raritan, NJ 08869-0602

Low-impact norethindrone

No significant change in mean values for total cholesterol, HDL, and LDL†



21- and 28-day regimens

ORTHO-NOVUM® 777 Tablets

norethindrone/ethinyl estradiol

†Serious as well as minor side effects have been reported with the use of oral contraceptives. The physician should remain alert to the earliest manifestations of any symptoms of serious disease and discontinue oral contraceptive therapy when appropriate.

Please see brief summary of complete Prescribing Information on the preceding page.

ORTHO PHARMACEUTICAL CORPORATION
Raritan, New Jersey 08869-0602

© OPC 1988

OJ-377R

11. 12. 98

December 1989
in two parts, part 1
volume 161, *number* 6
Index number

American Journal of OBSTETRICS AND GYNECOLOGY

Copyright © 1989 by The C.V. Mosby Company

Editor in Chief
JOHN I. BREWER

Editors
FREDERICK P. ZUSPAN • E. J. QUILLIGAN

Associate Editor
ALBERT B. GERBIE

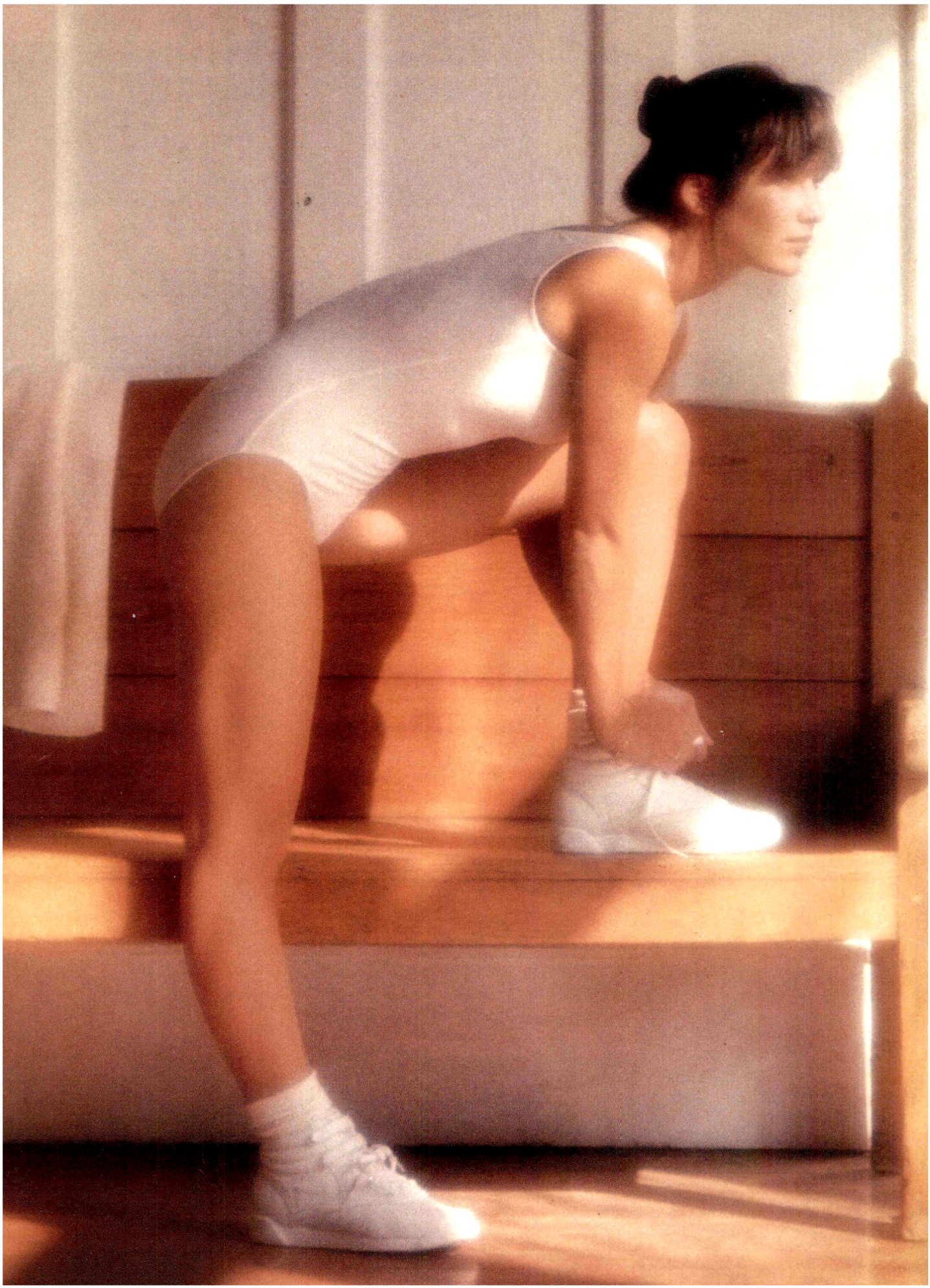
Official Publication

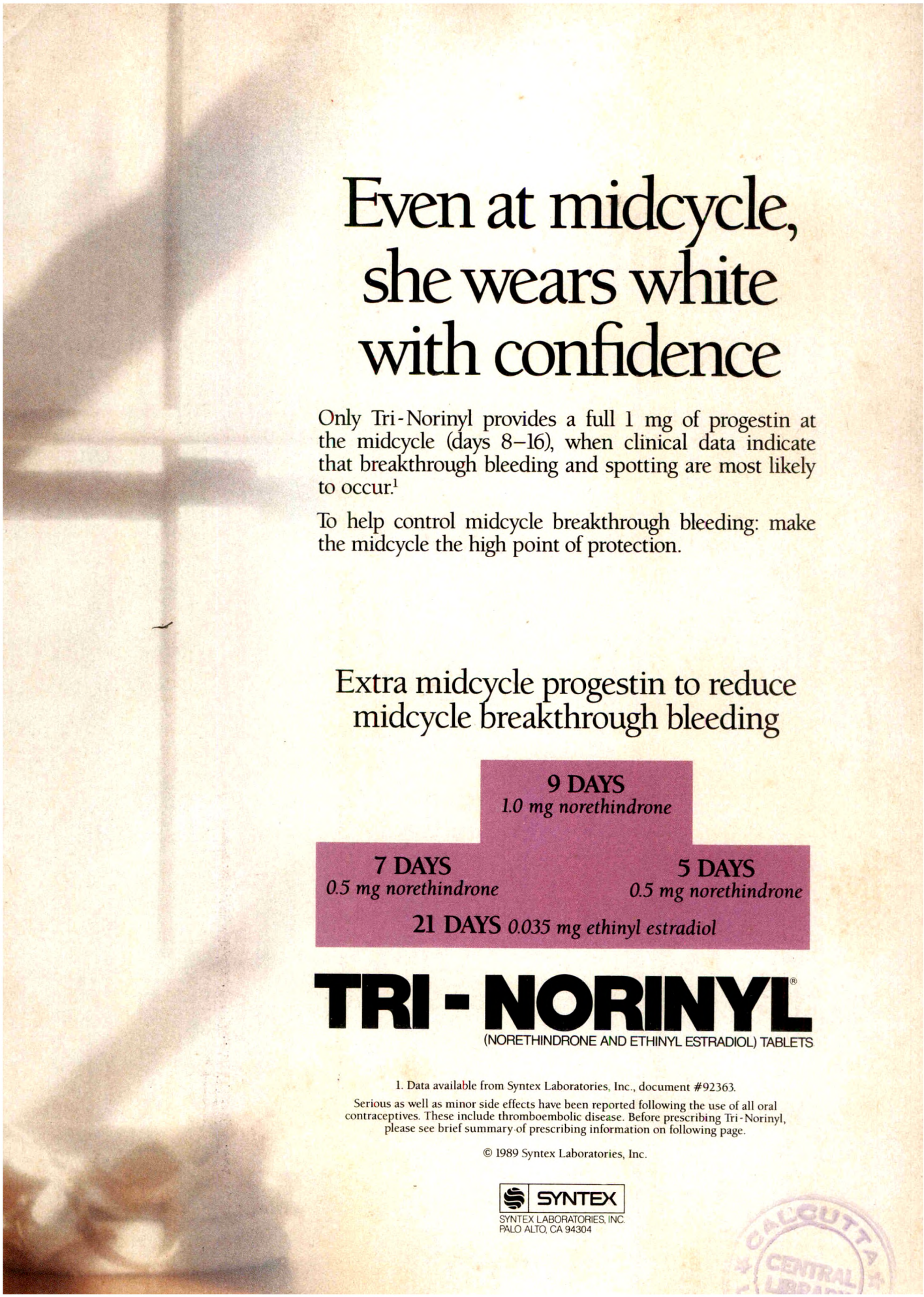
AMERICAN GYNECOLOGICAL AND OBSTETRICAL SOCIETY
CENTRAL ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS
SOUTH ATLANTIC ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS
PACIFIC COAST OBSTETRICAL AND GYNECOLOGICAL SOCIETY
AMERICAN BOARD OF OBSTETRICS AND GYNECOLOGY
SOCIETY FOR GYNECOLOGIC INVESTIGATION
SOCIETY OF PERINATAL OBSTETRICIANS

Published by

THE C.V. MOSBY COMPANY
St. Louis, Missouri 63146-3318

ISSN 0002-9378





Even at midcycle, she wears white with confidence

Only Tri-Norinyl provides a full 1 mg of progestin at the midcycle (days 8–16), when clinical data indicate that breakthrough bleeding and spotting are most likely to occur.[1]

To help control midcycle breakthrough bleeding: make the midcycle the high point of protection.

Extra midcycle progestin to reduce midcycle breakthrough bleeding

9 DAYS
1.0 mg norethindrone

7 DAYS
0.5 mg norethindrone

5 DAYS
0.5 mg norethindrone

21 DAYS *0.035 mg ethinyl estradiol*

TRI-NORINYL®

(NORETHINDRONE AND ETHINYL ESTRADIOL) TABLETS

1. Data available from Syntex Laboratories, Inc., document #92363.

Serious as well as minor side effects have been reported following the use of all oral contraceptives. These include thromboembolic disease. Before prescribing Tri-Norinyl, please see brief summary of prescribing information on following page.

© 1989 Syntex Laboratories, Inc.

SYNTEX

SYNTEX LABORATORIES, INC.
PALO ALTO, CA 94304

CALCUTTA CENTRAL LIBRARY

TRI - NORINYL®

(NORETHINDRONE AND ETHINYL ESTRADIOL) TABLETS

TRI-NORINYL® 21-Day Tablets (7 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg, 9 yellow-green tablets of norethindrone 1.0 mg with ethinyl estradiol 0.035 mg, 5 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg)
TRI-NORINYL® 28-Day Tablets (7 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg, 9 yellow-green tablets of norethindrone 1.0 mg with ethinyl estradiol 0.035 mg, 5 blue tablets of norethindrone 0.5 mg with ethinyl estradiol 0.035 mg, 7 inert orange tablets)
BREVICON® 21-Day Tablets (norethindrone 0.5 mg with ethinyl estradiol 0.035 mg)
BREVICON® 28-Day Tablets (21 norethindrone 0.5 mg with ethinyl estradiol 0.035 mg tablets followed by 7 inert tablets)
NORINYL® 1 + 35 21-Day Tablets (norethindrone 1 mg with ethinyl estradiol 0.035 mg)
NORINYL® 1 + 35 28-Day Tablets (21 norethindrone 1 mg with ethinyl estradiol 0.035 mg tablets followed by 7 inert tablets)
NORINYL® 1 + 50 21-Day Tablets (norethindrone 1 mg with mestranol 0.05 mg)
NORINYL® 1 + 50 28-Day Tablets (21 norethindrone 1 mg with mestranol 0.05 mg tablets followed by 7 inert tablets)
NOR-Q.D.® (norethindrone) Tablets 0.35 mg
ORAL CONTRACEPTIVE (O.C.) AGENTS

Indications: Prevention of pregnancy.

CONTRAINDICATIONS

Oral contraceptives should not be used in women who have the following conditions:
- Thrombophlebitis or thromboembolic disorders
- A past history of deep vein thrombophlebitis or thromboembolic disorders
- Cerebral vascular or coronary artery disease
- Known or suspected carcinoma of the breast
- Carcinoma of the endometrium, and known or suspected estrogen-dependent neoplasia
- Undiagnosed abnormal genital bleeding
- Cholestatic jaundice of pregnancy or jaundice with prior pill use
- Hepatic adenomas, carcinomas or benign liver tumors
- Known or suspected pregnancy

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia and gallbladder disease, although the risk of serious morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, hypercholesterolemia, obesity and diabetes.

Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

The information contained in this package insert is principally based on studies carried out in patients who used oral contraceptives with formulations containing 0.05 mg or higher of estrogen. The effects of long term use with lower dose formulations of both estrogens and progestogens remain to be determined.

Throughout this labeling, epidemiological studies reported are of two types: retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of a disease. Relative risk, the *ratio* of the incidence of a disease among oral contraceptive users to that among non-users cannot be assessed directly from case control studies, but the odds ratio obtained is a measure of relative risk. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide not only a measure of the relative risk but a measure of attributable risk, which is the *difference* in the incidence of disease between oral contraceptive users and non-users. The attributable risk does provide information about the actual occurrence of a disease in the population.

1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS

a. Myocardial Infarction

An increased risk of myocardial infarction has been attributed to oral contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary artery disease such as hypertension, hypercholesterolemia, morbid obesity and diabetes. The relative risk of heart attack for current oral contraceptive users has been estimated to be 2 to 6. The risk is very low under the age of 30. However, there is the possibility of a risk of cardiovascular disease even in very young women who take oral contraceptives.

Smoking in combination with oral contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women 35 or older, with smoking accounting for the majority of excess cases.

Mortality rates associated with circulatory disease have been shown to increase substantially in smokers over the age of 35 and non-smokers over the age of 40 among women who use oral contraceptives.

Oral contraceptives may compound the effects of well-known risk factors for coronary artery disease, such as hypertension, diabetes, hyperlipidemias, hypercholesterolemia, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and impair oral glucose tolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see **WARNINGS**, section 9). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors.

b. Thromboembolism

An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to non-users to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. One cohort study has shown the relative risk to be somewhat lower, about 3 for new cases (subjects with no past history of venous thrombosis or varicose veins) and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease due to oral contraceptives is not related to length of use and disappears after pill use is stopped.

A 2- to 6-fold increase in relative risk of post-operative thromboembolic complications has been reported with the use of oral contraceptives. If feasible, oral contraceptives should be discontinued at least 4 weeks prior to and for 2 weeks after elective surgery and during and following prolonged immobilization. Since the immediate postpartum period also is associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than 4 to 6 weeks after delivery in women who elect not to breast feed.

c. Cerebrovascular diseases

An increase in both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes) has been shown in users of oral contraceptives. In general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and non-users for both types of strokes while smoking interacted to increase the risk for hemorrhagic strokes.

In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for non-smokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk also is greater in women 35 or older and among smokers.

d. Dose-related risk of vascular disease from oral contraceptives

A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high density lipoproteins (HDL) has been reported with some progestational agents. A decline in serum high density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the nature and absolute amount of progestogens used in the contraceptives. The amount of both hormones should be considered in the choice of an oral contraceptive.

Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral contraceptive agents should be started on preparations containing the lowest estrogen content that produces satisfactory results for the individual.

e. Persistence of risk of vascular disease

There are three studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40-49 years who had used oral contraceptives for 5 or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. Subarachnoid hemorrhage also has a significantly increased relative risk after termination of use of oral contraceptives. However, these studies were performed with oral contraceptive formulations containing 0.05 mg or higher of estrogen.

2. ESTIMATES OF MORTALITY FROM CONTRACEPTIVE USE

One study gathered data from a variety of sources which have estimated the mortality rates associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is low and below that associated with childbirth. However, smokers 35 and older and non-smokers 40 and older who use oral contraceptives have an increase in mortality greater than those using other methods of birth control. These facts must be weighed in conjunction with failure rates for other methods and the risk associated with subsequent pregnancy.

3. CARCINOMA OF THE BREAST AND REPRODUCTIVE ORGANS

Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. The evidence in the literature suggests that use of oral contraceptives is not associated with an increase in the risk of developing breast cancer, regardless of the age and parity of first use or with most of the marketed brands and doses. The Cancer and Steroid Hormone study also showed no latent effect on the risk of breast cancer for at least a decade following long term use. A few studies have shown a slightly increased relative risk of developing breast cancer, although the methodology of these studies, which included differences in examination of users and non-users and differences in age at start of use, has been questioned.

Some studies suggest that oral contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors.

In spite of many studies of the relationship between oral contraceptive use and breast or cervical cancers, a cause and effect relationship has not been established.

4. HEPATIC NEOPLASIA

Benign hepatic adenomas are associated with oral contraceptive use although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases per 100,000 for users, a risk that increases after 4 or more years of use. Rupture of rare, benign, hepatic adenomas may cause death through intra-abdominal hemorrhage.

Studies in the United States and Britain have shown an increased risk of developing hepatocellular carcinoma in long term (>8 years) oral contraceptive users. However, these cancers are extremely rare in the United States and the attributable risk (the excess incidence) of liver cancers in oral contraceptive users is less than 1 per 1,000,000 users.

5. OCULAR LESIONS

There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately.

6. ORAL CONTRACEPTIVE USE BEFORE OR DURING EARLY PREGNANCY

Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. More recent studies do not suggest a teratogenic effect, particularly insofar as cardiac anomalies and limb reduction defects are concerned, when taken inadvertently during early pregnancy.

The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion.

It is recommended that for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing oral contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral contraceptive use should be discontinued if pregnancy is confirmed.

7. GALLBLADDER DISEASE

Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral contraceptive formulations containing lower hormonal doses of estrogens and progestogens.

8. CARBOHYDRATE AND LIPID METABOLIC EFFECTS

Oral contraceptives have been shown to impair oral glucose tolerance. Oral contraceptives containing greater than 0.075 mg of estrogen cause glucose intolerance with impaired insulin secretion, while lower doses of estrogen may produce less glucose intolerance. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the non-diabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects, prediabetic and diabetic women should be carefully observed while taking oral contraceptives.

Some women may develop persistent hypertriglyceridemia while on the pill. As discussed earlier (see **WARNINGS**, sections 1a. and 1d.), changes in serum triglycerides and lipoprotein levels have been reported in oral contraceptive users.

9. ELEVATED BLOOD PRESSURE

An increase in blood pressure has been reported in women taking oral contraceptives. The incidence of risk also was reported to increase with continued use and among older women. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing concentrations of progestogens.

Women with a history of hypertension or hypertension-related diseases or renal disease should be encouraged to use another method of contraception. If women elect to use oral contraceptives, they should be monitored closely and if significant elevation of blood pressure occurs oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives and there is no difference in the occurrence of hypertension among ever- and never-users.

10. HEADACHE

The onset or exacerbation of migraine or development of headache with a new pattern which is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause.

11. BLEEDING IRREGULARITIES

Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first 3 months of use. Non-hormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding, as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out.

Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was pre-existent.

PRECAUTIONS

1. PHYSICAL EXAMINATION AND FOLLOW-UP

A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology and relevant laboratory tests. In case of undiagnosed, persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care.

2. LIPID DISORDERS

Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult.

3. LIVER FUNCTION

If jaundice develops in any woman receiving oral contraceptives the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function.

4. FLUID RETENTION

Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention.

5. EMOTIONAL DISORDERS

Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree.

6. CONTACT LENSES

Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist.

7. DRUG INTERACTIONS

Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar association though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines.

8. INTERACTIONS WITH LABORATORY TESTS

Certain endocrine and liver function tests and blood components may be affected by oral contraceptives:

a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.

b. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG. Free T4 concentration is unaltered.

c. Other binding proteins may be elevated in serum.

d. Sex steroid binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged.

e. Triglycerides may be increased.

f. Glucose tolerance may be decreased.

g. Serum folate levels may be depressed by oral contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives.

9. CARCINOGENESIS

See **WARNINGS** section.

10. PREGNANCY

Pregnancy Category X. See **CONTRAINDICATIONS** and **WARNINGS** sections.

11. NURSING MOTHERS

Small amounts of oral contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child.

INFORMATION FOR THE PATIENT

See **PATIENT LABELING**.

ADVERSE REACTIONS

An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see **WARNINGS** section):

• Thrombophlebitis • Arterial thromboembolism • Pulmonary embolism • Myocardial infarction • Cerebral hemorrhage • Cerebral thrombosis • Hypertension • Gallbladder disease • Hepatic adenomas, carcinomas or benign liver tumors

There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed:

• Mesenteric thrombosis • Retinal thrombosis

The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related:

• Nausea • Vomiting • Gastrointestinal symptoms (such as abdominal cramps and bloating) • Breakthrough bleeding • Spotting • Change in menstrual flow • Amenorrhea • Temporary infertility after discontinuation of treatment • Edema • Melasma which may persist • Breast changes: tenderness, enlargement, secretion • Change in weight (increase or decrease) • Change in cervical erosion and secretion • Diminution in lactation when given immediately postpartum • Cholestatic jaundice • Migraine • Rash (allergic) • Mental depression • Reduced tolerance to carbohydrates • Vaginal candidiasis • Change in corneal curvature (steepening) • Intolerance to contact lenses

The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted:

• Pre-menstrual syndrome • Cataracts • Changes in appetite • Cystitis-like syndrome • Headache • Nervousness • Dizziness • Hirsutism • Loss of scalp hair • Erythema multiforme • Erythema nodosum • Hemorrhagic eruption • Vaginitis • Porphyria • Impaired renal function • Hemolytic uremic syndrome • Budd-Chiari syndrome • Acne • Changes in libido • Colitis

OVERDOSAGE

Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

NON-CONTRACEPTIVE HEALTH BENEFITS

The following non-contraceptive health benefits related to the use of oral contraceptives are supported by epidemiological studies which largely utilized oral contraceptive formulations containing estrogen doses exceeding 0.035 mg of ethinyl estradiol or 0.05 mg of mestranol.

Effects on menses:

• Increased menstrual cycle regularity • Decreased blood loss and decreased incidence of iron deficiency anemia • Decreased incidence of dysmenorrhea

Effects related to inhibition of ovulation:

• Decreased incidence of functional ovarian cysts • Decreased incidence of ectopic pregnancies

Effects from long-term use:

• Decreased incidence of fibroadenomas and fibrocystic disease of the breast • Decreased incidence of acute pelvic inflammatory disease • Decreased incidence of endometrial cancer • Decreased incidence of ovarian cancer

Rev. August 1989

SYNTEX

Mosby

American Journal of Obstetrics and Gynecology

Founded in 1920

Copyright © 1989 by The C.V. Mosby Company

Contents

December
Part 1
1989

CONSULTANTS

Consultants for the American Journal of Obstetrics and Gynecology 1431

PAPERS OF THE SOCIETY FOR GYNECOLOGIC INVESTIGATION

Central hemodynamic assessment of normal term pregnancy 1439
Steven L. Clark, MD, David B. Cotton, MD, Wesley Lee, MD, Clark Bishop, MD, Tracy Hill, MD, James Southwick, MD, James Pivarnik, PhD, Thomas Spillman, PhD, Greggory R. DeVore, MD, Jeffrey Phelan, MD, Gary D. V. Hankins, MD, Thomas J. Benedetti, MD, and Dennis Tolley, PhD
Provo, Utah, Houston and San Antonio, Texas, Los Angeles, California, and Seattle, Washington

Central hemodynamic parameters were assessed with pulmonary artery catheterization in 10 normal patients during the third trimester and postpartum periods.

The central hemodynamics of severe preeclampsia 1443
William C. Mabie, MD, Thomas E. Ratts, MD, and Baha M. Sibai, MD
Memphis, Tennessee

The failure of the circulation to dilate in the setting of increasing cardiac output appears to be a characteristic feature of preeclampsia.

Cardiovascular changes in early phase of pregnancy 1449
Eleanor L. Capeless, MD, and James F. Clapp, MD
Burlington, Vermont

The majority of the pregnancy-induced changes in cardiac output and systemic vascular resistance occur during the embryonic period, as compared with the preconceptional baseline.

(Contents continued on page 5A)

Vol. 161, No. 6, Part 1, December 1989. The American Journal of Obstetrics and Gynecology (ISSN 0002-9378) is published monthly (six issues per volume, two volumes per year) by The C.V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318. Second-class postage paid at St. Louis, Missouri, and additional mailing offices. POSTMASTER: Send address changes to The C.V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318, (314) 872-8370, ext. 351. Annual subscription rates for 1990: domestic, $71.50 for individuals and $130.00 for institutions. Printed in the U.S.A. Copyright © 1989 by The C.V. Mosby Company.

To be continued...



Continuation...an excellent indicator of patient satisfaction.

The Ortho ALL-FLEX* Arcing Spring Diaphragm

- Easy to insert and position
- Easy to clean and maintain
- Easy to use with unscented, colorless, stainless GYNOL II* (2% nonoxynol-9) Contraceptive Jelly
- The combination for continuation—with 4 out of 5 patients continuing after 1 year†

Continuation...it's one reason why physicians continue to make ORTHO ALL-FLEX and GYNOL II the #1 prescribed diaphragm combination in America.‡

Ortho ALL-FLEX*

Arcing Spring

DIAPHRAGM



†Number of patients continuing the method for 12 consecutive months; data on file, Ortho Pharmaceutical Corporation.

‡Data on file, Ortho Pharmaceutical Corporation.

Consult the patient brochure for information on toxic shock syndrome (TSS) and wearing time.

ORTHO PHARMACEUTICAL CORPORATION
Raritan, New Jersey 08869-0602

© OPC 1989 *Trademark OJ-319

ORTHO

Contents continued from page 3A

The effects of maternal exercise on early pregnancy outcome 1453
James F. Clapp III, MD
Burlington, Vermont

Running and aerobic dancing at high intensity at the time of conception and throughout early pregnancy did not influence early pregnancy outcome.

Oxygen consumption during treadmill exercise before, during, and after pregnancy 1458
James F. Clapp III, MD
Burlington, Vermont

The efficiency of low- and moderate-intensity treadmill exercise is increased early in pregnancy, maintained throughout pregnancy in most subjects, and enhanced by continued physical training during pregnancy.

Exercise prescription in pregnancy: Weight-bearing versus non-weight-bearing exercise 1464
Raul Artal, MD, Damon I. Masaki, MD, Nazareth Khodiguian, PhD, Yitzhak Romem, MD, Susan E. Rutherford, MD, and Robert A. Wiswell, PhD
Los Angeles, California

Weight-bearing exercise was compared with non-weight-bearing exercise in pregnant women by indirect calorimetry, which indicates preferential use of carbohydrates during non-weight-bearing exercise.

Human fetal behavioral states after vibratory stimulation 1470
Robert Gagnon, MD, Cora Hunse, RN, and John Foreman, PEng
London, Ontario, Canada

External vibratory stimulation of healthy term fetuses was associated with a transition from a quiet to an active sleep state.

(Contents continued on page 7A)

1990 Annual subscription rates	*U.S.A.*	*International (surface mail) All regions*	*International (airmail)°*				
			Canada	*Mexico*	*Region 1*	*Region 2*	*Region 3*
Institutions†	$130.00	$156.00	$182.00	$188.25	$200.50	$242.25	$284.00
Individuals‡	$ 71.50	$ 97.50	$123.50	$129.75	$142.00	$183.75	$225.50
Students, residents‡	$ 38.00	$ 64.00	$ 90.00	$ 96.25	$108.50	$150.25	$192.00

Subscription rates include supplements. Single copies are $7.00. Remittances should be made by check, draft, or post office or express money order, in U.S. funds drawn through a U.S. bank, payable to this JOURNAL. Claims for missing issues will be serviced only within six months of cover date. Single copy prices will be charged on missing issue claims older than six months from cover date. Back issues generally are available for the previous five years. Contact the Publisher to confirm availability of specific issues.

°*Airmail breakdown—Domestic:* First-class and Priority rates for the U.S. and possessions are available upon request. *Canada:* All provinces of Canada. *Mexico:* All localities within Mexico. *Region 1:* Colombia, Venezuela, Central America, Caribbean Islands, Bahamas, Bermuda, and St. Pierre and Miquelon (also from American Samoa to Western Samoa and from Guam to the Philippines). *Region 2:* South America (except Colombia and Venezuela), Europe (except Estonia, Latvia, Lithuania, and U.S.S.R), and North Africa (Morocco, Algeria, Tunisia, Libya, and Egypt). *Region 3:* Estonia, Latvia, Lithuania, U.S.S.R., Asia, Australia, New Zealand, Pacific Ocean Islands, Africa (other than North Africa), Indian Ocean Islands, and the Middle East.

†Institutional (multiple-reader) subscriptions are available to public and private libraries, schools, hospitals, and clinics; city, county, state, provincial, and national government bureaus and departments; and all commercial and private institutions and organizations.

‡Individual subscriptions and all student-rate subscriptions must be in the names of, billed to, and paid by individuals. All student-rate requests must indicate training status and name of institution.

Subscriptions may begin at any time.

THE PERMANENTE MEDICAL GROUP

NORTHERN CALIFORNIA



QUALITY

STABILITY

LEADERSHIP

GROWTH

SUPPORT

REWARDS

The Permanente Medical Group, the largest multispecialty group practice in the U.S., is undergoing explosive growth in its Northern California region: the San Francisco Bay Area, Sacramento and the Central Valley. Our rapid increase in membership has created opportunities throughout the region for additional BC/BE Obstetricians and Gynecologists.

As part of a comprehensive health care system made up of the Kaiser Foundation Health Plan, Kaiser Foundation Hospitals, and our physician-managed group, you will have access to the latest medical technology and resources, the support of colleagues in all subspecialties — and the opportunity to provide excellent health care without the burdens of managing a practice.

Join us in one of these facilities:

Richmond — Small, congenial clinic setting staffed by five physicians and five nurse practitioners with ready consultation for other services, including perinatology. Practice general OB/GYN, high-risk obstetrics and infertility. Expertise in colposcopy, laparoscopy, hysteroscopy and laser welcome. Resident teaching opportunities exist. Staff call at Kaiser Hospital in Oakland, shared with resident staff.

Oakland — Twenty physicians, 12 residents offer services in oncology, infertility, maternal-fetal medicine and a large ICN.

Redwood City — Specializes in family-centered maternity care; offers modern LDR rooms and full range of gynecological surgery. With ten physicians, 24-hour call is required three times each month. Beautiful Peninsula location, near San Francisco and the Pacific Ocean.

Stockton — Small four physician department in a new clinic building with new equipment, including laser. Stockton's lifestyle is affordable and attractive, offering recreational activities such as boating on the inland waterways. Centrally located, with mountains to the east and San Francisco Bay Area to the west.

Roseville — Eight physicians and two nurse practitioners in clinic setting. Patients are hospitalized for delivery and surgery in Sacramento where physicians take call for L&D and emergency about 5 times each month.

San Francisco — Full range of services including large high-risk, tertiary care OB service. Eighteen physicians, including GYN Oncologist. Residency program. Clinical research and academic teaching appointments available.

Sacramento — Growing metropolitan area between San Francisco Bay Area and Sierra Nevada mountains. Up-to-date facility with active staff of 14 and excellent residency program affiliated with the University of California at Davis. Flexible call averaging five shifts per month covers L&D, emergency room and inpatient services.

Pleasanton — Staffed by four physicians and three nurse practitioners, clinic provides general OB/GYN services, non-stress tests, ultrasound and cystoscopy. Consultant support, including a Perinatologist, readily available. Share call with staff of Walnut Creek Medical Center. Affiliated with the University of California at San Francisco as clinical instructors.

Fresno — Ground-floor opportunities in growing area for clinically-oriented individuals. Provide general OB/GYN; special interests encouraged. Planning new $26 million outpatient facility to accompany current medical office building. On call every fourth night and weekend. Local residency program at Valley Medical Center affiliated with the University of California at San Francisco. Teaching positions possible.

South San Francisco — Full service except L&D. Nine physicians, six nurse practitioners. On San Francisco Peninsula, 15 minutes from downtown.

KAISER PERMANENTE
Good People. Good Medicine.

Member physicians share TPMG's many benefits, from our leading quality assurance and continuing education to malpractice coverage and substantial retirement programs. For application information, call or send CV to: The Permanente Medical Group, Inc., Richmond Prescott, M.D., Physician Recruitment Services, 1814 Franklin, 4th Floor, Oakland, CA 94612. 800-777-4912. EOE

Contents continued from page 5A

The effect of vibroacoustic stimulation on the nonstress test at gestational ages of thirty-two weeks or less 1476
Maurice L. Druzin, MD, Terri G. Edersheim, MD, J. M. Hutson, MD, and Annette L. Bond, MD
New York, New York

The incidence of reactive nonstress tests after vibroacoustic stimulation was significantly increased after 26 weeks' gestation, and this finding may have clinical applicability.

Effects of low-frequency vibration on human term fetuses 1479
Robert Gagnon, MD, John Foreman, PEng, Cora Hunse, RN, and John Patrick, MD
London, Ontario, Canada

External low-frequency vibratory stimulation of healthy term fetuses was associated with changes in fetal movement and fetal heart rate patterns suggesting a switch from a state of quiet sleep to a state of active sleep.

Nifedipine pharmacokinetics during preterm labor tocolysis 1485
James E. Ferguson II, MD, Thomas Schutz, BA, Robert Pershe, BS, David K. Stevenson, MD, and Terrence Blaschke, MD
Stanford, California

Tocolysis with sublingual and oral nifedipine resulted in variable but usually measurable maternal blood levels; placental transfer of nifedipine occurs.

Prediction of hematocrit decline after intravascular fetal transfusion 1491
Scott N. MacGregor, DO, Michael L. Socol, MD, Bruce W. Pielet, MD, John S. Sholl, MD, and Richard K. Silver, MD
Chicago and Evanston, Illinois

Fetal hematocrit decline after intravascular transfusion may be predicted to determine the optimum timing of repeat transfusion.

Effect of acute intravascular volume expansion on human fetal prostaglandin concentrations 1494
Carl P. Weiner, MD, and Jean E. Robillard, MD
Iowa City, Iowa

Intravenous transfusion of the anemic human fetus stimulates the release of prostaglandin E_2 and prostacyclin.

The effect of intravascular transfusion on umbilical venous pressure in anemic fetuses with and without hydrops 1498
Carl P. Weiner, MD, Gay D. Pelzer, RN, Joni Heilskov, RN, Katharine D. Wenstrom, MD, and Roger A. Williamson, MD
Iowa City, Iowa

The anemic human fetus tolerates direct intravascular transfusion with only a modest increase in umbilical venous pressure.

(Contents continued on page 8A)

Contents continued from page 7A

Duplex Doppler ultrasonographic evaluation of the fetal renal artery in normal and abnormal fetuses 1502
J. C. Veille, MD, and C. Kanaan, MD
Cleveland, Ohio

Duplex ultrasonography of the fetal renal artery was obtained in normal fetuses and in fetuses with intrauterine growth retardation; fetuses with intrauterine growth retardation have an increased vascular resistance within this bed.

Intrapartum Doppler velocimetry, amniotic fluid volume, and fetal heart rate as predictors of subsequent fetal distress. I. An initial report 1508
Albert P. Sarno, Jr., MD, MAJ, USA, Myoung Ock Ahn, MD, MPH, Harbinder S. Brar, MD, Jeffrey P. Phelan, MD, and Lawrence D. Platt, MD
Los Angeles, California

Amniotic fluid volume and the initial fetal heart rate tracing in early labor may be resonable predictors of subsequent fetal condition, whereas Doppler velocimetry alone was not.

Doppler assessment of the fetal and uteroplacental circulation during nifedipine therapy for preterm labor 1514
Giancarlo Mari, MD, Brian Kirshon, MD, Kenneth J. Moise, Jr., MD, Wesley Lee, MD, and David B. Cotton, MD
Houston, Texas

Nifedipine, when used for the treatment of preterm labor, did not alter the fetal or uteroplacental circulation as evaluated by the Doppler technique at 5 hours after the initiation of therapy.

Maternal and fetal blood flow velocity waveforms in patients with preterm labor: Relationship to outcome 1519
Harbinder S. Brar, MD, Arnold L. Medearis, MD, Greggory R. DeVore, MD, and Lawrence D. Platt, MD
Los Angeles, California

Abnormal pretherapy umbilical and/or uterine systolic to diastolic ratios in patients with preterm labor predict increased risk of preterm birth and poor pregnancy outcome.

Clinical management of the fetus with markedly diminished umbilical artery end-diastolic flow 1523
Michael Y. Divon, MD, Barbara A. Girz, DO, Richard Lieblich, MD, and Oded Langer, MD
Bronx, New York

Delivery of the preterm fetus with diminished end-diastolic flow may not be mandatory; the use of fetal biophysical testing, coupled with commonly used criteria for delivery, results in acceptable fetal outcome and prolongation of gestational age.

Contents

Doppler assessment of the pulsatility index of the middle cerebral artery during constriction of the fetal ductus arteriosus after indomethacin therapy 1528

Giancarlo Mari, MD, Kenneth J. Moise, Jr., MD, Russell L. Deter, MD, Brian Kirshon, MD, James C. Huhta, MD, Robert J. Carpenter, Jr., MD, and David B. Cotton, MD

Houston, Texas

A response to indomethacin therapy sufficient to cause fetal ductal constriction and tricuspid insufficiency decreased the pulsatility index of the middle cerebral artery.

Human fetal tricuspid and mitral deceleration time: Changes with normal pregnancy and intrauterine growth retardation 1532

Kathryn L. Reed, MD, Christopher P. Appleton, MD, David J. Sahn, MD, and Caroline F. Anderson, RDMS

Tucson, Arizona, and San Diego, California

Doppler echocardiography was used to show abnormal prolongation of the atrioventricular deceleration time in fetuses with intrauterine growth retardation and absent end-diastolic velocities in the umbilical artery.

Effect of placental laterality on uterine artery resistance and development of preeclampsia and intrauterine growth retardation 1536

Alexander D. Kofinas, MD, Mary Penry, RN, Melissa Swain, RN, and Christos G. Hatjis, MD

Winston-Salem, North Carolina

Placental laterality is associated with increased uterine artery resistance and almost a threefold increase in the incidence of preeclampsia and intrauterine growth retardation.

Ultrasonographic criteria for the prenatal diagnosis of placental chorionicity in twin gestations 1540

Hung N. Winn, MD, Sandro Gabrielli, MD, E. Albert Reece, MD, J. Andres Roberts, MD, Carolyn Salafia, MD, and John C. Hobbins, MD

New Haven, Connecticut

Quantitative assessment of the dividing membranes is helpful in the prenatal diagnosis of placental chorionicity by ultrasonography.

Esterase activity in second- and third-trimester amniotic fluid: An indicator of chorioamnionitis 1543

Iffath Abbasi Hoskins, MD, Joseph Katz, PhD, Steven A. Ordorica, MD, and Bruce K. Young, MD

New York, New York

An in vitro leukocyte esterase assay is a reliable alternative for the laboratory diagnosis of chorioamnionitis.

Rapid chromosome analysis with the use of spontaneously dividing cells derived from umbilical cord blood (fetal and neonatal) 1546

Robert E. Tipton, MD, Avirachan T. Tharapel, PhD, Hua-Hua T. Chang, BS, Joe Leigh Simpson, MD, and Sherman Elias, MD

Memphis, Tennessee

Rapid chromosome analysis with the use of umbilical cord blood provides high-quality metaphases that can be used to facilitate management of fetuses or neonates with malformations or intrauterine growth retardation.

(Contents continued on page 10A)

Contents continued from page 9A

Effects of fetal sex and dexamethasone on preterm maternal serum concentrations of human chorionic gonadotropin, progesterone, estrone, estradiol, and estriol 1549
Ray V. Haning, Jr., MD, Luis B. Curet, MD, W. Kenneth Poole, PhD, Lynn M. Boehnlein, BS, Donna L. Kuzma, BS, and Sandra M. Meier, BS
Providence, Rhode Island, Madison, Wisconsin, and Research Triangle Park, North Carolina

The serum human chorionic gonadotropin concentration was significantly higher in female pregnancies than in male pregnancies at an average gestational age of 32 weeks.

High glucose levels decrease proliferation of cultured human fetal cells from placenta 1553
D. Michael Nelson, MD, PhD, and Edward M. Curran, MS
St. Louis, Missouri

The proliferation of cultured human fetal cells from the placenta is decreased by elevated glucose levels in the range observed in some pregnant diabetic women.

Stimuli for fetal swallowing: Systemic factors 1559
Michael G. Ross, MD, Dan J. Sherman, MD, M. Gore Ervin, PhD, Linda Day, and James Humme
Torrance and Los Angeles, California, and Tel Aviv, Israel

Fetal electromyographic swallowing activity and volume swallowed increased significantly in response to acute plasma hyperosmolality.

Effect of whole-body pH changes on thoracic duct lymph flow in anesthetized sheep 1566
Guillermo J. Valenzuela, MD, Hugh W. Forbes, and Justo G. Alonso, MD
Loma Linda, California

Significant cardiovascular and lymph flow effects were noted after the whole-animal pH was changed by 0.15 units.

Does furosemide alter the hemodynamic response to rapid intravascular transfusion of the anemic fetal lamb? 1571
David H. Chestnut, MD, Kenneth L. Pollack, MD, Carl P. Weiner, MD, Jean E. Robillard, MD, Christine S. Thompson, and Craig S. DeBruyn
Iowa City, Iowa

Rapid intravascular transfusion of anemic fetal lambs resulted in modest increases in fetal central venous pressure and mean arterial pressure, with or without furosemide.

Intrauterine growth retardation and the circulatory responses to acute hypoxemia in fetal sheep 1576
Barry S. Block, MD, Donald H. Schlafer, DVM, PhD, Richard A. Wentworth, PhD, Lois A. Kreitzer, MS, and Peter W. Nathanielsz, MD, PhD
Chicago, Illinois, and Ithaca, New York

Despite chronic hypoxemia, fetuses with early intrauterine growth retardation have a regional blood flow distribution response to acute imposed hypoxemia similar to that of normally grown fetuses.

Contents

Prostaglandin E_2 metabolism in the human fetal membranes 1580
Paul Y. C. Cheung, BSc, and John R. G. Challis, PhD, DSc
London, Ontario, Canada

Conversion of prostaglandin E_2 to prostaglandin $F_{2\alpha}$ can occur in the human fetal membranes and decidua but does not normally contribute significantly to prostaglandin $F_{2\alpha}$ output by intrauterine tissues at labor.

Increased placental progesterone may cause decreased placental prostacyclin production in preeclampsia 1586
Scott W. Walsh, PhD, and Stephanie Coulter, BS
Houston, Texas

Addition in vitro of progesterone at a concentration equivalent to that present in the incubation medium of placentas from women with preeclampsia inhibits prostacyclin production by normal placentas to a rate characteristic of preeclampsia.

Aspirin differentially affects thromboxane and prostacyclin production by trophoblast and villous core compartments of human placental villi 1593
D. Michael Nelson, MD, PhD, and Scott W. Walsh, PhD
St. Louis, Missouri, and Houston, Texas

Aspirin can selectively inhibit thromboxane production in whole placental villi and can differentially affect thromboxane and prostacyclin production by the trophoblast and villous core compartments.

In vitro release of endothelium-derived relaxing factor by acetylcholine is increased during the guinea pig pregnancy 1599
Carl Weiner, MD, Ernest Martinez, BA, Liu Kang Zhu, MD, Abdi Ghodsi, BS, and David Chestnut, MD
Iowa City, Iowa

Pregnancy is associated with a systemic increase in endothelium-derived relaxing factor activity.

Effect of pregnancy on uterine and carotid artery response to norepinephrine, epinephrine, and phenylephrine in vessels with documented functional endothelium 1605
Carl P. Weiner, MD, Ernest Martinez, BA, David H. Chestnut, MD, and Abdi Ghodsi, BS
Iowa City, Iowa

Pregnancy is associated with a decrease in uterine artery response, efficacy, and sensitivity to catecholamines.

Evaluation of pulsed Doppler echocardiography for measurement of aortic blood flow in the fetal lamb 1610
Jean-Claude Veille, MD, Michael Tavill, BA, Mark Sivakoff, MD, Ilan Cohen, MD, Moshe Ben-Ami, MD, Yuh-Cheng Yang, MD, and Vasil Jovkovsky, MD
Cleveland, Ohio

Validation of pulsed Doppler echocardiography was done in the fetal lamb; invasive and noninvasive methods were found to be highly correlated ($r = 0.93$).

(Contents continued on page 12A)

Contents continued from page 11A

Lymph flow rate response to angiotensin II is decreased in pregnant sheep 1615
Guillermo J. Valenzuela, MD, and Lawrence D. Longo, MD
Loma Linda, California

Lymph flow response to angiotensin II was decreased in pregnant sheep; this characteristic may play a role in edema formation of pregnancy.

Atrial natriuretic factor: The effects of pregnancy and a high-salt diet on atrial levels 1620
Lony C. Castro, MD, Chander P. Arora, PhD, Jane L. Davis, MD, Calvin J. Hobel, MD, and Hassan Parvez, PhD
Los Angeles, California, and Paris, France

The concentration of atrial natriuretic factor was found to be elevated in the right and left atria of postpartum rats and in the left atria of term pregnant rats on a high-salt diet.

Is a decrease in plasma oncotic pressure enough to explain the edema of pregnancy? 1624
Guillermo J. Valenzuela, MD
Loma Linda, California

Decrease of the plasma protein produces a decrease in capillary filtration, thus maintaining a stable oncotic pressure difference across the capillary wall.

Treatment of oophorectomized guinea pigs with intrauterine 17β-estradiol pellets may modulate myometrial β-adrenergic receptor binding properties 1628
Christos G. Hatjis, MD, David M. Grogan, BA, and Donald R. Koritnik, PhD
Winston-Salem, North Carolina, and Columbus, Ohio

17β-Estradiol treatment in pharmacologic doses may result in a significant increase in the relative concentration of guinea pig myometrial β-adrenergic receptors capable of forming the "high affinity" state.

Pregnancy-induced changes in the interaction of guinea pig myometrial β-adrenergic receptors with *l*-isoproterenol 1633
Christos G. Hatjis, MD, and David M. Grogan, BA
Winston-Salem, North Carolina, and Columbus, Ohio

The ability of guinea pig myometrial β-adrenergic receptors to form a "high-affinity" state with *l*-isoproterenol is enhanced in pregnant animals at ≥0.9 of gestation.

Vasopressin receptors in human pregnant myometrium and decidua: Interactions with oxytocin and vasopressin agonists and antagonists 1637
Marina Ivanisevic, MD, Oliver Behrens, MD, Hanns Helmer, MD, Keith Demarest, PhD, and Anna-Riitta Fuchs, DSc
New York, New York, and Raritan, New Jersey

High concentrations of vasopressin receptors in decidua and myometrium with high affinity also to oxytocin and oxytocin antagonists may contribute significantly to uterine stimulation during labor.

Contents

The antagonistic effect of oxytocin and relaxin on rat uterine segment contractility 1644

Laura T. Goldsmith, PhD, Joan H. Skurnick, PhD, Andrea S. Wojtczuk, MS, Martin Linden, PhD, Mary Jeanne Kuhar, MD, and Gerson Weiss, MD

Newark, New Jersey

Relaxin and oxytocin have antagonistic effects on contractions in an in vitro system with rat uterine segments.

Decreased β-carotene tissue levels in uterine leiomyomas and cancers of reproductive and nonreproductive organs 1649

Prabhudas R. Palan, PhD, Magdy Mikhail, MD, and Seymour L. Romney, MD

Bronx, New York

Tissue β-carotene levels are significantly lower in uterine fibroids and malignant neoplasms than in adjacent normal sites.

Compensatory ovarian hypertrophy occurs by a mechanism distinct from compensatory growth in the regenerating liver 1653

Ronald D. Alvarez, MD, William E. Grizzle, MD, PhD, Lori J. Smith, and Donald M. Miller, MD, PhD

Birmingham, Alabama

A significant increase in proto-oncogene expression or deoxyribonucleic acid synthesis occurs in the contralateral ovary after hemioophorectomy in rats.

Assessment of epidermal growth factor in the healing process of clean full-thickness skin wounds 1658

Alfredo J. Jijon, MD, Donald G. Gallup, MD, M. Ali Behzadian, PhD, and William P. Metheny, PhD

Augusta, Georgia

A trend toward faster wound healing was noted with use of epidermal growth factor compared with Ringer's lactate solution in female pigs.

Immunocytochemical study of *ras* and *myc* proto-oncogene polypeptide expression in the human menstrual cycle 1663

Lawrence D. Odom, MD, J. Michael Barrett, PhD, Cooley G. Pantazis, MD, Leland D. Stoddard, MD, and Paul G. McDonough, MD

Augusta, Georgia

Evaluation of c-*ras* and c-*myc* polypeptide expression reveals no cyclic variation for c-*ras*, but the c-*myc* product is reduced in the secretory phase.

Comparison of Cytobrush and cervicovaginal lavage sampling methods for the detection of genital human papillomavirus 1669

Gary L. Goldberg, MD, Sten H. Vermund, MD, Mark H. Schiffman, MD, Diane B. Ritter, MD, Carol Spitzer, BS, MS, and Robert D. Burk, MD

Bronx, New York, and Bethesda, Maryland

Cervicovaginal lavage, together with human papillomavirus deoxyribonucleic acid hybridization, is the most sensitive noninvasive method of harvesting cells for detection of human papillomavirus.

(Contents continued on page 14A)

Contents continued from page 13A

Spontaneously resorbing ectopic pregnancy: Preservation of human chorionic gonadotropin bioactivity despite declining steroid hormone levels 1673

Mark V. Sauer, MD, Robert E. Anderson, MD, Michael Vermesh, MD, Bronte A. Stone, PhD, and Richard J. Paulson, MD

Los Angeles, California

Corpora lutea of spontaneously resorbing ectopic gestations produce very low levels of steroids despite continued serum human chorionic gonadotropin bioactivity.

Decreased in vitro production of 6-keto-prostaglandin $F_{1\alpha}$ by uterine arteries from postmenopausal women 1677

Alex Steinleitner, MD, Frank Z. Stanczyk, PhD, Jay H. Levin, MD, Gerritt d'Ablaing III, MD, Marcela A. Vijod, BS, Violette L. Shahbazian, BS, and Rogerio A. Lobo, MD

Los Angeles, California

In vitro production of prostacyclin by uterine arteries was significantly decreased in specimens obtained from postmenopausal women.

Depressive episodes in premenstrual syndrome 1682

Joseph F. Mortola, MD, Lyda Girton, PhD, and Samuel S. C. Yen, MD, DSc

La Jolla, California

Premenstrual depressive episodes are shown to be distinct from major depression with both psychometric measures and cortisol pulsatile activity used as indices.

Peri-implantation phase endometrial estrogen and progesterone receptors: Effect of ovulation induction with clomiphene citrate 1688

Bryan R. Hecht, MD, Firyal S. Khan-Dawood, PhD, and M. Yusoff Dawood, MD

Chicago, Illinois

Clomiphene citrate (50 and 150 mg) treatment did not significantly affect the concentrations and binding affinities of peri-implantation phase endometrial estrogen and progesterone receptors.

Aromatase in human fetal tissues 1694

Kevin J. Doody, MD, and Bruce R. Carr, MD

Dallas, Texas

The presence of aromatase in various human fetal tissues is shown.

Comparison of in vivo bioavailability of progestational agents into the rat uterus and liver and the effect of plasma protein binding 1698

Bradley Van Voorhis, MD, Dennis W. Matt, PhD, Maria L. Tedeschi, and Kenneth A. Steingold, MD

Richmond, Virginia

Compared with progesterone or 19-nortestosterone, medroxyprogesterone acetate exhibited greater uterine bioavailability as a result of decreased plasma protein binding.

Contents

Androgen sulfate and glucuronide conjugates in nonhirsute and hirsute women with polycystic ovarian syndrome 1704

Robert K. Matteri, MD, Frank Z. Stanczyk, PhD, Elisabet E. Gentzschein, BA, Clara Delgado, BA, and Rogerio A. Lobo, MD

Los Angeles, California

Several C_{19} conjugates that reflect peripheral androgen activity were measured; these conjugates (in particular, androsterone glucuronide) were elevated only in hirsute women.

In vivo administration of allopurinol affects ovulation and early embryonic development in rabbits 1709

Toyohiko Miyazaki, MD, PhD, Tsung-Cheng Kuo, MD, PhD, Arunasalam M. Dharmarajan, PhD, Susan J. Atlas, PhD, and Edward E. Wallach, MD

Baltimore, Maryland

Allopurinol, administered to rabbits along with an ovulation-inducing dose of human chorionic gonadotropin, significantly reduced the percent of large follicles ovulating, cumulus dispersal, and early embryonic development.

Effects of platelet activating factor on mouse oocyte fertilization in vitro 1714

Brijinder S. Minhas, PhD, Raj Kumar, PhD, Deborah D. Ricker, MS, William E. Roudebush, PhD, Melvin G. Dodson, MD, PhD, and Stephen J. Fortunato, MD

Johnson City, Tennessee, and San Antonio, Texas

In vitro fertilization of mouse oocytes is enhanced by platelet activating factor treatment of spermatozoa.

Release of tumor necrosis factor-α by human peritoneal macrophages in vivo and in vitro 1718

Jouko Halme, MD, PhD

Los Angeles, California, and Chapel Hill, North Carolina

Peritoneal macrophages released tumor necrosis factor activity in vitro and thus are a likely source of the activity in peritoneal fluid of patients with endometriosis.

Isolation and sequencing of a complementary deoxyribonucleic acid clone encoding human placental 17β-estradiol dehydrogenase: Identification of the putative cofactor binding site 1726

Michael J. Gast, MD, PhD, Harold F. Sims, BS, Gary L. Murdock, PhD, Peter M. Gast, and Arnold W. Strauss, MD

St. Louis, Missouri

We have isolated and cloned a complementary deoxyribonucleic acid encoding estradiol dehydrogenase from human placenta and delineated its primary protein structure and several important functional portions of the molecule.

Molecular scanning of Yq11 (interval 6) in men with Sertoli-cell-only syndrome 1732

Mark D. Johnson, MD, Sandra P. T. Tho, MD, Ali Behzadian, PhD, and Paul G. McDonough, MD

Augusta, Georgia

Probing Yq11 (interval 6) in six men with Sertoli-cell-only syndrome revealed absence of 50f2/C in one individual and conservation of pDP105/B in all subjects.

(Contents continued on page 16A)

Contents continued from page 15A

Steroid biosynthesis in the Sertoli-Leydig cell tumor: Effects of insulin and luteinizing hormone 1738
Manubai Nagamani, MD, Charles A. Stuart, MD, and Tung Van Dinh, MD
Galveston, Texas

A Sertoli-Leydig cell tumor of the ovary was found to have specific binding sites for insulin and the tumor tissue responded to luteinizing hormone and insulin with release of androstenedione.

CORRESPONDENCE

Fetomaternal hemorrhage—Possible cause of fetal distress and death Michael S. Cardwell, MD, and Scott W. Snyder, MD *Columbia, Missouri* 1744

Reply Paul W. Biddinger, MD, Patricia A. Stafford, MD, and Ross E. Zumwalt, MD *Cincinnati, Ohio* 1744

Dosage and duration differences lead to diverse conclusions Sidney Shulman, PhD *New York, New York* 1744

Reply Mary G. Hammond, MD *Chapel Hill, North Carolina* 1745

Number clarification requested Christos G. Hatjis, MD *Columbus, Ohio* 1746

Reply Isabelle Wilkins, MD, and Lauren Lynch, MD *New York, New York* 1746

Pulmonary artery agenesis Marion S. Verp, MD *Chicago, Illinois* 1746

Other routes charted after description of adventurous journey of CA 125 L. C. Fuith, MD *Innsbruck, Austria* 1747

Reply J. Gerald Quirk, Jr., MD, PhD, and Timothy J. O'Brien, PhD *Little Rock, Arkansas* 1747

Perinatal death as a result of social factors Sylvain Fribourg, MD *Panorama City, California* 1748

Reply Isaac Delke, MD, and Howard L. Minkoff, MD *Brooklyn, New York* 1748

Techniques differ in use of Doppler ultrasonography Thomas R. Easterling, MD, and Thomas J. Benedetti, MD *Seattle, Washington* 1748

Reply Wesley Lee, MD, and Roxann Rokey, MD *Royal Oak, Michigan, and Houston, Texas* 1749

Response by Drs. Easterling and Benedetti 1750

Maternal hematocrit and premature labor Robert C. Goodlin, MD *Denver, Colorado* 1750

Reply Ellice Lieberman, MD, DrPH, Kenneth J. Ryan, MD, Richard R. Monson, MD, ScD, and Stephen C. Schoenbaum, MD, MPH *Boston and Brookline, Massachusetts* 1750

Multiple gestation with intrauterine insemination and menotropin therapy Gregory H. Corsan, MD, Robert M. Shelden, PhD, and Ekkehard Kemmann, MD *New Brunswick, New Jersey* 1751

Reply William C. Dodson, MD, Claude L. Hughes, PhD, MD, and A. F. Haney, MD *Durham, North Carolina* 1752

Allergic vaginitis Murray Dworetzky, MD *New York, New York* 1752

Reply Leo Galland, MD *New York, New York* 1753

Results of uterine activity studies in agreement, but methods differ Miklós Török, MD, and István Gáti, PhD, DSc *Budapest, Hungary* 1753

Reply Michael P. Nageotte, MD *Long Beach, California* 1754

Contents

Effect of magnesium and preeclampsia on plasma cholinesterase activity Gertie F. Marx, MD *Bronx, New York* 1754

Reply Jay Kambam, MD, Stephanie M. Perry, MD, and Bradley E. Smith, MD *Nashville, Tennessee* 1754

ITEMS

Items 1756

INDEX

Author index 1759

Subject index 1780

CORRECTION

Metabolic changes during medical treatment of endometriosis: Nafarelin acetate versus danazol 1755
(Burry KA, Patton PE, Illingworth DR. 1989;160:1454-61)

INFORMATION FOR AUTHORS

Information for authors 25A

Checklist and copyright statement 29A

Index to advertisers on page 58A

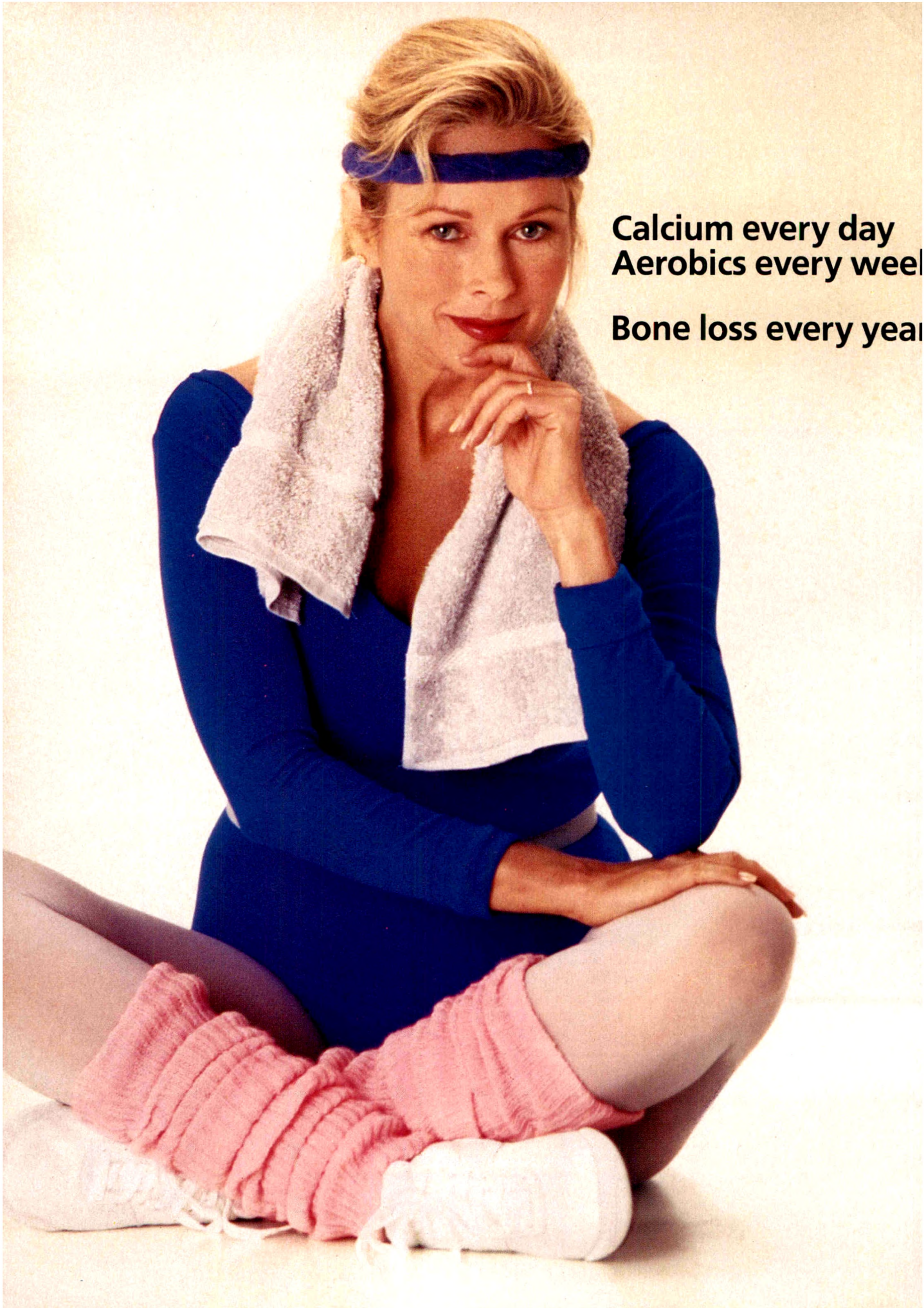
Make researching easier with the Ten-Year Cumulative Index (1978-1987) to the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY

The Ten-Year Cumulative Index is a complete guide to more than 30,000 pages of original articles, case reports, letters, and editorials published in the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY from 1978 through 1987 (volumes 130 to 157). Numbering more than 800 pages, the Cumulative Index includes both a subject and an author index. This hard-cover volume is $64.50 ($69.00 international). Prices include shipping. *Payment must accompany all orders and must be in U.S. funds, drawn on a U.S. bank.*

Contact The C.V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318 USA; phone (314) 872-8370, ext. 394. In the U.S., call toll-free: 1-800-325-4177, ext. 394. Please allow six weeks for delivery.

The JOURNAL is abstracted and/or indexed in *Index Medicus, Science Citation Index, Current Contents/Clinical Medicine, Current Contents/Life Sciences, Excerpta Medica, BRS Colleague,* and MEDLINE.

Authorization to photocopy items for internal or personal use, or the internal or personal use of specific clients, is granted by The C.V. Mosby Company for libraries and other users registered with the Copyright Clearance Center (CCC) Transactional Reporting Service, provided that 10¢ per page per copy is paid directly to CCC, 27 Congress St., Salem, MA 01970; phone (508)744-3350. The U.S. Government retains a nonexclusive, royalty-free license in and to any copyright on articles authored by government employees. 0002-9378/89 $00.00 + .10.


Calcium every day
Aerobics every week
Bone loss every year

She needs PREMARIN to help prevent further bone loss

Calcium[1] and exercise are not enough to prevent post-menopausal osteoporosis, since estrogen deficiency is the primary cause.[2] PREMARIN is the only brand of estrogen indicated to prevent further bone loss and in a recently published study has been shown to reduce the risk of hip fractures by as much as 66%.[3]

Early PREMARIN therapy offers the best protection

The most significant rate of bone loss occurs in early menopause. PREMARIN can help maintain bone mass, preventing the progression of osteoporosis and its consequences.

Protection continues as long as estrogen therapy continues

Clinical studies show that within three years of menopause when therapy is withdrawn, bone loss begins again.[4]

PREMARIN®

(conjugated estrogens tablets)

Proven benefits for menopause and beyond

See important information on adjacent page.

For moderate-to-severe vasomotor symptoms and for osteoporosis

PREMARIN®
(conjugated estrogens tablets)



0.3 mg 0.625 mg 0.9 mg 1.25 mg 2.5 mg

The appearance of these tablets is a trademark of Wyeth-Ayerst Laboratories.

For atrophic vaginitis

PREMARIN®
(conjugated estrogens)

Vaginal Cream

0.625 mg/g



BRIEF SUMMARY *(FOR FULL PRESCRIBING INFORMATION AND PATIENT INFORMATION, SEE PACKAGE CIRCULARS.)*

PREMARIN® Brand of conjugated estrogens tablets, USP
PREMARIN® Brand of conjugated estrogens Vaginal Cream, in a nonliquefying base

1. ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.
Three independent, case-controlled studies have reported an increased risk of endometrial cancer in postmenopausal women exposed to exogenous estrogens for more than one year. This risk was independent of the other known risk factors for endometrial cancer. These studies are further supported by the finding that incidence rates of endometrial cancer have increased sharply since 1969 in eight different areas of the United States with population-based cancer reporting systems, an increase which may be related to the rapidly expanding use of estrogens during the last decade. The three case-controlled studies reported that the risk of endometrial cancer in estrogen users was about 4.5 to 13.9 times greater than in nonusers. The risk appears to depend on both duration of treatment and on estrogen dose. In view of these findings, when estrogens are used for the treatment of menopausal symptoms, the lowest dose that will control symptoms should be utilized and medication should be discontinued as soon as possible. When prolonged treatment is medically indicated, the patient should be reassessed on at least a semi-annual basis to determine the need for continued therapy. Although the evidence must be considered preliminary, one study suggests that cyclic administration of low doses of estrogen may carry less risk than continuous administration; it therefore appears prudent to utilize such a regimen. Close clinical surveillance of all women taking estrogens is important. In all cases of undiagnosed persistent or recurring abnormal vaginal bleeding, adequate diagnostic measures should be undertaken to rule out malignancy. There is no evidence at present that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equi-estrogenic doses.
2. ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.
The use of female sex hormones, both estrogens and progestogens, during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have an increased risk of developing, in later life, a form of vaginal or cervical cancer that is ordinarily extremely rare. This risk has been estimated as not greater than 4 per 1,000 exposures. Furthermore, a high percentage of such exposed women (from 30% to 90%) have been found to have vaginal adenosis, epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether they are precursors of malignancy. Although similar data are not available with the use of other estrogens, it cannot be presumed they would not induce similar changes. Several reports suggest an association between intrauterine exposure to female sex hormones and congenital anomalies, including congenital heart defects and limb-reduction defects. One case-controlled study estimated a 4.7-fold increased risk of limb-reduction defects in infants exposed in utero to sex hormones (oral contraceptives, hormone withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some of these exposures were very short and involved only a few days of treatment. The data suggest that the risk of limb-reduction defects in exposed fetuses is somewhat less than 1 per 1,000. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective for these uses. If PREMARIN is used during pregnancy, or if the patient becomes pregnant while taking this drug, she should be apprised of the potential risks to the fetus, and the advisability of pregnancy continuation.

DESCRIPTION: PREMARIN (conjugated estrogens, USP) contains a mixture of estrogens, obtained exclusively from natural sources, blended to represent the average composition of material derived from pregnant mares' urine. It contains estrone, equilin, and 17α-dihydroequilin, together with smaller amounts of 17α-estradiol, equilenin, and 17α-dihydroequilenin as salts of their sulfate esters. Tablets are available in 0.3 mg, 0.625 mg, 0.9 mg, 1.25 mg, and 2.5 mg strengths of conjugated estrogens. Cream is available as 0.625 mg conjugated estrogens per gram.

INDICATIONS AND USAGE: PREMARIN (conjugated estrogens tablets, USP): Moderate-to-severe *vasomotor* symptoms associated with the menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression without associated vasomotor symptoms and they should not be used to treat such conditions.) Osteoporosis (abnormally low bone mass). Atrophic vaginitis. Kraurosis vulvae. Female castration.

PREMARIN (conjugated estrogens) Vaginal Cream is indicated in the treatment of atrophic vaginitis and kraurosis vulvae.

PREMARIN HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETUS (SEE BOXED WARNING).

CONTRAINDICATIONS: Estrogens should not be used in women (or men) with any of the following conditions: 1. Known or suspected cancer of the breast except in appropriately selected patients being treated for metastatic disease. 2. Known or suspected estrogen-dependent neoplasia. 3. Known or suspected pregnancy (see Boxed Warning). 4. Undiagnosed abnormal genital bleeding. 5. Active thrombophlebitis or thromboembolic disorders. 6. A past history of thrombophlebitis, thrombosis, or thromboembolic disorders associated with previous estrogen use (except when used in treatment of breast or prostatic malignancy).

WARNINGS: Estrogens have been reported to increase the risk of endometrial carcinoma (see Boxed Warning). However, a recent large, case-controlled study indicated no increase in risk of breast cancer in postmenopausal women. A recent study has reported a 2- to 3-fold increase in the risk of surgically confirmed gallbladder disease in women receiving postmenopausal estrogens.

Adverse effects of oral contraceptives may be expected at the larger doses of estrogen used to treat prostatic or breast cancer or postpartum breast engorgement; it has been shown that there is an increased risk of thrombosis in men receiving estrogens for prostatic cancer and women for postpartum breast engorgement. Users of oral contraceptives have an increased risk of diseases, such as thrombophlebitis, pulmonary embolism, stroke, and myocardial infarction. Cases of retinal thrombosis, mesenteric thrombosis, and optic neuritis have been reported in oral contraceptive users. An increased risk of postsurgery thromboembolic complications has also been reported in users of oral contraceptives. If feasible, estrogen should be discontinued at least 4 weeks before surgery of the type associated with an increased risk of thromboembolism, or during periods of prolonged immobilization. Estrogens should not be used in persons with active thrombophlebitis, thromboembolic disorders, or in persons with a history of such disorders in association with estrogen use. They should be used with caution in patients with cerebral vascular or coronary artery disease. Large doses (5 mg conjugated estrogens per day), comparable to those used to treat cancer of the prostate and breast, have been shown to increase the risk of nonfatal myocardial infarction, pulmonary embolism, and thrombophlebitis. When doses of this size are used, any of the thromboembolic and thrombotic adverse effects should be considered a clear risk.

Benign hepatic adenomas should be considered in estrogen users having abdominal pain and tenderness, abdominal mass, or hypovolemic shock. Hepatocellular carcinoma has been reported in women taking estrogen-containing oral contraceptives. Increased blood pressure may occur with use of estrogens in the menopause and blood pressure should be monitored with estrogen use. A worsening of glucose tolerance has been observed in patients on estrogen-containing oral contraceptives. For this reason, diabetic patients should be carefully observed. Estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases.

PRECAUTIONS: Physical examination and a complete medical and family history should be taken prior to the initiation of any estrogen therapy with special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolaou smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed. Conditions influenced by fluid retention, such as asthma, epilepsy, migraine, and cardiac or renal dysfunction, require careful observation. Certain patients may develop manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding, mastodynia, etc. Prolonged administration of unopposed estrogen therapy has been reported to increase the risk of endometrial hyperplasia in some patients. Oral contraceptives appear to be associated with an increased incidence of mental depression. Patients with a history of depression should be carefully observed. Pre-existing uterine leiomyomata may increase in size during estrogen use. The pathologist should be advised of estrogen therapy when relevant specimens are submitted. If jaundice develops in any patient receiving estrogen, the medication should be discontinued while the cause is investigated. Estrogens should be used with care in patients with impaired liver function, renal insufficiency, metabolic bone diseases associated with hypercalcemia, or in young patients in whom bone growth is not yet complete. If concomitant progestin therapy is used, potential risks may include adverse effects on carbohydrate and lipid metabolism.

Concomitant Progestin Use: The lowest effective dose appropriate for the specific indication should be utilized. Studies of the addition of a progestin for 7 or more days of a cycle of estrogen administration have reported a lowered incidence of endometrial hyperplasia. Morphological and biochemical studies of the endometrium suggest that 10 to 13 days of progestin are needed to provide maximal maturation of the endometrium and to eliminate any hyperplastic changes. Whether this will provide protection from endometrial carcinoma has not been clearly established. There are possible additional risks which may be associated with the inclusion of progestin in estrogen replacement regimens. The choice of progestin and dosage may be important in minimizing possible adverse effects.

The following changes may be expected with larger doses of estrogen:

a. Increased sulfobromophthalein retention.

b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.

c. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by PBI, T_4 by column, or T_4 by radioimmunoassay. Free T_3 resin uptake is decreased, reflecting the elevated TBG; free T_4 concentration is unaltered.

d. Impaired glucose tolerance.

e. Decreased pregnanediol excretion.

f. Reduced response to metyrapone test.

g. Reduced serum folate concentration.

h. Increased serum triglyceride and phospholipid concentration.

As a general principle, the administration of any drug to nursing mothers should be done only when clearly necessary since many drugs are excreted in human milk.

Long-term, continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver. However, in a recent, large case-controlled study of postmenopausal women there was no increase in risk of breast cancer with use of conjugated estrogens.

ADVERSE REACTIONS: The following have been reported with estrogenic therapy, including oral contraceptives: breakthrough bleeding; spotting; change in menstrual flow; dysmenorrhea; premenstrual-like syndrome; amenorrhea during and after treatment; increase in size of uterine fibromyomata; vaginal candidiasis; change in cervical erosion and in degree of cervical secretion; cystitis-like syndrome; tenderness, enlargement, secretion (of breasts); nausea, vomiting, abdominal cramps, bloating; cholestatic jaundice; chloasma or melasma which may persist when drug is discontinued; erythema multiforme; erythema nodosum; hemorrhagic eruption; loss of scalp hair; hirsutism; steepening of corneal curvature; intolerance to contact lenses; headache, migraine, dizziness, mental depression, chorea; increase or decrease in weight; reduced carbohydrate tolerance; aggravation of porphyria; edema; changes in libido.

ACUTE OVERDOSAGE: May cause nausea, and withdrawal bleeding may occur in females.

DOSAGE AND ADMINISTRATION:

PREMARIN® Brand of conjugated estrogens tablets, USP

1. *Given cyclically for short-term use only.* For treatment of moderate-to-severe *vasomotor* symptoms, atrophic vaginitis, or kraurosis vulvae associated with the menopause (0.3 mg to 1.25 mg or more daily). The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible. Administration should be cyclic (eg, three weeks on and one week off). Attempts to discontinue or taper medication should be made at three- to six-month intervals.

2. *Given cyclically:* Osteoporosis. Female castration. Osteoporosis —0.625 mg daily. Administration should be cyclic (eg, three weeks on and one week off). Female castration—1.25 mg daily, cyclically. Adjust upward or downward according to response of the patient. For maintenance, adjust dosage to lowest level that will provide effective control.

Patients with an intact uterus should be monitored for signs of endometrial cancer and appropriate measures taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

PREMARIN® Brand of conjugated estrogens Vaginal Cream

Given cyclically for short-term use only. For treatment of atrophic vaginitis or kraurosis vulvae.

The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible.

Administration should be cyclic (eg, three weeks on and one week off).

Attempts to discontinue or taper medication should be made at three- to six-month intervals.

Usual dosage range: 2 g to 4 g daily, intravaginally, depending on the severity of the condition.

Treated patients with an intact uterus should be monitored closely for signs of endometrial cancer and appropriate diagnostic measures should be taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

Revised October 1987

References:

1. Nilas L, Christiansen C, Rødbro P: Calcium supplementation and postmenopausal bone loss. *Br Med J* 1984; 289:1103-1106. **2.** Riggs BL: Research Directions in Osteoporosis. Scientific Workshop, National Institutes of Health, February 9-11, 1987. **3.** Kiel DP, Felson DT, Anderson JJ, et al: Hip fracture and the use of estrogens in postmenopausal women. *N Engl J Med* 1987;317:1169-1174. **4.** Christiansen C, Christensen MS, Transbøl I: Bone mass in postmenopausal women after withdrawal of oestrogen/gestagen replacement therapy. *Lancet* 1981;1:459-461.

© 1989 Wyeth-Ayerst Laboratories

WYETH-AYERST LABORATORIES
Philadelphia, PA 19101

Editors

Editors

JOHN I. BREWER, *Editor in Chief*
Sandra Fernandez, *Senior Editorial Assistant*

FREDERICK P. ZUSPAN, *Editor*
Donna Peterson, *Editorial Assistant*

E. J. QUILLIGAN, *Editor*
Sherry A. Merryman, *Editorial Assistant*

ALBERT B. GERBIE, *Associate Editor*

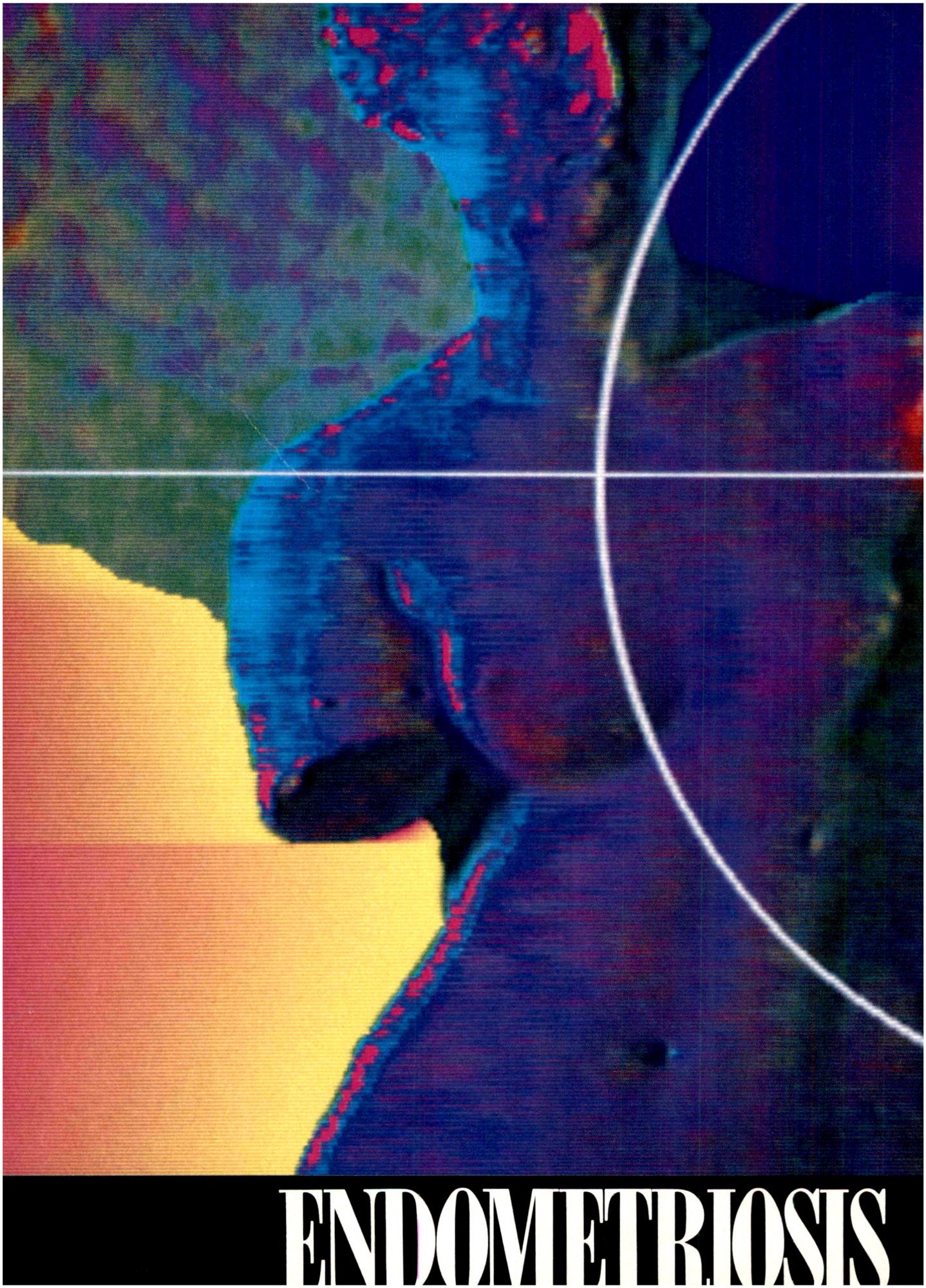
Advisory committee on policy

Garland Anderson
William R. Anderson
Mason C. Andrews
E. Forrest Boyd
Robert C. Cefalo
Donald R. Coustan
David C. Figge
A. Jerome Freeland
Edgar O. Horger III
W. Glenn Hurt
James M. Ingram
Walter S. Keifer
Emmet J. Lamb
Roger A. Lobo
Douglas J. Marchant
James H. Maxwell
Arthur M. McCausland
Allan G. W. McLeod
James A. Merrill
Daniel R. Mishell, Jr.
John C. Morrison
Carl J. Pauerstein
Richard Paul
Karl C. Podratz
Ruth W. Schwartz
James R. Scott
Henry A. Thiede
Carol Trumbold

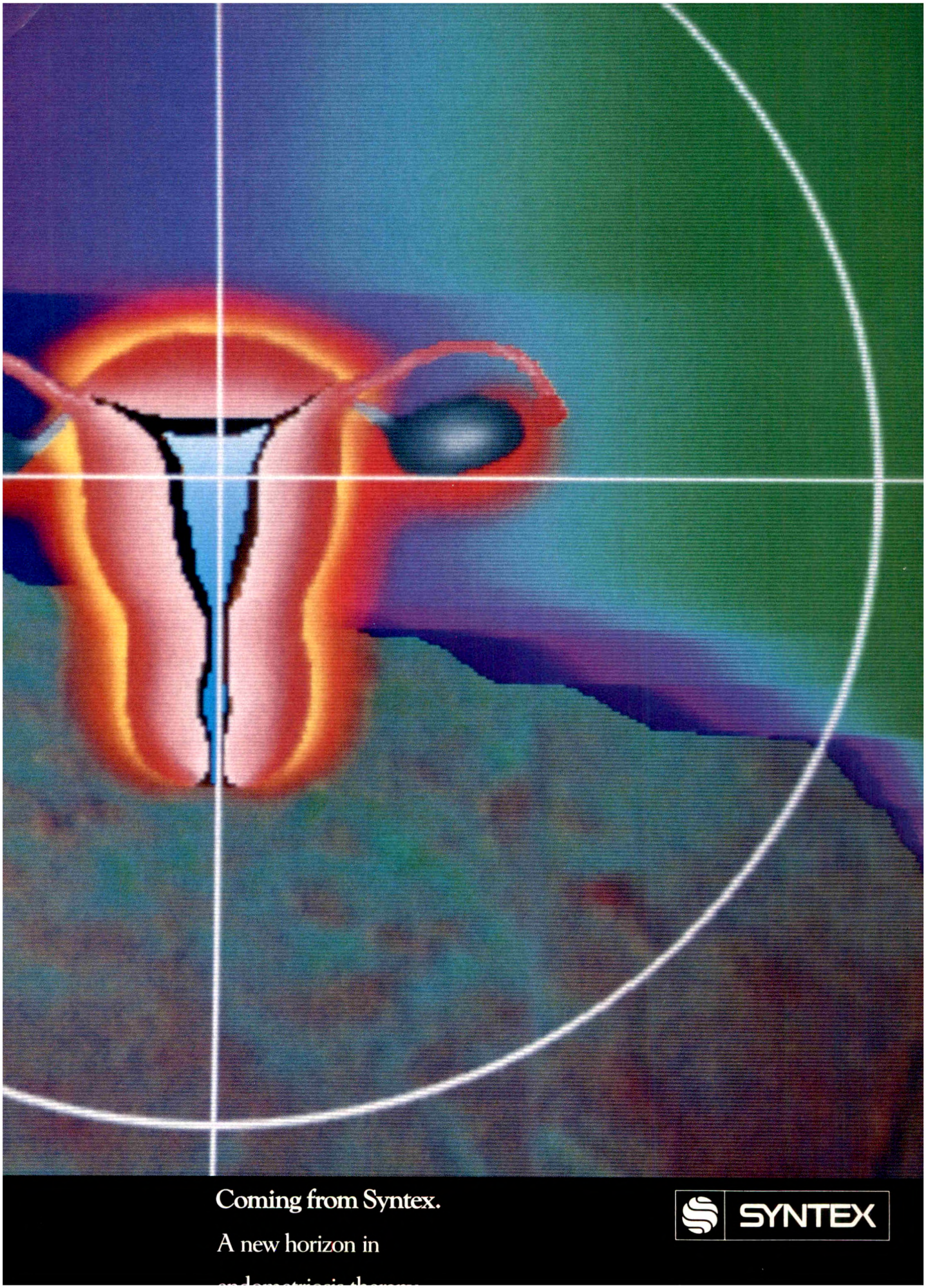
Board of corresponding editors

Luigi Carenza, *Rome, Italy*
T. K. A. B. Eskes, *Nijmegen, The Netherlands*
Wolfgang Künzel, *Giessen, West Germany*
Matsuto Mochizuki, *Kobe, Japan*
M. Reuben Peyser, *Tel Aviv, Israel*
Nils-Otto Sjöberg, *Malmö, Sweden*
Gordon M. Stirrat, *Bristol, England*
Claude Sureau, *Paris, France*
Carl Wood, *Melbourne, Victoria, Australia*
Olavi Ylikorkala, *Helsinki, Finland*

Publisher
THE C.V. MOSBY COMPANY, 11830 Westline Industrial Drive,
St. Louis, Missouri 63146-3318
Kay G. Goehler, *Senior Manuscript Editor*



ENDOMETRIOSIS


Coming from Syntex.
A new horizon in
SYNTEX

American Journal of Obstetrics and Gynecology

Societies

in addition to those listed on the front cover
the Journal is the official publication
of the following societies

NEW YORK OBSTETRICAL SOCIETY

OBSTETRICAL SOCIETY OF PHILADELPHIA

BROOKLYN GYNECOLOGICAL SOCIETY

ST. LOUIS GYNECOLOGICAL SOCIETY

NEW ORLEANS GYNECOLOGICAL AND OBSTETRICAL SOCIETY

THE OBSTETRICAL AND GYNECOLOGICAL SOCIETY OF MARYLAND

CHICAGO GYNECOLOGICAL SOCIETY

CINCINNATI OBSTETRICAL AND GYNECOLOGICAL SOCIETY

WASHINGTON GYNECOLOGICAL SOCIETY

PITTSBURGH OBSTETRICAL AND GYNECOLOGICAL SOCIETY

BOSTON OBSTETRICAL SOCIETY

LOUISVILLE OBSTETRICAL AND GYNECOLOGICAL SOCIETY

SEATTLE GYNECOLOGICAL SOCIETY

ALABAMA ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS

AKRON OBSTETRICAL AND GYNECOLOGICAL SOCIETY

KANSAS CITY GYNECOLOGICAL SOCIETY

CENTRAL NEW YORK ASSOCIATION OF GYNECOLOGISTS
AND OBSTETRICIANS

NEW JERSEY OBSTETRICAL AND GYNECOLOGICAL SOCIETY

IOWA OBSTETRIC AND GYNECOLOGIC SOCIETY

THE TEXAS ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS

OKLAHOMA CITY OBSTETRICAL AND GYNECOLOGICAL SOCIETY

MEMPHIS OBSTETRICAL AND GYNECOLOGICAL SOCIETY

UTAH OBSTETRICAL AND GYNECOLOGICAL SOCIETY

ROCHESTER OBSTETRICAL AND GYNECOLOGICAL SOCIETY

ARKANSAS OBSTETRICAL AND GYNECOLOGICAL SOCIETY

TENNESSEE STATE OBSTETRICAL AND GYNECOLOGICAL SOCIETY

NEW YORK GYNECOLOGICAL SOCIETY

PACIFIC NORTHWEST OBSTETRICAL AND GYNECOLOGICAL ASSOCIATION

BUFFALO OBSTETRICAL AND GYNECOLOGICAL SOCIETY

SAN FRANCISCO GYNECOLOGICAL SOCIETY

JACKSON GYNECIC SOCIETY

INDIANA OBSTETRICAL AND GYNECOLOGICAL SOCIETY

THE MINNESOTA OBSTETRICAL AND GYNECOLOGICAL SOCIETY

COLUMBUS OBSTETRIC AND GYNECOLOGIC SOCIETY

GREATER HARTFORD OBSTETRICAL AND GYNECOLOGICAL SOCIETY

American Journal of Obstetrics and Gynecology

Copyright © 1989 by The C.V. Mosby Company

Information for Authors

Editors

John I. Brewer, *Editor in Chief*
710 North Fairbanks Court, Suite 8300,
Chicago, IL 60611

Sandra Fernandez, *Senior Editorial Assistant*
Tel.: (312) 908-5266

Frederick P. Zuspan, *Editor*
The Ohio State University, Fifth Floor, Means Hall,
1654 Upham Dr., Columbus, OH 43210

Donna Peterson, *Editorial Assistant*
Tel.: (614) 293-8798

E. J. Quilligan, *Editor*
University of California, Irvine, Medical Center,
Department of Obstetrics and Gynecology, 101 The
City Drive, South, Building 26, Orange, CA 92668

Sherry A. Merryman, *Editorial Assistant*
Tel.: (714) 634-6572

Albert B. Gerbie, *Associate Editor*
710 North Fairbanks Court, Suite 8300,
Chicago, IL 60611

Publisher
THE C.V. MOSBY COMPANY, 11830 Westline
Industrial Drive, St. Louis, Missouri 63146-3318

Kay G. Goehler, *Senior Manuscript Editor*
Tel.: (314) 872-8370 (ext. 591)

INFORMATION FOR AUTHORS

Editorial policies

The requirements for manuscripts submitted to the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY conform to the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" established by the International Committee of Medical Journal Editors and published in *Annals of Internal Medicine* 1988;108:258-65. Certain requirements unique to our JOURNAL are provided in Information for Authors, published in each issue of the JOURNAL, and in more detail in the *Guide to Writing for the American Journal of Obstetrics and Gynecology*. The latter may be obtained from The C.V. Mosby Company or the Editors on request.

Manuscript submission. Manuscripts should be submitted to one of the three Editors as follows:

1. **Dr. Brewer**—all manuscripts originating from the southeastern quadrant of the United States or Canada and those presented before one of the official sponsoring societies, except the Society for Gynecologic Investigation and The Society of Perinatal Obstetricians.

2. **Dr. Zuspan**—manuscripts from the northeastern quadrant of the United States and from Japan, Israel, Italy, and England, manuscripts written for Clinical Opinion and Current Development, and Letters to the Editors. Dr. Zuspan is responsible for manuscripts from The Society of Perinatal Obstetricians. Manuscripts from The Society of Perinatal Obstetricians should be submitted to Dr. Donald R. Coustan, Department of Obstetrics and Gynecology, Women and Infants Hospital of Rhode Island, 101 Dudley St., Providence, RI 02905.

3. **Dr. Quilligan**—manuscripts from the north central states (including Ohio), states west of the Mississippi River, Hawaii, Alaska, and abroad (except Japan, Israel, Italy, and England). Dr. Quilligan is responsible for manuscripts from the Society for Gynecologic Investigation. Manuscripts from the Society for Gynecologic Investigation should be submitted to Dr. Roger A. Lobo, Women's Hospital, 1240 North Mission Road, Room 1M2, Los Angeles, CA 90033.

Author's designation of reviewers. When authors submit their manuscripts, they may provide the names and addresses of three reviewers for consideration by the Editors.

Copyright statement. The specified copyright statement that follows the Information for Authors in each issue of the JOURNAL must be completed, signed by all authors, and submitted with the manuscript. (The Checklist and Copyright Statement may be torn from the JOURNAL or photocopied for submission.) If not completed in full, it will be returned to the author for completion.

Checklist. The checklist that appears with the copyright statement in each issue of the JOURNAL must be fully completed by the author(s) and sent with each submitted manuscript. A more complete description of each item that must be checked is provided under the appropriate heading in the Information for Authors, which is published in each issue of the JOURNAL. If the checklist is not completed in full, the deficiencies will be noted, and the form, as well as the manuscript when necessary, will be returned to the author for completion.

Previous publication. If a report by the same author(s) has been previously published in any medium that deals in any respect whatever with the same patients, same animals, same laboratory experiments, or same data, in part or in full, as those reported in the manuscript being submitted, two reprints of the article or two copies of the manuscript, be it a full-length report or an abstract, must be submitted with the manuscript. The author(s) should inform the Editor of the circumstances of the two reports. This requirement also applies to the submission of a manuscript in which a few different patients, animals, laboratory experiments, or data were added to those reported in a previous publication or in a submitted or

accepted manuscript. Articles previously published in another language will not be considered.

Human and animal experimentation. It is assumed by the Editors that manuscripts emanating from a particular institution are submitted with the approval of the requisite authority. Human experimentation that requires local institutional approval must have this approval *before the experiment is started* and approval must be so indicated in the Methods section of the submitted manuscript. Reports of experiments on animals must *state in the Methods section of the manuscript* that the guidelines for the care and use of the animals *approved by the local institution* were followed.

Authorship. For manuscripts with two or more authors, each author must qualify by having participated actively and sufficiently in the study that is being performed and reported. The inclusion of each author in the authorship list of a report is based only (1) on substantial contributions to (a) concept and design, or analysis and interpretation of data and (b) drafting the manuscript or revising it critically for important intellectual content; and (2) on final approval by each author of the version of the manuscript. Conditions 1 (a and b) and 2 must both be met. Others contributing to the work should be recognized separately in an Acknowledgment. In the covering letter that accompanies the submitted manuscript, it must be confirmed that all authors fulfilled both conditions.

Conflict of interest. Authors are expected to inform the Editor, in a letter accompanying the submitted manuscript, of any commercial association that might pose a conflict of interest, such as ownership, stock holdings, equity interests and consultant activities, or patent-licensing situations. Such information is confidential, is not given to the consultants, and does not play a part in the decision of the quality or timeliness of the manuscript. If the manuscript is accepted, the author and the Editor will determine how best to release the information. The usual and customary listing of sources of support and institutional affiliations on the title page is proper and does not imply a conflict of interest; only where there is a possible conflict of interest is the author(s) expected to inform the Editor.

Disclaimer. Statements and opinions expressed in articles and communications herein are those of the author(s) and not necessarily those of the Editor(s) or publisher, and the Editor(s) and publisher disclaim any responsibility or liability for such material. Neither the Editor(s) nor the publisher guarantees, warrants, or endorses any product or service advertised in this publication, nor do they guarantee any claim made by the manufacturer of such product or service.

General requirements for preparation of manuscripts

The original and two good-quality photocopies of the manuscript and three sets of glossy prints of illustrations are required.

Manuscripts must be typed double-spaced on one side only of 22 × 28 cm (8½ × 11 inch) white bond paper with 1-inch margins at top, bottom, and sides. Number pages consecutively in the upper right-hand corner in the following order: title page, condensation, abstract, body of text, acknowledgments, references, legends, and tables.

Title page. The title page (page 1) should contain in sequence the title (concise and suitable for indexing purposes); author line with first name, middle initial, and last name of each author and each author's highest academic degree (both MD and PhD are acceptable); city(ies), state(s) in which the study was conducted; divisional, or departmental, and institutional affiliations at the time the study was performed; source(s) of financial support; presented line, if applicable; disclaimers, if any; name, address, and business and home telephone numbers of author to whom requests for reprints should be addressed (if reprints will not be available, it should be so stated); and name, address, and business and home telephone numbers of author responsible for correspondence concerning the manuscript if different from author to whom reprint requests are addressed. At the bottom of the title page supply a short title for the running head not exceeding 52 characters (including word spaces).

Condensation. On page 2 of the manuscript provide a brief, concise condensation, typed double-spaced, that will appear with the title in publication of the Contents pages of the JOURNAL. It should be a single sentence, limited to a maximum of 25 words, delineating the essential point(s) made in the manuscript.

Abstract page and key words/phrases. On manuscript page 3 type the abstract, double-spaced, with the required margins and headed by the title of the article and name(s) of author(s). Abstracts for regular articles, Current Investigation, Clinical Opinion, and Current Development may not exceed 150 words. Abstracts for case reports and brief communications may not exceed 50 words. Below the abstract list 3 to 5 *key words* or short phrases for indexing purposes.

Text. Do not hesitate to write your manuscript in the first-person, active voice if it is more appropriate to the information you wish to convey. The passive voice is generally more effective for describing techniques or observations, since the emphasis is on the "action" rather than on the person performing the action.

Only standard abbreviations are to be used. Consult the *Council of Biology Editors Style Manual* or the AMA's *Manual for Authors and Editors*. Abbreviations in the title are not acceptable. They should be avoided, if possible, in the abstract. In the text they should be kept to a practical minimum. The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement.

Either the generic, chemical, or proprietary names of drugs may be used. If the generic or chemical name is used, authors may, if they desire, insert the proprietary name in parentheses after the first mention in the text, with the name of the manufacturer and city and state.

Regular articles are customarily organized into the following sections: an introduction and headings that identify Material and Methods, Results, and Comment. Authors may wish to summarize their findings in a short paragraph at the end of the Comment section. This format may not be appropriate for some types of articles.

In the introduction, state concisely the purpose and rationale for the study and cite only the most pertinent references as background.

In the *Material and Methods* section describe briefly (but in sufficient detail to permit other workers to evaluate and reproduce the results) the plan, patients and/or experimental animals and controls, methods and procedures utilized, and statistical method(s) employed.

In the *Results* section present the detailed findings. Include mentions of all tables and/or figures. Avoid

Estimating length of manuscripts

The length of text material (introduction through Comment section) in regular manuscripts accepted for publication normally ranges from 750 to 4200 words (an average of 2000 words). A 4200-word text can seldom be accepted, especially if tables and figures are included. The average manuscript of 2000 words of text with abstract, 3 tables with captions, 2 figures with legends, and references makes a 5.7-page article in the JOURNAL. The 2000 words of text alone make approximately 8 pages of manuscript typed double-spaced with the required 1-inch margins (approximately 250 words per page). A table or figure that occupies both columns of half a JOURNAL page is equivalent to approximately 500 words in manuscript. Thus, if a greater number of illustrations and tables are used, the length of the text should be adjusted accordingly.

duplication of text and supporting material. Emphasize only your important observations; do not compare your observations with those of others. Such comparisons and comments are reserved for the Comment section.

In the *Comment* section state the importance and significance of your findings but do not repeat the details given in the Results section. Limit your opinions to those strictly indicated by the facts in your report. Compare your findings with those of others. No new data should be presented in this section.

Acknowledgments. Acknowledge only persons who have made substantive contributions to the study.

References. A reasonable number are allowed, except in case reports and brief communications (limited to 2) and in manuscripts for the Current Development section (for which there is no limit). Number references consecutively in the order in which they are mentioned in the text. Use the format of the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" (Vancouver style) (Ann Intern Med 1988;108:258-65). Journal titles should conform to abbreviations used in *Cumulated Index Medicus.*

Examples (if six or fewer authors, list all; if seven or more authors, list three then et al.):

JOURNAL: Flamm BL, Fischermann E, Quilligan EJ, et al. Vaginal delivery following cesarean section—use of oxytocin augmentation and epidural anesthesia. AM J OBSTET GYNECOL 1984;148:759-63.

BOOKS: James VHT, Folkerd EJ, Bonney RC, Beranek PA, Reed MJ. Factors influencing estrogen production and metabolism in postmenopausal women with endocrine cancer. In: van Herendael HB, Riphagen FE, Goessens L, van der Pas H, eds. The climacteric, an update. Lancaster, England: MTP Press, 1983:29.

Personal communications and unpublished data, if essential, may be used but not as numbered references. If they are used, they are to be referred to, within parentheses, at the appropriate location in the text. If used, the author(s) must obtain written and signed permission for their use from the individual being quoted. This signed permission must accompany the manuscript when it is submitted to the Editor. Abstracts are not acceptable as numbered references.

Illustrations and tables. Illustrations and tables should supplement, not duplicate, the text; presentation of data in either one or the other will suffice.

A reasonable number of halftone and line illustrations will be reproduced without charge, but special arrangements must be made with the Editors for *color illustrations* at a cost of $525 per page (one side).

For *color photographs* submit original transparencies and two sets of unmounted prints on glossy (smooth-surface) paper. Polaroid prints are not acceptable. Color transparencies must have a color balance (consistency in lighting and film speed) that is acceptable to the author and Editors before acceptance for publication. Please note that 35 mm transparencies are enlarged to twice their original size. If it is important to deviate from this standard, please so indicate when the material is submitted. The *top, first author's last name,* and *figure number* must be indicated on the front of each transparency and the back of each print. Consistency in size of illustrations within the article is strongly preferred.

For *black-and-white illustrations* submit three sets of 3×4 inch (minimum) to 5×7 inch (maximum) unmounted, glossy photographic prints. All lettering must be done with commercially available paste-on letters (or numbers) or by a professional; typed or freehand lettering is not acceptable. All lettering must be in proportion to the drawing, graph, or photograph. Original drawings, appropriately done in black India ink, roentgenograms, and other material must be submitted as glossy photographic prints with good black-and-white contrast. Consistency in size within the article is strongly preferred. Any special instructions regarding sizing should be clearly noted.

Do not use paper clips or mar the surface of prints in any way.

Figures must be cited consecutively in the text in Arabic numerals and identified thusly on the back of the print (gummed label with): author(s) name(s), title of article, number, and top marked clearly.

Figures will be returned only on request by the author.

Tables should be typed on separate sheets of paper, one table to a page, and included at the end of the text. They should be numbered in Roman numerals. Each table must be cited in sequence at an appropriate point in the text. Captions should be brief yet indicate clearly the purpose or content of each table, and each column should be precisely defined by headings. Abbreviations and special designations should be explained in a footnote to the table. *If a table or any part thereof has been taken from copyrighted material, a legend to the table must give full credit to the original source.* Special arrangements must be made with the Editors for elaborate tables because of space limitations.

Legends to illustrations. Legends for all figures must be typed double-spaced on paper separate from the text of the

manuscript, and these pages must be numbered in sequence after the references. Titles should be included in the legend; *not* on the print. Original magnifications should be provided. *If an illustration has been taken from copyrighted material, the legend must give full credit to the original source.*

Computer-generated illustrations. *Black-and-white illustrations* submitted must be legible and clearly printed in jet-black ink on heavy coated paper with either a glossy or dull finish. Any patterns or shadings must be dark enough for reproduction and must be distinguishable from each other. Lines, symbols, and letters should be both smooth and complete. The legend for the illustration should not appear on the print. On the back of each print the name of the first author and the figure number should be given and the top indicated. Original individual laser or plotter prints are to be submitted *unmounted* with the manuscript. Laser prints should be full size at 300 dots per inch (DPI) or greater full-page resolution; multiple illustrations on a page cannot be accepted. Dot matrix prints and photographic halftones are not acceptable. *Color illustrations* are acceptable, but special arrangements must be made with the Editors. The colors used must be dark enough and of sufficient contrast for reproduction. With the exception of fluorescent colors, all colors can be reproduced in four-color illustrations. The preparation and submitting of color prints should follow the preceding guidelines for black-and-white computer-generated illustrations.

Permissions. Direct quotations, tables, or illustrations that have appeared in copyrighted material must be accompanied by *written permission* for their use from the copyright owner and original author along with complete information as to source. Photographs of identifiable persons must be accompanied by signed releases or else all recognizable features masked.

Requirements for special sections

Case reports and brief clinical and basic science communications. Limit of 700 words, 2 references. Include abstract of 50 words maximum, 3 to 5 key words/phrases for indexing purposes, and short title. If tables and/or figures are used, an equivalent number of words must be deducted from the total (see "Estimating Length of Manuscript").

Current Investigation. Same requirements as for regular article.

Clinical Opinion. Limit of 3000 words. Include abstract of 50 to 150 words, 3 to 5 key words/phrases, and short title. Submit to Dr. Zuspan.

Current Development. Limit of 6000 words. Include abstract of 50 to 150 words, 3 to 5 key words/phrases, and short title. Submit to Dr. Zuspan.

Correspondence. Two types of correspondence will be considered for publication. (1) A Letter to the Editors commenting on an article that has appeared in the JOURNAL should be brief and directly related to the published article. The editorial staff reserves the right to shorten letters if necessary and to make minor editorial alterations without reference to the writer. Letters may be published together with a reply from the original author. If the original author does not respond, a notation indicating "Response declined" will be published. As space for letters is limited, only a selection of letters submitted may be published. (2) A brief case presentation or a short report of a pertinent observation in the form of a Letter to the Editors will be considered for publication. All letters should be typed double-spaced. The original and a good photocopy must be submitted. Letters should be sent to Dr. Zuspan.

Announcements. Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60 U.S., and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, The C.V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, MO 63146-3318.

Books. Books received will be listed in the JOURNAL. They should be sent to Dr. Gerbie. No books will be returned.

Reprints

Reprints of articles must be obtained from the author. The corresponding author will receive a price schedule and order form at the time of publication. Reprints in quantities must be ordered from the publisher with the author's consent.

Business communications

Communications of a business nature and all advertising communications should be addressed to: Journal Publisher, The C.V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, MO 63146-3318, or call Journal Advertising Production Manager (314) 872-8370.

CHECKLIST AND COPYRIGHT STATEMENT

Both the checklist and the copyright statement forms **must be completed** and sent with each submitted manuscript. If **not** completed, these forms will be returned to the author for completion.

These items appear in each monthly issue of the JOURNAL. A photocopy may be used. An exposition of the requirements given in the checklist is in the Information for Authors, which is published in each issue of the JOURNAL.

CHECKLIST

General

—The original and two copies of the manuscript are submitted.
—The appropriate signed completed checklist and assignment of copyright signed by **all** authors accompany the manuscript.
—Three sets of unmounted glossy photographic prints are submitted, properly numbered and labeled on the back of each print.
—Three sets of computer-generated figures printed on heavy coated paper with either a glossy or dull finish, unmounted, each one on a page, and properly labeled on the back are submitted. None are dot matrix or photographic prints.
—Human experimentation has been approved by the local institution as stated in the Methods section.
—Guidelines for the care and use of animals approved by the institution have been followed as indicated in the Methods section.
—The covering letter with essential information is with the manuscript.
—All elements of the manuscript are typed double-spaced on bond paper with 1-inch margins at top, bottom, and sides.
—All pages are numbered in the following order: title page, condensation, abstract, body of the text, acknowledgments only of persons who have made substantive contributions to the study, references, legends, and tables.
—Signed, written permission from both the copyright holder and the original author for the use of tables, figures, or quotations previously published and their complete references are enclosed with the manuscript.
—Signed, written permission for the use of quotations of personal communications and unpublished data has been obtained from the person(s) being quoted and is enclosed.

Authorship

—In the covering letter that accompanies the submitted manuscript I/we have confirmed that all authors fulfilled both conditions required for authorship.

Conflict of interest

—In the covering letter that accompanies the submitted manuscript the commercial association of the author or of any co-authors that might pose a conflict of interest is described.

Previous publications

—Enclosed with the submitted manuscript are two reprints each of articles the author or co-authors have previously published or have in manuscript form dealing with same patients, same animals, same laboratory experiment, or same data, in part or in full, as those reported in the submitted manuscript. Further explanation is provided in the covering letter that accompanies the submitted manuscript.

Title page

—These elements are given in the following sequence, and are typed double-spaced.
—Title
—Author(s) name(s) and highest academic degree(s)
—City(ies), state(s), and country other than U.S. in which the study was conducted are given
—The name(s) of the institution(s) section(s), division(s), department(s) in which the study was performed is provided and the author(s) institutional affiliation at the time of the study is indicated
—Acknowledgment of financial support is cited
—Name, address, and business and home telephone numbers of author to whom requests for reprints are to be sent
—Reprints will not be available. This has been stated on the title page
—Since the corresponding author is different from the author to whom reprint requests are to be sent, his/her name, address, and business and home telephone numbers have been added
—At the bottom of the page, a running head not exceeding 52 characters (including word spaces) has been typed

Condensation

—On a separate sheet of paper, page 2 of the manuscript, a single sentence limited to 25 words delineating the essential point(s) is typed double-spaced.

Abstract

—The abstract is typed double-spaced with required margins (on page 3) headed by the title and the author(s) name, not exceeding 150 words. For case reports and brief communications the limit of 50 words is not exceeded.
—Three to five key words or short phrases are placed beneath the abstract on page 3.

Tables

—Each table headed by a caption and numbered in Roman numerals is typed double-spaced on a separate page.
—Tables are cited in sequence in the text.

Figures

—Are numbered in Arabic numerals and are cited in numerical sequence in the text.

—Original transparencies of **color photographs** appropriately numbered and identified are submitted along with two sets of unmounted prints on glossy paper, numbered and identified on the back.

References

—Are typed double-spaced.

—Are numbered consecutively in the order they are cited in the text.

—The format of the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" is used. Examples shown in Information for Authors were followed.

—Abstracts, personal communications, and unpublished work are not used as numbered references but are mentioned in the text with the written approval of the person being quoted and the signed approval is enclosed.

AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY

COPYRIGHT STATEMENT

Must be signed by ALL authors

"The undersigned author(s) transfers all copyright ownership of the manuscript entitled

to The C.V. Mosby Company in the event the work is published. The undersigned author(s) warrants that the article is original; is not under consideration by another publication; and its essential substance, tables, or figures have not been previously published. This restriction does not apply to abstracts or press reports published in connection with scientific meetings. The author(s) confirms the final manuscript has been read and each author's contribution has been approved by the appropriate author."

The responsible author(s) must be named_____ _____
(Type name) Signature

Each author's name must be typed underneath the signature.

_____ _____

_____ _____

_____ _____

_____ _____

Date: _____

American Journal of Obstetrics and Gynecology

Founded in 1920

volume 161 *number* 6 *part* 1 DECEMBER 1989

CONSULTANTS

Consultants for the American Journal of Obstetrics and Gynecology

September 1, 1988—August 31, 1989

The Editors and The C.V. Mosby Company take this opportunity to acknowledge those reviewers who have contributed many hours and much effort in the evalution of manuscripts submitted to the JOURNAL during the past year. Without their interest and help, publication of the JOURNAL would be very difficult indeed. We also continue to seek advice from reviewers and readers alike as regards their overall evaluation of the JOURNAL.

Abdella, Thomas, *Memphis, Tennessee*
Abel, Ernest L., *Buffalo, New York*
Abraham, Guy, *Torrance, California*
Abrams, Robert M., *Gainesville, Florida*
Adams, Eve, *Irvine, California*
Adashi, Eli Y., *Baltimore, Maryland*
Aksel, Sezer, *Mobile, Alabama*
Albers, John J., *Seattle, Washington*
Albrecht, Eugene D., *Baltimore, Maryland*
Amsel, Rhonda, *Montreal, Quebec, Canada*
Amstey, Marvin S., *Rochester, New York*
Ances, Isadore G., *Camden, New Jersey*
Anderson, Barrie T., *Iowa City, Iowa*
Anderson, John, *London, England*
Anderson, Ted, *Norfolk, Virginia*
Anderson, William D, *Boston, Massachusetts*
Andrews, Mason, *Norfolk, Virginia*
Angell, Rosyln R., *Edinburgh, Scotland*
Ansbacher, Rudi, *Ann Arbor, Michigan*
Anton-Culver, Hoda, *Irvine, California*
Archer, David, *Norfolk, Virginia*
Arias, Fernando, *St. Louis, Missouri*
Arquilla, Edward R., *Irvine, California*
Artal, Raul, *Los Angeles, California*
Asch, Ricardo, *Garden Grove, California*
Ashmead, Graham, *Cleveland, Ohio*
Assali, Nicholas S., *Los Angeles, California*
Aubry, Richard H., *Syracuse, New York*
Ayers, Leona, *Columbus, Ohio*
Baggish, Michael S., *Syracuse, New York*
Baker, David, *Stoneybrook, New York*
Ballon, Samuel C., *San Jose, California*
Bancroft, John, *Edinburgh, Scotland*
Barbieri, Robert, *Boston, Massachusetts*
Barden, Tom, *Cincinnati, Ohio*
Barltrop, Donald, *London, England*
Barrow, William, *Chicago, Illinois*
Bassell, Gerry, *Wichita, Kansas*
Bast, Robert, *Durham, North Carolina*
Bates, George William, *Charleston, South Carolina*
Battaglia, Frederick C., *Denver, Colorado*
Beck, Peter, *Edmonton, Alberta, Canada*
Beer, Alan E., *North Chicago, Illinois*
Begin, Louis R., *Montreal, Quebec, Canada*
Beller, Fritz K., *Iowa City, Iowa*
Benacerraf, Beryl, *Boston, Massachusetts*
Benedetti, Thomas, *Seattle, Washington*
Berek, Jonathan S., *Los Angeles, California*
Berendes, Heinz W., *Bethesda, Maryland*
Berkowitz, Gertrud S., *New York, New York*
Berkowitz, Richard, *New York, New York*
Bernfield, Merton R., *Stanford, California*
Bernstein, Renee, *Orange, California*
Bibbo, Marluce, *Chicago, Illinois*
Birnholz, Jason C., *Highland Park, Illinois*
Bissonnette, John M., *Portland, Oregon*
Blackwell, Richard E., *Birmingham, Alabama*
Blanc, William A., *New York, New York*
Blonsky E. Richard, *Chicago, Illinois*
Bobitt, John R., *El Paso, Texas*
Bocian, Maureen, *Orange, California*
Boehm, Frank H., *Nashville, Tennessee*
Bolognese, Ronald J., *Philadelphia, Pennsylvania*
Bonnar, John, *Dublin, Ireland*
Bottoms, Sidney, *Detroit, Michigan*
Boughton, B. J., *Birmingham, England*

Bowes, Watson, Jr., *Chapel Hill, North Carolina*
Bowman, John M., *Winnipeg, Manitoba, Canada*
Boyers, Stephen P., *Davis, California*
Brace, Robert A., *La Jolla, California*
Braly, Patricia S., *San Diego, California*
Brame, Robert G., *Greenville, North Carolina*
Brann, Alfred W., *Atlanta, Georgia*
Brans, Yves W., *Detroit, Michigan*
Brar, Harbinder, *Los Angeles, California*
Breen, James L., *Livingston, New Jersey*
Bremner, I., *Aberdeen, Scotland*
Brenner, Paul F., *Los Angeles, California*
Brent, Robert, *Philadelphia, Pennsylvania*
Breslow, Lester, *Los Angeles, California*
Brewer, John I., *Chicago, Illinois*
Brinkman, Charles R., *Torrance, California*
Brinton, Louise, *Bethesda, Maryland*
Brown, Gregory, *Hamilton, Ontario, Canada*
Brown, Zane A., *Seattle, Washington*
Brunage, Philip, *Durham, North Carolina*
Buchanan, Thomas A., *Los Angeles, California*
Buchsbaum, Herbert J., *Milwaukee, Wisconsin*
Buchsbaum, Monte S., *Irvine, California*
Bump, Richard, *Richmond, Virginia*
Burk, Billy D., *Rome, Georgia*
Burrow, Gerard N., *La Jolla, California*
Burton, Barbara, *Chicago, Illinois*
Bush, Trudy L., *Baltimore, Maryland*
Buster, John E., *Memphis, Tennessee*
Butler, William J., *Albany, New York*
Buttram, Veasy C. B., *Houston, Texas*
Cabaniss, Micki S., *Mobile, Alabama*
Cameron, Alan D., *Glasgow, Scotland*
Caritis, Steven N., *Pittsburgh, Pennsylvania*
Carpenter, Robert, *Columbus, Georgia*
Carpenter, Robert J., *Houston, Texas*
Carr, Bruce R., *Dallas, Texas*
Carsten, Mary, *Los Angeles, California*
Cartwright, Peter Scot, *Nashville, Tennessee*
Casper, Robert F., *London, Ontario, Canada*
Castillo, Ramon, *Augusta, Georgia*
Cederblad, Gitten *Huddinge, Sweden*
Cefalo, Robert C., *Chapel Hill, North Carolina*
Cetrulo, Curtis, *Boston, Massachusetts*
Challis, John R. G., *London, Ontario, Canada*
Chambers, Charles B., *Pomona, California*
Chard, Tim, *London, England*
Chaudhuri, Gautam, *Los Angeles, California*
Cherry, Flora F., *New Orleans, Louisiana*
Chervenak, Frank A., *New York, New York*
Chesley, Leon, *Valley Stream, New York*
Chestnut, David H., *Iowa City, Iowa*
Cheung, Cecelia, *La Jolla, California*
Chez, Ronald A., *Secaucus, New Jersey*
Chisolm, John Cobeen, *Memphis, Tennessee*
Cho, Zang-Hee, *Irvine, California*
Chotiner, Harold C., *Sparks, Nevada*
Christenson, R. K., *Clay Center, Nebraska*
Chrousos, Geo Panagiotis, *Bethesda, Maryland*
Cibils, Luis, *Chicago, Illinois*
Cines, D. B., *Philadelphia, Pennsylvania*
Clapp, James Ford, III, *Burlington, Vermont*
Clark, David A., *Hamilton, Ontario, Canada*
Clark, Kenneth E., *Cincinnati, Ohio*
Clark, Martin, *Chapel Hill, North Carolina*
Clark, Stephen, *Provo, Utah*
Clausen, Kathryn, *Columbus, Ohio*
Clewell, William H., *Phoenix, Arizona*
Cohen, Carmel J., *New York, New York*
Cole, Laurence A., *New Haven, Connecticut*
Collea, Joseph V., *Potomac, Maryland*
Connell, Elizabeth B., *Atlanta, Georgia*
Copel, Joshua A., *New Haven, Connecticut*
Copeland, Larry, *Columbus, Ohio*
Cordero, Leandro, *Columbus, Ohio*
Corson, Stephen, *Philadelphia, Pennsylvania*
Cotton, David B., *Houston, Texas*
Coulam, Carolyn Bedke, *Indianapolis, Indiana*
Cousins, Larry, *Loma Linda, California*
Coustan, Donald R., *Providence, Rhode Island*
Coutinho, Elsimar M., *Bahia, Brazil*
Cowchock, Suasn, *Philadelphia, Pennsylvania*
Crane, James P., *St. Louis, Missouri*
Creasman, William T., *Charleston, South Carolina*
Creasy, Robert K., *Houston, Texas*
Crenshaw, Carlyle, *Baltimore, Maryland*
Crosby, Warren M., *Oklahoma City, Oklahoma*
Cruikshank, Dwight, *Iowa City, Iowa*
Cruz, Amelia, *Gainesville, Florida*
Csernansky, John, *Stanford, California*
Cunningham, Douglas, *Laguna Hills, California*
Cunningham, F. Gary, *Dallas, Texas*
Curet, Luis B., *Albuquerque, New Mexico*
Cusson, Jean R., *Montreal, Quebec, Canada*
Daffos, Fernand, *Paris, France*
Dancis, Joseph, *New York, New York*
Danforth, David N., *Evanston, Illinois*
Davajan, Val, *Los Angeles, California*
Davies, K. J. A., *Los Angeles, California*
Davis, Ann, *Canton, Massachusetts*
Davis, Richard, *Birmingham, Alabama*
Dawes, Geoffrey, *Oxford, England*
Dawood, Mohamed Yusoff, *Chicago, Illinois*
De la Camara, Crisogono, *Madrid, Spain*
DeCherney, Alan H., *New Haven, Connecticut*
DeVane, Gary W., *Orlando, Florida*
DeVoe, Larry, *Augusta, Georgia*
DeVore, Greggory R., *Salt Lake City, Utah*
Deddish, Ruth, *Chicago, Illinois*
Deter, Russell L., *Houston, Texas*
DiSaia, Philip J, *Orange, California*
DiZerega, Gere S., *Los Angeles, California*
Diamond, Edward, *Irvington, New Jersey*
Dignam, William, *Los Angeles, California*
Dilts, Preston V., *Ann Arbor, Michigan*
Divon, Michael Y., *Bronx, New York*
Dodds, William, *Columbus, Ohio*
Dodson, William, *Durham, North Carolina*
Dombrowski, Mitchell, *Detroit, Michigan*
Donaldson, J. O., *Farmington, Connecticut*

Dooley, Sharon, *Chicago, Illinois*
Droegemueller, William, *Chapel Hill, North Carolina*
Druzin, Maurice, *New York, New York*
Ducsay, Charles, *Loma Linda, California*
Duff, W. Patrick, *Tacoma, Washington*
Dumars, Kenneth, *Orange, California*
Durst, Matthias, *Heidelberg, Federal Republic of Germany*
Edelstone, Daniel I., *Pittsburg, Pennsylvania*
Edwards, Miles, *Portland, Oregon*
Ehrlich, Clarence E., *Indianapolis, Indiana*
Elias, Sherman, *Memphis, Tennessee*
Elliott, John P., *Phoenix, Arizona*
Enkin, Murray, *Hamilton, Ontario, Canada*
Entman, Steven, *Nashville, Tennessee*
Erickson, Gregory F., *La Jolla, California*
Eschenbach, David A., *Seattle, Washington*
Evans, Mark I., *Detroit, Michigan*
Fadel, Hossam, *Augusta, Georgia*
Fairweather, Denys, *London, England*
Fantl, John, *Richmond, Virginia*
Farmakides, George, *Mineola, New York*
Farrar, William, *Columbus, Ohio*
Feichter, G. E., *Heidelberg, Federal Republic of Germany*
Feingold, Michael, *Boston, Massachusetts*
Feinstein, Alvan, *New Haven, Connecticut*
Ferenczy, Alex, *Montreal, Quebec, Canada*
Ferrazzi, Enrico, *Milan, Italy*
Ferris, Thomas F., *Minneapolis, Minnesota*
Fishburne, John I., *Oklahoma City, Oklahoma*
Fisher, Delbert, *Torrance, California*
Flamm, Bruce, *Anaheim, California*
Fleischer, Adiel, *New Hyde Park, New York*
Flickinger, George L., *Philadelphia, Pennsylvania*
Fowler, Wesley, *Chapel Hill, North Carolina*
Fraser, William D., *Quebec, Canada*
Freda, Vincent J., *New York, New York*
Frederiksen, Marilynn C., *Chicago, Illinois*
Freeman, Roger, *Long Beach, California*
Freese, Uwe E., *Chicago, Illinois*
Freinkel, Norbert, *Chicago, Illinois*
Friedman, Chad, *Columbus, Ohio*
Friedman, Emanuel, *Boston, Massachusetts*
Frigoletto, Frederick D., *Boston, Massachusetts*
Fukushima, Teiichiro, *Los Angeles, California*
Fuller, Arlan, *Boston, Massachusetts*
Gabbe, Steven G., *Columbus, Ohio*
Galask, Rudolph P., *Iowa City, Iowa*
Gall, Stanley, *Chicago, Illinois*
Gambrell, R. Don, *Augusta, Georgia*
Gant, Norman F., *Dallas, Texas*
Gardin, Julius M., *Orange, California*
Gardner, Debra K., *Columbus, Ohio*
Gardner, Vance O., *Orange, California*
Garfield, Robert E., *Hamilton, Ontario, Canada*
Garite, Thomas, *Orange, California*
Gazzaniga, Alan B., *Orange, California*
Gerbie, Melvin, *Chicago, Illinois*
Gersony, Welton M., *New York, New York*
Gibbons, William E., *Houston, Texas*
Gibbs, Charles, *Denver, Colorado*
Gibbs, Ronald S., *San Antonio, Texas*
Gibson, Mark, *Burlington, Vermont*
Gidwani, Gita, *Cleveland, Ohio*
Gilbert, Raymond D., *Loma Linda, California*
Giles, Harlan R., *Pittsburgh, Pennsylvania*
Gilstrap, Larry C., III, *Dallas, Texas*
Givens, James R., *Memphis, Tennessee*
Glasser, Stanley, *Houston, Texas*
Gleicher, Norbert, *Chicago, Illinois*
Gluck, Louis, *Orange, California*
Golbus, Mitchell S., *San Francisco, California*
Goldberg, Jeff, *Columbus, Ohio*
Golde, Steven H., *Burbank, California*
Goldstein, Allan, *Washington, D.C.*
Goldstein, Steven, *New York, New York*
Goldzieher, Joseph, *Houston, Texas*
Gomel, Victor, *Vancouver, British Columbia, Canada*
Goodlin, Robert C., *Denver, Colorado*
Goodstein, Richard K., *Belle Mead, New Jersey*
Goplerud, Clifford P., *Iowa City, Iowa*
Gordon, Myron, *Albany, New York*
Gorman, John G., *New York, New York*
Grannum, Peter, *New Haven, Connecticut*
Grant, Adrian, *Oxford, England*
Gravett, Michael, *Boise, Idaho*
Gray, Elizabeth S., *Aberdeen, Scotland*
Greenberg, Raymond S., *Atlanta, Georgia*
Greene, John, *Lexington, Kentucky*
Greenspoon, Jeffrey S., *Los Angeles, California*
Greiss, Frank, *Mooresville, North Carolina*
Grimes, David A., *Los Angeles, California*
Grossman, John H., III, *Washington, D.C.*
Grumbach, Melvin, *San Francisco, California*
Gupta, Sudhir, *Orange, California*
Gurpide, Emilio, *New York, New York*
Gusdon, John P., *Winston-Salem, North Carolina*
Gustavii, Bjorn, *Lund, Sweden*
Guthenberg, Claes, *Stockholm, Sweden*
Gwinup, Grant, *Orange, California*
Hadlock, Frank, *Houston, Texas*
Hahn, Bevra H., *Los Angeles, California*
Hahn, Do W., *Raritan, New Jersey*
Hale, Ralph W., *Honolulu, Hawaii*
Haluska, George J., *Beaverton, Oregon*
Hammond, Charles B., *Durham, North Carolina*
Hammond, Mary G., *Chapel Hill, North Carolina*
Haney, Arthur F., *Durham, North Carolina*
Haning, Ray V., Jr., *Providence, Rhode Island*
Hankins, Gary, *San Antonio, Texas*
Hansen, Thomas N., *Houston, Texas*
Harbert, Guy, Jr., *Charlottesville, Virginia*
Hargens, Alan R., *Moffett Field, California*
Harger, James H., *Pittsburgh, Pennsylvania*
Hargrove, Joel T., *Columbia, Tennessee*
Harlass, Frederick, *Tacoma, Washington*
Harris, E. Nigel, *Louisville, Kentucky*
Harrison, Deborah M., *New Haven, Connecticut*
Harrison, Gail G., *Tucson, Arizona*
Haseltine, Florence, *Bethesda, Maryland*
Hasselmeyer, Elaine, *Bethesda, Maryland*

Hatjis, Christos G., *Columbus, Ohio*
Hauth, John C., *Birmingham, Alabama*
Hayashi, Robert, *Ann Arbor, Michigan*
Hayashi, T. Terry, *Pittsburgh, Pennsylvania*
Haynes, R. B., *Hamilton, Ontario, Canada*
Head, Judith, *Dallas, Texas*
Healy, David L., *Melbourne, Australia*
Heinrichs, W. LeRoy, *Stanford, California*
Henderson, Brian, *Los Angeles, California*
Hendricks, Charles H., *Chapel Hill, North Carolina*
Hensleigh, Paul A., *Stanford, California*
Herbst, Arthur L., *Chicago, Illinois*
Herrmann, Walter, *Geneva, Switzerland*
Hertelendy, Frank, *St. Louis, Missouri*
Hertz, Roger H., *Southfield, Michigan*
Hibbard, Lester T., *Carmel, California*
Hill, Joseph A., III, *Boston, Masschusetts*
Hill, Lyndon M., *Pittsburgh, Pennsylvania*
Hill, Washington C., *Omaha, Nebraska*
Hobart, John M., *Evanston, Illinois*
Hobbins, John C., *New Havaen, Connecticut*
Hobel, Calvin J., *Los Angeles, California*
Hodgen, Gary D., *Norfolk, Virginia*
Hofmann, Glen E., *Norfolk, Virginia*
Hogge, W. Allen, *Charlottesville, Virginia*
Hollingsworth, Dorothy, *San Diego, California*
Hollingsworth, M., *Manchester, England*
Holmes, King Kennard, *Seattle, Washington*
Homesley, Howard D., *Winston-Salem, North Carolina*
Hon, Edward, *Bradbury, California*
Horger, Edgar O., III, *Charleston, South Carolina*
Horton, Richard, *Los Angeles, California*
Hoskins, Iffath Abbasi, *New York, New York*
Hsueh, Aaron, *La Jolla, California*
Huddleston, John F., *Atlanta, Georgia*
Hulka, Jaroslav, *Chapel Hill, North Carolina*
Hunt, Carl E., *Toledo, Ohio*
Hurt, Glen, *Richmond, Virginia*
Huszar, Gabor Bela, *New Haven, Connecticut*
Iams, Jay, *Columbus, Ohio*
Iams, Pat, *Columbus, Ohio*
Iannaccone, Philip, *Chicago, Illinois*
Iffy, Leslie, *Newark, New Jersey*
Isada, Nelson B., *Washington. D.C.*
Israel, Robert, *Los Angeles, California*
Itoh, Kyogo, *Houston, Texas*
Iturbe-Alessio, Ignacio, *Chula Vista, California*
Iversen, Ole-Erik, *Haukeland Sykeus, Norway*
Jackson, Laird, *Philadelphia, Pennsylvania*
Jenkins, Susan, *Durham, North Carolina*
Jewelewicz, Raphael, *New York, New York*
Johnson, J. W. C., *Gainesville, Florida*
Johnson, Mary Ann, *Vancouver, Br. Columbia, Canaaa*
Johnson, Peter, *Liverpool, England*
Johnson, Spencer F., *Baltimore, Maryland*
Johnson, Timothy R. B., *Baltimore, Maryland*
Johnson, William M., *Birmingham, Alabama*
Jones, C. R., *Glasgow, Scotland*
Jones, Georgeanna, *Norfolk, Virginia*
Jones, Kenneth Lyons, *San Diego, California*
Jones, Malcolm D., *Orange, California*
Jones, Robert B., *Indianapolis, Indiana*
Jones, Terry, *London, England*
Josimovich, John B., *Newark, New Jersey*
Jouppila, P., *Oulu, Finland*
Jovanovic, Lois, *Santa Barbara, California*
Juchau, M. R., *Seattle, Washington*
Judd, Howard L., *Los Angeles, California*
Junqueira, L. C. U., *Sao Paulo, Brazil*
Katz, David, *Orange, California*
Katz, Michael, *San. Francisco, California*
Kaufman, Raymond H., *Houston, Texas*
Kauppila, Antti, *Oulu, Finland*
Kawada, Charles, *Newton, Massachusetts*
Kazer, Ralph R., *Chicago, Illinois*
Keegan, Kirk A., *Orange, California*
Keith, Louis, *Chicago, Illinois*
Kelly, John V., *Phoenix, Arizona*
Kennedy, Melanie, *Columbus, Ohio*
Keren, Andre, *Jerusalem, Israel*
Kerin, John F., *Los Angeles, California*
Keye, William R., *Salt Lake City, Utah*
Khan-Dawood, Firyal S., *Chicago, Illinois*
Killam, Allen P., *Durham, North Carolina*
Kim, Moon H., *Columbus, Ohio*
Kirkinen, P., *Oulu, Finland*
Kirschbaum, Thomas, *Los Angeles, California*
Kirz, Donna S., *Chicago, Illinois*
Kitay, David, *Daytona Beach, Florida*
Kitridou, Rodanthi C., *Los Angeles, California*
Kitzmiller, John L., *San Francisco, California*
Kletzky, Oscar A., *Torrance, California*
Knapp, Robert C., *Boston, Massachusetts*
Knopp, Robert H.. *Seattle, Washington*
Knuppel, Robert A., *Tampa, Florida*
Kochenour, Neil K., *Salt Lake City, Utah*
Kohorn, Ernest I., *New Haven, Connecticut*
Kopelman, Jerome, *Kailua, Hawaii*
Kramer, Michael S., *Montreal, Quebec, Canada*
Krantz, Kermit E., *Kansas City, Kansas*
Krauss, Ronald, *Berkeley, California*
Kruskall, Margot S., *Boston, Massachusetts*
Kryc, Joseph, *Canton, Ohio*
Kuhlman, Kathleen, *Philadelphia, Pennsylvania*
Kuhnert, Betty R., *Philadelphia, Pennsylvania*
Kurman, Robert J., *Baltimore, Maryland*
Lacey, J. Hubert, *London, England*
Lagasse, Leo, *Los Angeles, California*
Landon, Mark B., *Columbus, Ohio*
Landy, Helain J., *Washington, D.C.*
Langer, O., *San Antonio, Texas*
Laros, Russell K., *Tiburon, California*
Larsen, John, *Washington, D.C.*
Larson, B., *Huntington, West Virginia*
Lau, Rebecca Jane, *Evansville, Indiana*
Lauersen, Niels Helth, *New York, New York*
Lawson, Edward, *Chapel Hill, North Carolina*
LeMaire, Wiliam, *Miami, Florida*
Leake, Rosemary D., *Torrance, California*
Lebherz, Thomas E. K., *Los Angeles, California*

Ledger, William J., *New York, New York*
Lee, Milton, *Los Angeles, California*
Lee, Raymond A., *Rochester, Minnesota*
Leegaard, Merete, *Niva, Denmark*
Leffler, Charles W., *Memphis, Tennessee*
Lenke, Roger, *Toledo, Ohio*
Leppert, Phyllis, *New York, New York*
Leung, Benjamin S., *Minneapolis, Minnesota*
Leveno, Kenneth J., *Dallas, Texas*
Lewandowski, George, *Columbus, Ohio*
Lewis, John, *New York, New York*
Liggins, G. C., *Auckland, New Zealand*
Lin, Chin C., *Chicago, Illinois*
Lindheimer, Marshall, *Chicago, Illinois*
Lindsay, Robert, *West Haverstraw, New York*
Little, Brian A., *Montreal, Quebec, Canada*
Liu, James H., *Cincinnati, Ohio*
Lobo, Rogerio A., *Los Angeles, California*
Lomano, Jack, *Westerville, Ohio*
Longo, Lawrence D., *Loma Linda, California*
Lopez, Luis, *Columbus, Ohio*
Lossick, Joseph G., *Atlanta, Georgia*
Low, James, *Kingston, Ontario, Canada*
Lucey, Jerold F., *Burlington, Vermont*
Lucky, Ann, *Cincinnati, Ohio*
Lurain, John R., *Chicago, Illinois*
Mabie, William, *Memphis, Tennessee*
MacDonald, Paul C., *Dallas, Texas*
MacGillivray, Ian, *Bristol, England*
MacKay, H. Trent, *Sacramento, California*
MacLaughlin, David T., *Boston, Massachusetts*
MacLusky, Neil J., *Toronto, Ontario, Canada*
Macnab, Joan C. M., *Glasgow, Scotland*
Magness, Ronald R., *Dallas, Texas*
Mahan, Charles S., *Gainesville, Florida*
Mahoney, Maurice J., *New Haven, Connecticut*
Makowski, Edgar L., *Denver, Colorado*
Malkasian, George, *Rochester, Minnesota*
Manetta, Alberto, *Orange, California*
Mann, Leon I., *Cleveland, Ohio*
Manning, Frank A., *Winnipeg, Manitoba, Canada*
March, Charles, *Los Angeles, California*
Marrs, Richard P., *Los Angeles, California*
Marshall, Jean, *Providence, Rhode Island*
Martin, Alice O., *Chicago, Illinois*
Martin, Chester B., Jr., *Madison, Wisconsin*
Marx, Gertie, *Bronx, New York*
Maski, Damon I., *Los Angeles, California*
Masterson, Byron Jack, *Gainesville, Florida*
Matkovic, Valimir, *Columbus, Ohio*
Maulik, Dev, *Kansas City, Missouri*
Mazor, Moshe, *Beer-Sheva, Israel*
McCarthy, Walter J., *Chicago, Illinois*
McCausland, Arthur M., *Sacramento, California*
McCowan, Lesley M., *Toronto, Ontario, Canada*
McDonald, John A., *Columbus, Ohio*
McDonough, Paul G., *Augusta, Georgia*
McGregor, James A., *Denver, Colorado*
McLaughlin, Margaret K., *Burlington, Vermont*
McLeod, Allan G. W., *Miami, Florida*
McVary, Kevin T., *Chicago, Illinois*
Medearis, Arnold L., *Rancho Palos Verdes, California*
Meis, Paul J., *Winston-Salem, North Carolina*
Meldrum, David R., *Redondo Beach, California*
Meltzer, Ronald M., *Chicago, Illinois*
Mennuti, Michael T., *Philadelphia, Pennsylvania*
Merkatz, Irwin R., *Bronx, New York*
Meschia, Giacomo, *Denver, Colorado*
Messer, Robert, *Lubbock, Texas*
Mestman, Jorge, *Los Angeles, California*
Michelassi, Febrizio, *Chicago, Illinois*
Mikuta, John J., *Philadelphia, Pennsylvania*
Milewich, Leon, *Dallas, Texas*
Miller, Davis S., *Chicago, Illinois*
Miller, Frank C., *Little Rock, Arkansas*
Miller, Kurt, *Columbus, Ohio*
Miller, R., *Rochester, New York*
Milunsky, Aubrey, *Boston, Massachusetts*
Minkoff, Howard, *Brooklyn, New York*
Miodovnik, Menachem, *Cincinnati, Ohio*
Mishell, Daniel R., *Los Angeles, California*
Mitchell, George W., *San Antonio, Texas*
Mitchell, Murray D., *Salt Lake City, Utah*
Miyazaki, Fred S., *Los Angeles, California*
Moawad, Atef H., *Chicago, Illinois*
Modanlou, Houchang D., *Long Beach, California*
Moghissi, Kamran S., *Detroit, Michigan*
Moise, Kenneth J., *Houston, Texas*
Mokhtari, A., *Orsay, France*
Moller, B. R., *Paris, France*
Moller, Birger R., *Aarhus, Denmark*
Montes, Gregorio S., *Sao Paulo, Brazil*
Moore, Donald E., *Seattle, Washington*
Moore, Ruth, *Detroit, Michigan*
Moore, Thomas R., *San Diego, California*
Morley, George, *Ann Arbor, Michigan*
Morris, Hugh J., *Cape Town, South Africa*
Morris, John McLean, *New Haven, Connecticut*
Morrison, John C., *Jackson, Mississippi*
Morrow, C. Paul, *Los Angeles, California*
Mortel, Rodrigue, *Hershey, Pennsylvania*
Mueller-Heubach, Eberhard, *Pittsburgh, Pennsylvania*
Muller, Miklos, *New York, New York*
Munsick, Robert, *Indianapolis, Indiana*
Murad, Tariq M., *Chicago, Illinois*
Murata, Yuji, *Orange, California*
Murphy, Beverly, *Montreal, Quebec*
Myatt, L., *Cincinnati, Ohio*
Myers, Stephen A., *Chicago, Illinois*
Naeye, Richard L., *Hershey, Pennsylvania*
Naftolin, Frederick, *New Haven, Connecticut*
Nageotte, Michael, *Long Beach, California*
Nagi, Saad, *Columbus, Ohio*
Nahhas, William, *Dayton, Ohio*
Nakamura, Robert M., *Los Angeles, California*
Nelson, D. Michael, *St. Louis, Missouri*
Nelson, George H., *Columbia, South Carolina*
Nelson, Karin B., *Bethesda, Maryland*
Nelson, Lewis W., *Winston-Salem, North Carolina*
Nesbitt, John, *Columbus, Ohio*

Nestler, John, *Richmond, Virginia*
Newman, Roger B., *Charleston, South Carolina*
Newton, Niles, *Chicago, Illinois*
Nichols, David, *Providence, Rhode Island*
Nicolaides, Kypros H., *London, England*
Nicoloff, Ruth L., *Los Angeles, California*
Niebyl, Jennifer R., *Iowa City, Iowa*
Niswander, Kenneth R., *Davis, California*
Nochimson, David J., *Farmington, Connecticut*
Noller, Kenneth L., *Worcester, Massachusetts*
Notelovitz, Morris, *Gainesville, Florida*
Novy, Miles, *Beaverton, Oregon*
Nuovo, Gerard J., *New York, New York*
O'Connor, Vincent J., *Chicago, Illinois*
O'Shaughnessy, Richard W., *Columbus, Ohio*
O'Sullivan, Mary Jo, *Miami, Florida*
O'Toole, Robert, *Columbus, Ohio*
Ober, Carole, *Chicago, Illinois*
Oh, William, *Providence, Rhode Island*
Olive, David L., *Little Rock, Arkansas*
Olson, Sandra, *Chicago, Illinois*
Oski, Frank, *Baltimore, Maryland*
Ostergard, Donald R., *Long Beach, California*
Paganini-Hill, Annlia, *Los Angeles, California*
Paneth, Nigel, *New York, New York*
Pardi, Giorgio, *Milan, Italy*
Parer, Julian T., *San Francisco, California*
Parisi, Valerie M., *Houston, Texas*
Parker, C. Richard, *Birmingham, Alabama*
Parmley, Timothy H., *Little Rock, Arkansas*
Parsons, Anna, *Tampa, Florida*
Pastorek, Joseph, *New Orleans, Louisiana*
Patillo, Roland A., *Milwaukee, Wisconsin*
Patrick, John E., *London, Ontario, Canada*
Pattillo, Roland A., *Milwaukee, Wisconsin*
Paul, Richard H., *Los Angeles, California*
Pearse, Warren H., *Washington, D.C.*
Pearson, Jack W., *Tucson, Arizona*
Pennell, Rebecca G., *Philadelphia, Pennsylvania*
Pepe, Gerald J., *Norfolk, Virginia*
Perkins, Richard, *Honolulu, Hawaii*
Pernoll, Martin L., *Kansas City, Kansas*
Perrella, Rita, *Los Angeles, California*
Peters, Ruth K., *Los Angeles, California*
Peterson, Herbert B., *Atlanta, Georgia*
Petitti, Diana B., *San Francisco, California*
Petrie, Roy H., *St. Louis, Missouri*
Peyser, M. R., *Tel-Aviv, Israel*
Phelan, Jeffrey P., *West Covina, California*
Pitkin, Roy M., *Los Angeles, California*
Pittaway, Donald Edward, *Clemmons, North Carolina*
Platt, Lawrence D., *Los Angeles, California*
Podratz, Karl, *Rochester, Minnesota*
Pomerance, Jeffrey, *Los Angeles, California*
Pope, J. Terrell, *Macon, Georgia*
Porges, Robert, *New York, New York*
Porter, D. G., *Guelph, Ontario, Canada*
Porto, Manuel, *Orange, California*
Powers, David, *Orange, California*
Powers, Gordon, *Loma Linda, California*
Prager, Kate, *Hyattsville, Maryland*
Prem, Konald, *Minneapolis, Minnesota*
Pryor, W. A., *Baton Rouge, Louisiana*
Queenan, John T., *Washington, D.C.*
Quigley, Martin M., *Cleveland, Ohio*
Quilligan, E. J., *Orange, California*
Quirk, J. Gerald, *Little Rock, Arkansas*
Rankin, John H. G., *Madison, Wisconsin*
Ranney, Brooks, *Yankton, South Dakota*
Rayburn, William F., *Omaha, Nebraska*
Read, John A., *Tacoma, Washington*
Read, M. D., *Gloucester, England*
Reading, Anthony, *Los Angeles, California*
Rebar, Robert W., *Cincinnati, Ohio*
Redman, Christopher, *Oxford, England*
Reece, E. Albert, *New Haven, Connecticut*
Reed, Kathryn, *Tucson, Arizona*
Reid, Richard, *Detroit, Michigan*
Reid, Robert L., *Kingston, Ontario, Canada*
Reindollar, Richard H., *Boston, Massachusetts*
Reiss, Rosemary E., *Columbus, Ohio*
Renkin, Eugene M., *Davis, California*
Resnik, Robert, *San Diego, California*
Rettenmaier, Mark A., *Newport Beach, California*
Reynolds, Telfer, *Los Angeles, California*
Richardson, Bryan, *London, Ontario, Canada*
Richardson, David A., *Detroit, Michigan*
Richart, Ralph M., *New York, New York*
Riddick, Daniel, *Burlington, Vermont*
Rightmire, Daniel A., *Springfield, Illinois*
Roberts, Daniel, *Wichita, Kansas*
Roberts, James M., *San Francisco, California*
Rock, John A., *Baltimore, Maryland*
Romero, Roberto, *New Haven, Connecticut*
Rose, James C., *Winston-Salem, North Carolina*
Rosen, Mortimer, *New York, New York*
Rosenfeld, Charles R., *Dallas, Texas*
Rosenfield, Allan, *New York, New York*
Rosenwaks, Zev, *New York, New York*
Rosner, Marvin A., *Chicago, Illinois*
Ross, Michael G., *Torrance, California*
Rothberg, Alan D., *Johannesburg, South Africa*
Rovner, Richard, *Chicago, Illinois*
Roy, Subir, *Los Angeles, California*
Rudolph, Abe, *San Francisco, California*
Rulin, Marvin C., *Pittsburgh, Pennsylvania*
Rurak, Danny W., *Vancouver, British Columbia, Canada*
Rutherford, A. F., *Hull, Humberside, England*
Ryan, Kenneth, *Boston, Massachusetts*
Sabbagha, Rudy E., *Chicago, Illinois*
Sachs, Benjamin P., *Boston, Massachusetts*
Sachs, Larry A., *Columbus, Ohio*
Saigel, Saroj, *Hamilton, Ontario, Canada*
Sakakini, Joseph, Jr., *El Paso, Texas*
Saldana-Gautier, Luis R., *Cincinnati, Ohio*
Saling, E., *Berlin, West Germany*
Samaan, Naguib, *Houston, Texas*
Samuels, Bernard, *Metairie, Louisiana*
Samuels, Philip, *Overbrookhills, Pennsylvania*
Sandberg, Finn, *Uppsala, Sweden*

Sanders, Christine C., *Omaha, Nebraska*
Sands, Peter, *Chicago, Illinois*
Sarto, Gloria E., *Albuquerque, New Mexico*
Scanlan, John, *Washington, D.C.*
Schaefer, George, *La Mesa, California*
Schifrin, Barry, *Tarzana, California*
Schlaerth, John B., *Los Angeles, California*
Schmidt, Grant, *Columbus, Ohio*
Schmidt-Sommerfeld, Eberhard, *Chicago, Illinois*
Schneider, Jack M., *Sacramento, California*
Schreiber, James, *Chicago, Illinois*
Schriock, Eldon, *Memphis, Tennessee*
Schulman, Harold, *Mineola, New York*
Schulman, Joseph D., *Fairfax, Virginia*
Schwartz, Marshall, *Sacramento, California*
Schwartz, Peter E., *New Haven, Connecticut*
Schwartz, Robert, *Providence, Rhode Island*
Scommegna, Antonio, *Chicago, Illinois*
Scott, James R., *Salt Lake City, Utah*
Seeds, John W., *Chapel Hill, North Carolina*
Segars, James, *Nashville, Tennessee*
Shah, Dinesh M., *Nashville, Tennessee*
Shah, K. V., *Baltimore, Maryland*
Shangold, Gary A., *Chicago, Illinois*
Shangold, Mona, *Washington, D.C.*
Shanklin, Douglas, *Memphis, Tennessee*
Shapiro, Sam, *Baltimore, Maryland*
Shenker, Lewis, *Tucscon, Arizona*
Sher, Geoffrey, *San Francisco, California*
Sherline, Donald M., *Augusta, Georgia*
Shime, J., *Toronto, Ontario, Canada*
Shingleton, Hugh M., *Birmingham, Alabama*
Shoupe, Donna, *Los Angeles, California*
Shulman, Sidney, *New York, New York*
Sibai, Baha, *Memphis, Tennessee*
Siiteri, Pentti, *San Francisco, California*
Siler-Khodr, Theresa M., *San Antonio, Texas*
Simpson, Joe Leigh, *Memphis, Tennessee*
Sjoberg, Nils Otto, *Malmo, Sweden*
Skinner, Donald G., *Los Angeles, California*
Smith, Helene S., *San Francisco, California*
Sobel, Jack D., *Philadelphia, Pennsylvania*
Socol, Michael L., *Chicago, Illinois*
Soderstrom, Richard M., *Seatile, Washington*
Sokol, Robert J., *Detroit, Michigan*
Solish, George I., *Brooklyn, New York*
Sonek, Jiri, *Columbus, Ohio*
Soules, Michael, *Seattle, Washington*
Spellacy, William N., *Tampa, Florida*
Speroff, Leon, *Portland, Oregon*
Spirtos, Nicholas Jack, *Akron, Ohio*
Spirtos, Nicola Michael, *Stanford, California*
Stafl, Adolf, *Milwaukee, Wisconsin*
Stallard, Richrd, *Columbus, Ohio*
Stanczyk, Frank Z., *Los Angeles, California*
Stanhope, R., *London, England*
Stark, Raymond, *New York, New York*
Starr, Arnold, *Irvine, California*
Steeper, Theresa Ann, *St. Paul, Minnesota*
Steinetz, B., *Tuxedo, New York*
Steisslinger, Hans Werner, *Baden, Federal Republic of Germany*
Stellato, Thomas A., *Cleveland, Ohio*
Stenchever, Morton, *Seattle, Washington*
Stevens, Vernon, *Columbus, Ohio*
Stewart, Donna E., *Toronto, Ontario, Canada*
Stirrat, Gordon, *Bristol, England*
Stone, Martin L., *Stony Brook, New York*
Stone, Sergio C., *Orange, California*
Stoughton, Richard B., *San Diego, California*
Strain, Lisa, *Edinburgh, Scotland*
Strassner, Howard, *Northbrook, Illinois*
Strauss, Jerome F., *Philadelphia, Pennsylvania*
Strickland, Daniel M., *Lackland AFB, Texas*
Stubblefield, Phillip G., *Portland, Maine*
Sullivan, Mary Jo, *Miami, Florida*
Sweet, Richard L., *San Francisco, California*
Symonds, E. Malcolm, *Nottingham, England*
Tabsh, Khalil M. A., *Los Angeles, California*
Taeusch, H. William, *Los Angeles, California*
Talalay, P., *Baltimore, Maryland*
Talbert, Luther M., *Chapel Hill, North Carolina*
Talbot, Christopher W., *Chicago, Illinois*
Tamura, Ralph K., *Chicago, Illinois*
Tatum, Howard James, *Atlanta, Georgia*
Taylor, Aubrey, *Mobile, Alabama*
Taylor, J. Bruce, *Charlotte, North Carolina*
Taylor, Peyton T., *Charlottesville, Virginia*
Temple, R. Henry, *Wilmington, North Carolina*
Teng, Nelson, *Stanford, California*
Teteris, Nicholas J., *Columbus, Ohio*
Thiede, Henry, *Rochester, New York*
Thomas, David J., *Omaha, Nebraska*
Thomason, Jessica Love, *Milwaukee, Wisconsin*
Thompson, Wm. Benbow, *Orange, California*
Thornburg, Kent L., *Portland, Oregon*
Thurnau, Gary, *Oklahoma City, Oklahoma*
Timor-Tritsch, Ilan E., *New York, New York*
Toomey, Kathleen E., *Atlanta, Georgia*
Townsend, Duane E., *Sacramento, California*
Tremper, Kevin K., *Orange, California*
Trudinger, Brian J., *Westmead, Australia*
Tseng, Linda, *Stony Brook, New York*
Tsibris, John C., *Tampa, Florida*
Tulenko, T. N., *Philadelphia, Pennsylvania*
Twiggs, Leo B., *Minneapolis, Minnesota*
Tyson, John, *Dallas, Texas*
Ueland, Kent, *Maui, Hawaii*
Underwood, Paul B., *Charlottesville, Virginia*
Utian, Wulf, *Moreland Hills, Ohio*
Valenzuela, Guillermo J., *Loma Linda, California*
Valle, Rafael F., *Chicago, Illinois*
Van Dorsten, James Peter, *Richmond, Virginia*
Van Nagell, John R., Jr., *Lexington, Kentucky*
Varner, Michael W., *Salt Lake City, Utah*
Verp, Marion S., *Chicago, Illinois*
Villar, Jose, *Geneva, Switzerland*
Vintzileos, Anthony, *Farmington, Connecticut*
Visser, G. H. A., *Groningen, The Netherlands*
Vorhees, C. V., *Cincinnati, Ohio*

Waffarn, Feizal, *Orange, California*
Waldstein, Sheldon, *Chicago, Illinois*
Walker, David W., *Melbourne, Australia*
Walker, Godfrey J. A., *Geneva, Switzerland*
Wallace, Louise, *West Midlands, England*
Wallach, Edward E., *Baltimore, Maryland*
Wallenburg, Henk C. S., *Rotterdam, The Netherlands*
Walsh, Scott, *Houston, Texas*
Warren, James, *St. Louis, Missouri*
Warsof, Steven L., *Norfolk, Virginia*
Wasmuth, John J., *Irvine, California*
Webster, James R., *Chicago, Illinois*
Wegmann, T., *Edmonton, Canada*
Wehmann, Robert E., *Bethesda, Maryland*
Weilander, Charles, *Atlanta, Georgia*
Weiner, Carl P., *Iowa City, Iowa*
Weingold, Allan B., *Washington, D.C.*
Weinstein, Gerald, *Irvine, California*
Weinstein, Louis, *Tucscon, Arizona*
Weiss, Gerson, *Newark, New Jersey*
Wendel, George D., *Dallas, Texas*
Wenstrom, Katharine, *Coralville, Iowa*
Wentz, Anne, *Chicago, Illinois*
Wheeless, Clifford, *Baltimore, Maryland*
White, Charles A., *New Orleans, Louisiana*
Whitehead, Malcolm, *London, England*
Whitman, Gail, *Simsbury, Connecticut*
Whittemore, Ruth, *New Haven, Connecticut*
Wied, George, *Chicago, Illinois*
Wilbanks, George D., *Chicago, Illinois*
Wile, Alan G., *Orange, California*
Williamson, H. Oliver, *Charleston, South Carolina*
Wilson, Emery A., *Lexington, Kentucky*
Winkel, Craig A., *Houston, Texas*
Winters, Chris J., *Little Rock, Arkansas*
Wolfe, Honor, *Detroit, Michigan*
Wolfson, Robert N., *Cleveland Heights, Ohio*
Woodruff, J. Donald, *Baltimore, Maryland*
Woods, James, *Cincinnati, Ohio*
Woods, James R., *Rochester, New York*
Worley, Richard J., *Denver, Colorado*
Worthington, Dennis, *Milwaukee, Wisconsin*
Yee, Bill, *Orange, California*
Yeh, Sze-Ya, *Allentown, Pennsylvania*
Yen, Samuel S. C., *La Jolla, California*
Ylikorkala, Olavi, *Helsinki, Finland*
Yonekura, Margaret Lynn, *Torrance, California*
Young, Bruce, K., *New York, New York*
Zackman, Richard D., *Madison, Wisconsin*
Zatuchni, Gerald I., *Chicago, Illinois*
Zuspan, Frederick P., *Columbus, Ohio*
Zuspan, Kathryn, *Edina, Minnesota*

Central hemodynamic assessment of normal term pregnancy

Steven L. Clark, MD, David B. Cotton, MD, Wesley Lee, MD, Clark Bishop, MD, Tracy Hill, MD, James Southwick, MD, James Pivarnik, PhD, Thomas Spillman, PhD, Greggory R. DeVore, MD, Jeffrey Phelan, MD, Gary D. V. Hankins, MD, Thomas J. Benedetti, MD, and Dennis Tolley, PhD

Provo, Utah, Houston and San Antonio, Texas, Los Angeles, California, and Seattle, Washington

Ten carefully screened primiparous patients between 36 and 38 weeks' gestation underwent pulmonary artery catheterization, arterial line placement, and central hemodynamic assessment in the left lateral recumbent position. Studies were repeated in the same patients between 11 and 13 weeks post partum. Compared with the nonpregnant state, there was a significant fall in systemic vascular resistance, pulmonary vascular resistance, colloid oncotic pressure, and colloid oncotic pressure–pulmonary capillary wedge pressure gradient by the late phase of the third trimester ($p < 0.05$). Pregnancy was associated with a significant rise in cardiac output and pulse in all patients ($p < 0.05$). There was no significant change in pulmonary capillary wedge pressure, central venous pressure, left ventricular stroke work index, or mean arterial pressure. Normally the late phase of the third trimester is not associated with hyperdynamic left ventricular function as assessed by the left ventricular stroke work index/pulmonary capillary wedge pressure ratio. (AM J OBSTET GYNECOL 1989;161:1439-42.)

Key words: Pregnancy, pulmonary artery catheter, critical care

During the past decade there has been a rising interest in the hemodynamic assessment of critically ill pregnant patients.[1-11] Whereas much has been documented with regard to the central hemodynamic profile of patients with severe preeclampsia, cardiac disease, and septic shock, interpretation of this information has been hampered by a lack of data that describe the central hemodynamic profile of the "normal" pregnant patient. Early work by both Bader and Ueland[12-14] involved the study of nonuniform patient populations with the use of investigational techniques rarely used in clinical practice today. Thus direct comparisons between their normal populations and recent investigations of critically ill pregnant patients may not be completely valid. This study was designed to define the central hemodynamic profile of a uniform population of normal third-trimester pregnant patients with contemporary clinical methods of hemodynamic assessment.

From the Utah Valley Regional Perinatal Center, Brigham Young University, Baylor College of Medicine, Queen of Angels Hospital, Wilford Hall United States Air Force Base, and the University of Washington.

Supported in part by a grant from Baxter Healthcare Corporation.

Presented at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.

Reprint requests: Steven L. Clark, MD, 1034 N. 500 W., Provo, UT 84604.

6/6/15665

Material and methods

Ten volunteers were studied. Each met criteria detailed in Table I.

Written informed consent was obtained. The study was approved by the hospital Institutional Review Board and an outside reviewer (pulmonologist/intensivist). Patients were reimbursed $200.00 for participation in each phase (pregnant and nonpregnant) of the study. Four patients who volunteered were excluded before initiation of the study because of borderline anemia, fetal intrauterine growth retardation, or the development of elevated blood pressure before the study. Patients were initially recruited from physicians' offices in Provo, Utah. The study was performed at the Utah Valley Regional Medical Center in Provo.

Each patient underwent pulmonary artery catheterization via the subclavian route and radial arterial line placement. Venous access was established by means of a peripheral heparin lock venous catheter. Baseline hemodynamic assessment was made with patients in the left lateral recumbent position after a 30-minute stabilization period. Cardiac output was measured with

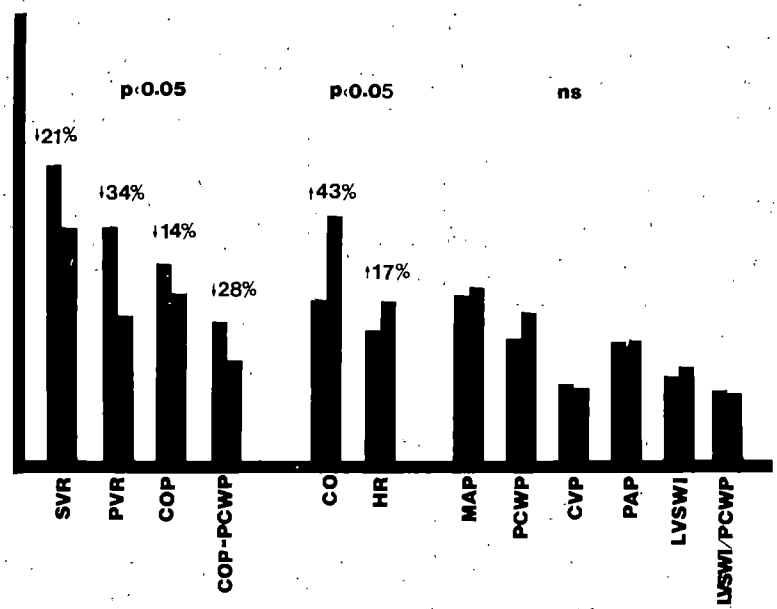


Fig. 1. Relative hemodynamic changes in pregnancy: nonpregnant versus late phase of third-trimester. *SVR*, systemic vascular resistance; *PVR*, pulmonary vascular resistance; *COP*, colloid oncotic pressure; *PCWP*, pulmonary capillary wedge pressure; *CO*, cardiac output; *HR*, heart rate; *MAP*, mean arterial pressure; *CVP*, central venous pressure; *PAP*, pulmonary artery pressure; *LVSWI*, left ventricular stroke work index.

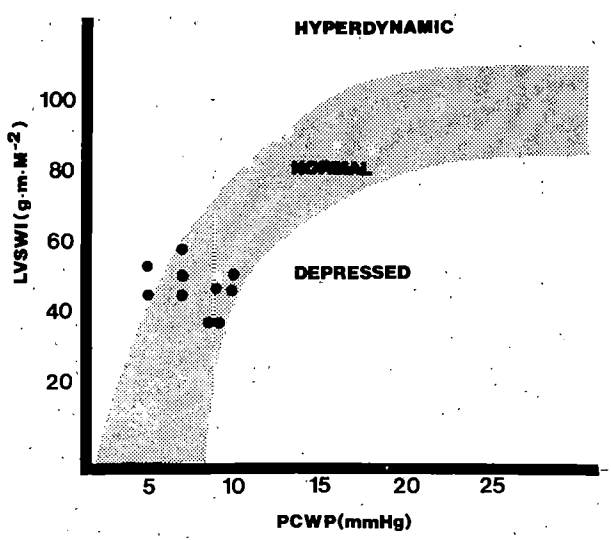


Fig. 2. Left ventricular function in late phase of third-trimester normotensive pregnant patients. *PCWP*, Pulmonary capillary wedge pressure; *LVSWI*, left ventricular stroke work index.

standard thermodilution technique with saline solution at 0° C (Cardiac output computer, American Edwards 18055 A, Santa Ana, Calif.). Reported cardiac outputs represent the mean of three of five independent measurements with high and low values discarded. Central pressures were measured and recorded with a 10-channel physiologic monitor-recorder (Hewlett Packard 7785 B, Waltham, Mass.). Central pressures represent the mean of three consecutive respiratory cycles as measured from the tracing. Monitors were re-zeroed in each position with transducer placement at the level of the left atrium confirmed by simultaneous real-time maternal echocardiography. Studies were repeated in each patient between 11 and 13 weeks post partum.

Statistical analysis was performed with a paired two-tailed Student *t* test.

Results

Table II presents the mean hemodynamic profiles for patients between 36 and 38 weeks' gestation and at 11 to 13 weeks post partum. We found the late phase of the third trimester to be associated with a 43% increase in cardiac output (4.3 ± 0.9 L/min to 6.2 ± 1.0 L/min), a 17% increase in pulse (71 ± 10.0 beats/min to 83 ± 10.0 beats/min), a 21% decline in systemic vascular resistance (1530 ± 520 dyne · cm · sec^{-5} to 1210 ± 266 dyne · cm · sec^{-5}), a 34% decline in pulmonary vascular resistance (119 ± 47 dyne · cm · sec^{-5} to 78 ± 22 dyne · cm · sec^{-5}), a 14% decline in colloid oncotic pressure (20.8 ± 1.0 mm Hg to 18.0 ± 1.5 mm Hg), and a 28% decline in colloid oncotic pressure–pulmonary capillary wedge pressure gradient (14.5 ± 2.5 mm Hg to 10.5 ± 2.7 mm Hg) ($p < 0.05$) (Fig. 1). There was no significant change between nonpregnant and third-trimester values with respect to mean arterial pressure, central venous pressure, pulmonary capillary wedge pressure, or left ventricular stroke work index.

Table I. Study inclusion criteria

1. Primiparous
2. Maternal age < 26 years
3. Singleton vertex fetus
4. Gestational age between 36 and 38 weeks confirmed by ultrasonography
5. No fetal intrauterine growth retardation or anomalies
6. Normal amniotic fluid volume
7. Normal maternal weight for height
8. Normal maternal hematocrit
9. No maternal alcohol, tobacco, or drug use
10. No maternal hypertension, medical illness, or medications

Table II. Central hemodynamic changes

	Nonpregnant	*Pregnant*
Cardiac output (L/min)	4.3 ± 0.9	6.2 ± 1.0
Heart rate (beats/min)	71 ± 10.0	83 ± 10.0
Systemic vascular resistance (dyne · cm · sec^{-5})	1530 ± 520	1210 ± 266
Pulmonary vascular resistance (dyne · cm · sec^{-5})	119 ± 47.0	78 ± 22
Colloid oncotic pressure (mm Hg)	20.8 ± 1.0	18.0 ± 1.5
Colloid oncotic pressure–pulmonary capillary wedge pressure (mm Hg)	14.5 ± 2.5	10.5 ± 2.7
Mean arterial pressure (mm Hg)	86.4 ± 7.5	90.3 ± 5.8
Pulmonary capillary wedge pressure (mm Hg)	6.3 ± 2.1	7.5 ± 1.8
Central venous pressure (mm Hg)	3.7 ± 2.6	3.6 ± 2.5
Left ventricular stroke work index (g · m · m^{-2})	41 ± 8	48 ± 6

Comment

More than 30 years ago Bader et al.[12] first described the hemodynamic profile of pregnant patients. Their study design differed from ours in several respects. First, there was no control for age, parity, smoking, maternal hematocrit, or obesity. Fetal age and appropriate growth were not documented. Further, 4 of 11 patients studied in the late third trimester had a mean arterial pressure ≥105 mm Hg. Cardiac output was assessed with the Fick principle. In subsequent studies by Ueland et al.[13, 14] central hemodynamic assessment was limited to measurement of central venous pressure, and cardiac output was assessed with a dye-dilution technique rarely used in clinical practice today. Thus, in these studies, patient populations were not strictly controlled and hemodynamic assessment was performed with older techniques not suitable for direct comparison with critically ill obstetric patients managed with pulmonary artery catheterization and thermodilution cardiac output assessment.

In the past decade numerous reports have appeared in the medical literature that document the central hemodynamic profiles of pregnant patients with a variety of pathologic conditions including pregnancy-induced hypertension, structural cardiac disease, amniotic fluid embolism, and septic shock.[1-11] Proper interpretation of such data is predicated upon knowledge of normal values. In the absence of such data, investigators heretofore have been forced to make assumptions with regard to what is normal on the basis of either the earlier studies with their methodologic limitations or data collected in nonpregnant subjects. We sought to correct this deficiency by designing a study that involved carefully screened normal pregnant patients and current clinical techniques with each patient serving as her own control during the postpartum period.

We documented a mean rise in cardiac output of 43% by the late phase of the third trimester in the lateral recumbent position ($p < 0.05$), as a result of significant changes in pulse (17%) and stroke volume (27%), the latter secondary to a previously documented increase in intravascular volume during pregnancy.[18] Both figures are in general agreement with measurements obtained by other techniques.[12, 13] We observed a significant fall in systemic vascular resistance (21%) and a more dramatic decrease in pulmonary vascular resistance (34%) ($p < 0.05$). Changes in pulmonary vascular resistance in pregnancy have not previously been described. Such alterations are not surprising and demonstrate increased vascular reactivity in the pulmonary circulation. In contrast, we documented no significant difference in intrinsic left ventricular contractility as assessed by left ventricular stroke work index. Such contractility when corrected for potential differences in Starling forces is assessed by the ratio left ventricular stroke work index/pulmonary capillary wedge pressure (Fig. 2). Thus normal pregnancy is not associated with hyperdynamic left ventricular function. These results confirm earlier observations obtained with noninvasive techniques.[16]

In general, a higher stroke volume (ventricular end-diastolic volume) should be reflected in a higher end-diastolic pressure; this assumption is implicit in the clinical use of the pulmonary capillary wedge pressure as an approximation to left ventricular end-diastolic volume. However, no increase in pulmonary capillary wedge pressure was found during pregnancy, as compared with the nonpregnant state. The explanation for this apparent paradox is ventricular dilatation, which accounts for the well-known increase in cardiac silhouette seen on x-ray film of the chest during pregnancy. The failure of pulmonary capillary wedge pressure or central venous pressure to increase in pregnant patients in the face of marked increases in intravascular volume also reflects the decreases in systemic vascular resistance and pulmonary vascular resistance, which allow both systemic and pulmonary vasculature to accommodate higher volumes at normal vascular pressures.

A pregnancy-associated fall in colloid oncotic pressure has previously been documented, and was con-

firmed by this study. Our documentation of a significant fall in the colloid oncotic pressure–pulmonary capillary wedge pressure gradient implies a greater propensity to pulmonary edema during pregnancy in the presence of any alteration in pulmonary capillary permeability or increase in cardiac preload.

The establishment for the first time of central hemodynamic parameters in the normal term primigravid patient has important research and clinical implications. It is anticipated that such information may facilitate the management of critically ill pregnant patients.

REFERENCES

1. Benedetti TJ, Cotton DB, Read JC, Miller FC. Hemodynamic observations in severe preeclampsia with a flow directed pulmonary artery catheter. Am J Obstet Gynecol 1980;136:465-70.
2. Hankins GDV, Wendel GD, Cunningham FG, Leveno KJ. Longitudinal evaluation of hemodynamic changes in eclampsia. Am J Obstet Gynecol 1984;150:506-12.
3. Clark SL, Divon MY, Phelan JP. Preeclampsia/eclampsia: hemodynamic and neurologic correlations. Obstet Gynecol 1985;66:337-40.
4. Clark SL, Horenstein JM, Phelan JP, Montag TW, Paul RH. Experience with the pulmonary artery catheter in obstetrics and gynecology. Am J Obstet Gynecol 1985; 152:374-8.
5. Clark SL, Greenspoon J, Phelan JP, Aldahl D, Horenstein J. Labor and delivery in the pressure of mitral stenosis—central hemodynamic observations. Am J Obstet Gynecol 1985;152:984-8.
6. Clark SL, Greenspoon JS, Aldahl D, Phelan JP. Severe preeclampsia with persistent oliguria: management of hemodynamic subsets. Am J Obstet Gynecol 1986;154: 490-4.
7. Cotton DB, Longmire S, Jones MM, Dorman KF, Tessem J, Joyce TH. Cardiovascular alterations in severe pregnancy-induced hypertension: effects of intravenous nitroglycerine coupled with blood volume expansion. Am J Obstet Gynecol 1986;154:1053-9.
8. Clark SL, Cotton DB, Gonik B, Greenspoon J, Phelan JP. Central hemodynamic alterations in amniotic fluid embolism. Am J Obstet Gynecol 1988;158:1124-6.
9. Clark SL, Cotton CB. Clinical indications for pulmonary artery catheterization in severe pregnancy-induced hypertension. Am J Obstet Gynecol 1988;158:453-8.
10. Cotton DB, Lee W, Huhta JC, Dorman KF. Hemodynamic profile of severe pregnancy-induced hypertension. Am J Obstet Gynecol 1988;158:523-9.
11. Lee WP, Clark SL, Cotton DB, et al. Septic shock in pregnancy. Am J Obstet Gynecol 1988;159:410-6.
12. Bader RA, Bader ME, Rose DJ, Braunwald E. Hemodynamics of rest and exercise in normal pregnancy as studied by cardiac catheterization. J Clin Invest 1955;34:1524-33.
13. Ueland K, Hansen JM. Maternal cardiovascular dynamics. III. Labor and delivery under local and caudal analgesia. Am J Obstet Gynecol 1969;103:8-18.
14. Ueland K, Hansen J, Eng M, Kalappa R, Parer JT. Maternal cardiovascular dynamics. V. Cesarean sections under thiopental, nitrous oxide, and succinylcholine anesthesia. Am J Obstet Gynecol 1970;108:615-22.
15. Pritchard JA. Changes in the blood volume during pregnancy and delivery. Anesthesiology 1965;26:393-9.
16. Katz R, Karliner J, Resnik R. Effects of a natural volume overload state (pregnancy) on left ventricular performance in normal human subjects. Circulation 1978;58: 434-40.

The central hemodynamics of severe preeclampsia

William C. Mabie, MD, Thomas E. Ratts, MD, and Baha M. Sibai, MD
Memphis, Tennessee

Swan-Ganz hemodynamic monitoring in 49 antepartum patients with severe preeclampsia revealed a variable hemodynamic profile. The majority of patients had normal left ventricular filling pressure (8.4 ± 0.2 mm Hg), normal to high cardiac index (4.4 ± 0.1 L · min^{-1} · m^2), and upper normal to moderately elevated systemic vascular resistance (1226 ± 37 dynes · sec · cm^{-5}). Eight patients had pulmonary edema and their findings included high wedge pressure (18 ± 1 mm Hg), upper normal to high cardiac index (4.9 ± 0.5 L · min^{-1} · m^2), and normal systemic vascular resistance (964 ± 50 dynes · sec · cm^{-5}). Left ventricular function was hyperdynamic in 73% of the 49 patients. Patients with chronic hypertension and superimposed preeclampsia were hemodynamically indistinguishable from patients with preeclampsia alone. We conclude that, in general, preeclampsia is a high cardiac output state associated with an inappropriately high peripheral resistance. The normal wedge and central venous pressures suggest central redistribution of intravascular volume if the generally accepted reports of decreased plasma volume in preeclampsia are correct. (Am J Obstet Gynecol 1989;161:1443-8.)

Key words: Preeclampsia, Swan-Ganz catheter, hemodynamic monitoring

The cardiovascular hemodynamics of preeclampsia have been reported to range from a low-output, high-resistance state to a high-output, low-resistance state. Hankins et al.[1] reviewed the six published studies and classified them according to therapy before insertion of the Swan-Ganz catheter. In the group without therapy there was disagreement. Cotton et al.[2] reported a high pulmonary capillary wedge pressure, high cardiac output, and minimally elevated systemic vascular resistance, whereas Groenendijk et al.[3] found a low pulmonary capillary wedge pressure, low cardiac output, and high systemic vascular resistance. In the group with fluid restrictions Benedetti et al.[4] found a normal pulmonary capillary wedge pressure, elevated cardiac output, and elevated systemic vascular resistance, whereas Hankins et al.[1] reported slightly different findings—a low pulmonary capillary wedge pressure, normal cardiac output, and elevated systemic vascular resistance. In the two studies in which there was liberal fluid administration, pulmonary capillary wedge pressure was normal to high, cardiac output was high, and systemic vascular resistance was normal to low in some patients.[5, 6]

Proposed explanations for the differences in the various hemodynamic studies have included: (1) small number of patients in each series (usually ≤10), (2) variable severity and duration of preeclampsia, (3) underlying medical problems such as cardiac or renal disease, and (4) therapeutic interventions before invasive monitoring. In addition, the dynamic fluctuation of the cardiovascular system makes it difficult to control for all the variables that can affect hemodynamics (e.g., gestational age, labor, pain, anxiety, hydralazine, intravenous fluids, analgesics, and anesthesia).

As a practical point, patients are often transferred to a tertiary care center several hours after initial therapy has been started. In addition, the clinical diagnosis of preeclampsia is often suspect, particularly in patients remote from term when underlying renal disease is likely.[7] In light of the heterogeneous circumstances under which these patients arrive at a referral center, we have assessed the hemodynamic profile of 49 patients first seen with severe preeclampsia. The purpose of our study was to determine whether there is a typical hemodynamic profile of patients with severe preeclampsia that requires delivery and to determine the frequency with which patients lie at the extremes of the hemodynamic spectrum.

Patients and methods

The study group consisted of 49 patients with severe preeclampsia who were managed at the E. H. Crump Women's Hospital between January 1, 1986, and March 1, 1988. The study was approved by the Institutional Review Board of the University of Tennessee, Memphis. A total of 38 patients had a clinical diagnosis of preeclampsia and 11 had a clinical diagnosis of chronic hypertension with superimposed preeclampsia. Criteria for entry to the study were (1) singleton pregnancy; (2) severe preeclampsia as evidenced by all three of the

From the Department of Obstetrics and Gynecology and the Cardiology Section of the Department of Internal Medicine, University of Tennessee, Memphis.
Presented in part at the Thirty-fifth Annual Meeting of the Society for Gynecologic Investigation, Baltimore, Maryland, March 17-20, 1988.
Reprint requests: William C. Mabie, MD, Department of Obstetrics and Gynecology, 853 Jefferson Ave., E-102, Memphis, TN 38103.
6/6/16511

Table I. Comparison of the hemodynamic profile of preeclamptic patients in this study versus the study of Cotton et al.

	*Mabie et al. (n = 41)**		*Cotton et al. (n = 45)†*	
	Mean ± SE	*Range*	*Mean ± SE*	*Range*
Heart rate (beats/min)	94 ± 2	(65-122)	95 ± 2	(69-129)
Systolic blood pressure (mm Hg)‡	175 ± 3	(132-219)	193 ± 3	(150-236)
Diastolic blood pressure (mm Hg)	106 ± 2	(74-130)	110 ± 2	(65-140)
Mean arterial pressure (mm Hg)‡	130 ± 2	(100-155)	138 ± 3	(97-171)
Pulse pressure (mm Hg)‡	70 ± 2	(36-97)	84 ± 2	(48-112)
Mean pulmonary artery pressure (mm Hg)	15 ± 0.5	(8-22)	17 ± 1	(8-34)
Pulmonary capillary wedge pressure (mm Hg)	8.3 ± 0.3	(3-13)	10 ± 1	(4-30)
Central venous pressure (mm Hg)	4.8 ± 0.4	(0-10)	4 ± 1	(0-12)
Cardiac output (L/min)‡	8.4 ± 0.2	(6.0-12.1)	7.5 ± 0.2	(4.4-10.6)
Cardiac index ($L \cdot min^{-1} \cdot m^2$)‡	4.4 ± 0.1	(3.1-6.2)	4.1 ± 0.1	(2.4-5.9)
Stroke volume (ml)‡	90 ± 2	(64-123)	79 ± 2	(44-113)
Stroke index ($ml \cdot beat \cdot m^2$)‡	48 ± 1	(33-65)	44 ± 1	(24-65)
Systemic vascular resistance ($dynes \cdot sec \cdot cm^{-5}$)‡	1226 ± 37	(852-1900)	1496 ± 64	(716-2734)
System vascular resistance index ($dynes \cdot sec \cdot cm^{-5} \cdot m^2$)‡	2293 ± 65	(1637-3336)	2726 ± 120	(1146-4996)
Pulmonary vascular resistance	65 ± 3	(16-109)	70 ± 5	(22-170)
Pulmonary vascular resistance index ($dynes \cdot sec \cdot cm^{-5}$)	121 ± 7	(33-207)	127 ± 9	(43-304)
Right ventricular stroke work index ($gm \cdot m \cdot m^2$)	10 ± 0.5	(4-17)	8 ± 1	(3-17)
Left ventricular stroke work index ($gm \cdot m \cdot m^2$)	84 ± 2	(55-117)	81 ± 2	(51-122)

*Eight patients with pulmonary edema were excluded.

†Included two patients with florid pulmonary edema.

‡ Statistically significant differences in means between the two groups.

Table II. Range of hemodynamic parameters in patients without pulmonary edema ($n = 41$)

	Low	*Normal*	*High*
Pulmonary capillary wedge pressure (mm Hg)	<6	6-12	>12
Number (%)	3 (7)	36 (88)	2 (5)
Cardiac index ($L \cdot min^{-1} \cdot m^2$)	<3	3-5	>5
Number (%)	0 (0)	33 (80)	8 (20)
Systemic vascular resistance ($dynes \cdot sec \cdot cm^{-5}$)	<800	800-1200	>1200
Number (%)	0 (0)	18 (44)	23 (56)

following: nondependent edema, diastolic blood pressure >110 mm Hg on two occasions 30 minutes apart, and 3+ to 4+ proteinuria by urine dipstick; and (3) a clinical decision to deliver.

All patients received intravenous magnesium sulfate given as a 6 gm loading dose over 20 minutes followed by a constant infusion of 2 to 3 gm per hour. Intravenous fluid consisted of 5% dextrose in lactated Ringer's solution adjusted to give a total fluid administration rate of 75 to 100 ml per hour. A total of 22 patients (45%) were in early labor, defined as cervical dilatation <4 cm, and 16 (33%) had received hydralazine within the preceding 7 hours (mean, 4.5 hours) before Swan-Ganz catheter insertion.

After written informed consent was obtained, a 20 gauge catheter (Arrow Corp.) was inserted into a radial artery for continuous blood pressure monitoring. After the administration of 25 to 50 mg of meperidine intravenously and 2 to 3 ml of 1% lidocaine locally, an 8.5F introducer (Arrow Corp.) was inserted into the right internal jugular vein. A 7.5F Swan-Ganz catheter (American Edwards Laboratories) was then positioned in the pulmonary artery and its location was documented by pressure waveform and roentgenogram of the chest. Pressures were measured with disposable transducers (Gould Corp.) with zero reference at the midchest level. Patients were positioned supine with a wedge under the right hip to prevent aortocaval compression. Electrocardiogram, blood pressure, and pulmonary artery pressure were continuously monitored with a multichannel scope (Hewlett-Packard model 78532B). Measurements of pulmonary artery and wedge pressures were obtained at end expiration from a strip chart recorder (Hewlett-Packard model 78572B). Cardiac output was measured by a thermodilution cardiac output computer (Hewlett-Packard model 78552A) with five (10 ml) injections of iced saline solution. The high and low values were discarded and the mean of the three remaining values was recorded. Hemodynamic measurements were made at least 30 minutes after insertion of the catheter to allow the patient to reach a steady state. The Swan-Ganz catheter was removed within 24 hours of insertion to decrease the risk of complications. The measured hemodynamic variables were heart rate, blood pressure, pulmonary artery pressure, pulmonary capillary wedge pressure, central venous pressure, and cardiac output. Computer-derived variables included cardiac index,

stroke volume and index, systemic vascular resistance and index, pulmonary vascular resistance and index, and left and right ventricular stroke work and indices. Values are reported as mean ± SE unless otherwise stated. Data were analyzed by the Student unpaired t test with significance levels taken at $p < 0.05$.

Results

The mean maternal age was 23.6 ± 1 years (range, 15 to 38). The mean gravidity was 2.2 ± 0.3 (range, 1 to 10) and the mean parity was 0.8 ± 0.2 (range, 0 to 6). The mean gestational age was 32.4 ± 0.5 weeks (range, 27 to 40). The mean birth weight was 1700 ± 112 gm; 10 babies (20%) weighed <1000 gm. Nine babies (18.4%) were growth-retarded.

A total of 30 patients (61%) were black. Four patients (8%) had eclampsia and eight patients (16%) had pulmonary edema. There were 27 patients (55%) who were maternal transfers from outside hospitals. A total of 41 (84%) were delivered of infants by cesarean section.

Abnormal laboratory values in the 49 study patients on admission were as follows: hematocrit <33% in 11 patients, serum creatinine >1.0 mg/dl in 12 patients, serum uric acid >6.0 mg/dl in 37 patients, serum alanine aminotransferase >40 U/L in 18 patients, serum albumin <3.2 gm/dl in 30 patients, and 24-hour urine protein >5 gm in 16 patients.

The eight patients with pulmonary edema on admission had clearly elevated pulmonary artery and wedge pressures and were therefore analyzed separately. When patients with pulmonary edema were excluded there was no significant difference in any hemodynamic parameter between patients with preeclampsia and those with chronic hypertension and superimposed preeclampsia. The hemodynamic parameters of the 41 patients without pulmonary edema are shown in Table I.

Although the normal hemodynamic parameters for pregnancy have not been clearly established, we selected the following ranges of normal on the basis of the results of others[3, 5-10]: (1) pulmonary capillary wedge pressure of 6 to 12 mm Hg, (2) cardiac index of 3 to 5 $L \cdot min^{-1} \cdot m^2$, and (3) systemic vascular resistance of 800 to 1200 $dynes \cdot sec \cdot cm^{-5}$. The number and percentage distribution of the study population in each of the categories are shown in Table II. With the use of these parameters 88% of the patients had a normal left ventricular filling pressure with only 7% low and 5% high. A total of 80% of patients had a normal cardiac index and the remaining patients had a high cardiac index. Approximately one half the patients had a normal systemic vascular resistance and the others had an elevated systemic vascular resistance.

The hemodynamic findings in the eight patients with pulmonary edema are shown in Table III. All had elevated wedge pressures, one half had normal and one half had elevated cardiac indices, and all had normal or low systemic vascular resistance. They were a heterogeneous group. Four were obese multiparous women over 30 years of age with chronic hypertension and superimposed preeclampsia whose pulmonary edema was multifactorial. These factors included left ventricular dilatation and hypertrophy, diastolic dysfunction as a result of impaired ventricular relaxation, and volume overload because of salt and water retention during the last weeks of pregnancy. A fifth patient was similar but also had echocardiographic evidence of systolic dysfunction. The sixth patient was a 17-year-old primigravida with worsening preeclampsia managed with escalating dosages of methyldopa and hydralazine in an outside hospital. The other two patients had been given copious amounts of fluid and blood because of oliguria during labor.

Table III. Hemodynamic profile of the eight patients with pulmonary edema

Variable	*Measurement*
Heart rate (beats/min)	106 ± 5
Mean arterial pressure (mm Hg)	136 ± 3
Mean pulmonary artery pressure (mm Hg)	27 ± 2
Pulmonary capillary wedge pressure (mm Hg)	18 ± 1
Central venous pressure (mm Hg)	11 ± 1
Cardiac output (L/min)	10.5 ± 0.6
Cardiac index ($L \cdot min^{-1} \cdot m^2$)	4.9 ± 0.5
Stroke volume (ml)	97 ± 7
Systemic vascular resistance ($dynes \cdot sec \cdot cm^{-5}$)	964 ± 50
Pulmonary vascular resistance ($dynes \cdot sec \cdot cm^{-5}$)	71 ± 9
Left ventricular stroke work index ($gm \cdot m \cdot m^2$)	87 ± 10

Values are mean ± SD.

Ventricular function as shown by the Starling plot of stroke work index versus pulmonary capillary wedge pressure in Fig. 1 was shifted upward and to the left, illustrating hyperdynamic function in 73% of the patients. In the subjects with pulmonary edema, function was normal in four, hyperdynamic in three, and depressed in the one subject who had echocardiographic evidence of systolic dysfunction.

Because our population was heterogeneous, we examined the hemodynamic variables in specific subsets. Sixteen patients treated with hydralazine were compared with 33 untreated patients. No differences in hemodynamics were found. Twenty-two patients in the latent phase of labor were compared to 27 patients not in labor. Small differences were found in heart rate (101 ± 2 versus 91 ± 3 beats/min, respectively, $p < 0.1$) and cardiac index (4.9 ± 0.2 versus 4.2 ± 0.1 $L \cdot min^{-1} \cdot m^2$, $p < 0.01$). Similar comparisons between

Table IV. Comparison of the hemodynamic profile of preeclamptic patients in this study versus the study of Wallenburg et al.

Variable	*Mabie et al. (n = 15)*		*Wallenburg et al. (n = 44)*	
	Median	*Range*	*Median*	*Range*
Heart rate (beats/min)	88	65-110	90	60-130
Mean arterial pressure (mm Hg)	126	100-150	120	95-143
Mean pulmonary artery pressure (mm Hg)	16	8-22	9	3-18
Pulmonary capillary wedge pressure (mm Hg)	8	3-13	4	0-13
Central venous pressure (mm Hg)	5	0-10	1	−2 to 6
Cardiac index ($L \cdot min^{-1} \cdot m^2$)	4.1	3.1-5.2	3	2.0-4.7
Stroke index ($ml \cdot beat \cdot m^2$)	45	33-65	35	20-57
System vascular resistance index ($dynes \cdot sec \cdot cm^{-5} \cdot m^2$)	2416	1660-3310	2970	2100-4585
Pulmonary vascular resistance index ($dynes \cdot sec \cdot cm^{-5}$)	122	62-207	82	25-133

patients received in transfer and those directly admitted did not reveal differences in hemodynamics. Last, we examined a subset of 15 patients from the group of 41 patients without pulmonary edema. The patients were nulliparous, not in labor, without preexisting hypertension, cardiac or renal disease, and had not received therapy other than magnesium sulfate and small amounts of intravenous fluid. Their heart rates (87 ± 3 versus 98 ± 2 beats/min, $p < 0.01$) and cardiac indices (4.1 ± 0.1 versus 4.7 ± 0.1 $L \cdot min^{-1} \cdot m^2$, $p < 0.05$) were slightly but significantly lower than those of the remaining 26 patients. However, there were no differences in their filling pressures (8.9 ± 0.6 versus 7.9 ± 0.4 mm Hg).

Comment

The results of this study demonstrate a spectrum of hemodynamic alterations in severe preeclampsia with the majority of patients having a normal pulmonary capillary wedge pressure, high cardiac output, and normal to moderately elevated systemic vascular resistance. These findings correlate with the physical examination of the patients who usually had tachycardia, bounding pulses, wide pulse pressure, a hyperdynamic precordium, systolic flow murmur, and warm extremities. The hemodynamics of patients with antecedent hypertension were similar to patients without prior hypertension. This observation has been reported by others.[11] There was significant variability in the cardiac output, ranging from 6.0 to 12.1 L/min (3.1 to 6.2 $L \cdot m^{-1} \cdot m^2$ index). The most surprising finding in this study was the normal pulmonary capillary wedge pressure, with only three patients with a low pulmonary capillary wedge pressure. Central venous pressure was also normal or increased in all subjects (normal ranging from 0 to 8 mm Hg).[12-14]

One potentially confounding variable in our study was fluid administration before insertion of the Swan-Ganz catheter. Because 55% of our patients were referred, it was difficult to ascertain fluid intake in them. We examined 34 patients in whom all intravenous fluid intake could be documented. The mean intake was 741 ± 760 ml (mean ± SD) over 10.1 ± 11.4 hours. We also correlated cardiac output with fluid intake for these patients and found no correlation ($r = -0.12$, $p = 0.51$). These observations suggest that, in the majority of patients, fluid administration was not excessive.

The patients with pulmonary edema were a heterogeneous group. They were clearly volume overloaded with high filling pressures and normal to high outputs. Five had preexisting hypertension and had underlying left ventricular hypertrophy with impaired relaxation and marked volume overload. Only one patient had evidence of decreased systolic function of unknown cause. Three patients had iatrogenic pulmonary edema principally as a result of fluid overload. All the patients improved dramatically with diuresis.

Cotton and his colleagues[11] have reported their results with regard to 45 preeclamptic patients in whom the initial hemodynamic measurements were obtained before any intervention other than magnesium sulfate and minimal intravenous fluids. Their results and ours are quite similar (Table I). The main differences are that our patients had lower systolic blood pressure and systemic vascular resistance with higher cardiac output. The differences may be related to intravenous meperidine given before insertion of the Swan-Ganz catheter in our patients. Together these studies which involve 94 subjects, allow a consensus view to emerge with regard to the hemodynamics of severe preeclampsia. Namely, that there is a variable hemodynamic profile with relatively high cardiac outputs and normal filling pressures, which reflect the volume loading of the circulation during pregnancy with inappropriately high systemic vascular resistance superimposed, resulting in hypertension. This variability is not surprising in that a spectrum of cardiac outputs and peripheral resistance is found in virtually every other type of experimental and naturally occurring hypertension.[15, 16] The mechanisms for the varied hemodynamics in hypertension

result from the complex interaction of multiple variables (cardiac output, total peripheral resistance, blood volume, central nervous system integration, and autonomic tone) and remain poorly defined.[16] The failure of the circulation to dilate in the setting of increasing cardiac output appears to be a characteristic feature of preeclampsia. Because resistance is calculated from the mean arterial pressure divided by the output, in a high output state the calculation results in a normal or even low resistance despite the elevated blood pressure.

Our results and those of Cotton et al.[11] contrast with the data reported from The Netherlands by Wallenburg[12] (Table IV). He found a variable pattern with a trend toward a low pulmonary capillary wedge pressure, low cardiac output, and high systemic vascular resistance in 44 untreated nulliparous, preeclamptic patients, whereas a wide range of hemodynamics was found in 22 patients who had received various therapies and were usually referred. He concluded that the untreated patient was significantly volume depleted and that the wide spectrum of hemodynamic findings present in the treated group resulted from prior therapy and the presence of other variables such as labor, multiparity, and preexisting hypertension. However, 15% or more of his untreated group had wedge pressures >8 mm Hg, which suggests a normal central blood volume. In emphasizing the predominant pattern of reduced filling pressures he minimized the variable hemodynamic presentations.

In an effort to obtain a population similar to Wallenburg's untreated group, we found 15 patients who were nulliparous, not in labor, had not received antihypertensives, and did not have a history or evidence of preexisting hypertension, heart, or renal disease. They had received magnesium sulfate and some intravenous fluids before invasive monitoring. Except for the slightly lower heart rates and outputs, the parameters of these 15 patients were not significantly different from those of the remaining 26 without pulmonary edema. The hemodynamic parameters of the 15 patients are listed in Table IV with each parameter presented as median and range in the manner of Wallenberg's data to allow comparison. Our patients tended to have high central pressures, higher outputs, and lower systemic resistances more frequently than did those of Wallenberg, but the range of values were similar.

Studies of plasma volume in preeclamptic patients have shown a 5% to 29% reduction in plasma volume with a mean reduction of 9%.[17] Whereas patients with preeclampsia may have reduced plasma and intravascular volume, the central blood volume as evidenced by central venous pressure and pulmonary capillary wedge pressure is usually normal, which suggests that the vascular capacitance is also reduced. Unfortunately,

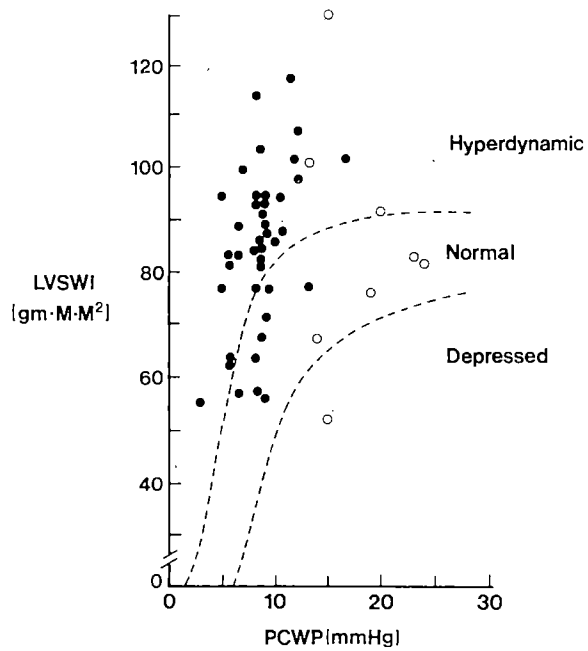


Fig. 1. Left ventricular function curve of left ventricular stroke work index *(LVSWI)* plotted against pulmonary capillary wedge pressure *(PCWP)* in 49 patients with severe preeclampsia. *Solid circles,* No pulmonary edema; *open circles,* eight patients with pulmonary edema.

venoconstriction cannot be easily and accurately assessed clinically. The immediate result of venoconstriction is to shift blood volume centrally from the splanchnic bed, which results in an increase in filling pressure.[18] With continued venoconstriction there is increased transudation of fluid with a decrease in plasma and intravascular volume.[16, 18] What role venoconstriction plays in the spectrum of hemodynamic abnormalities found in preeclampsia remains to be determined. Splanchnic venoconstriction and central relocation of intravascular volume also have been observed in other etiologic types of hypertension.[16] From a therapeutic and practical point of view, many of these patients behave as if they are venoconstricted and are quite susceptible to circulatory congestion and pulmonary edema with fluid administration.

Our study was not designed to determine the best treatment of preeclampsia. The normal pulmonary capillary wedge pressure with normal to elevated cardiac output would imply that neither volume expansion nor diuretic therapy is generally indicated. The findings support the currently used minimal intervention approach of bed rest, magnesium sulfate, and delivery. Attention to fluid balance with input minimally exceeding output can prevent circulatory congestion, especially in patients with left ventricular hypertrophy or chronic renal disease. Most patients with severe preeclampsia can be managed without a Swan-Ganz cath-

eter; however, in certain circumstances hemodynamic monitoring is potentially useful, namely, in sorting out cardiogenic from noncardiogenic pulmonary edema, in management of massive hemorrhage or refractory hypertension, and in induction of epidural anesthesia for cesarean section.

In summary, we have studied the hemodynamics of 49 subjects with severe preeclampsia at a large referral center. Despite a heterogeneous population of referred and nonreferred patients, pretreated and nonpretreated patients, a generally consistent profile emerged. A normal left ventricular filling pressure was present in 88%. A normal to high cardiac output for pregnancy was found with elevated resistances in one half the patients and normal resistances were found in the others. The normal resistances reflected higher cardiac outputs. Our patient population and results are similar to those of Cotton et al. and support the conclusion that, in general, preeclampsia is a normal to high output state with an inappropriately increased systemic resistance, which results in hypertension. The patients with pulmonary edema had either underlying heart disease or iatrogenic fluid overload. Even in patients in whom fluid administration was minimal, filling pressures were not low.

Our study was not designed to test the hypothesis that preeclampsia is a volume contracted state, but rather we wished to determine the central hemodynamics of patients with preeclampsia seen at a tertiary referral center. Because of the normal filling pressures in most and the propensity of pulmonary congestion to develop with fluid administration, we recommend fluid administration not to exceed output with emphasis on current minimal intervention and early delivery. We hope that our study provides insight for future studies of the treatment of preeclampsia.

REFERENCES

1. Hankins GDV, Wendel GD, Cunningham FC, Leveno KJ. Longitudinal evaluation of hemodynamic changes in eclampsia. AM J OBSTET GYNECOL 1984;150:506-12.
2. Cotton DB, Gonik B, Dorman KF. Cardiovascular alterations in severe pregnancy-induced hypertension: acute effects of magnesium sulfate. AM J OBSTET GYNECOL 1984;148:162-5.
3. Groenendijk R, Trimbos MJ, Wallenburg HCS. Hemodynamic measurements in preeclampsia: preliminary observations. AM J OBSTET GYNECOL 1984;150:232-6.
4. Benedetti TJ, Cotton DB, Read JC, Miller FC. Hemodynamic observations in severe preeclampsia with a flow-directed pulmonary artery catheter. AM J OBSTET GYNECOL 1980;136:465-70.
5. Rafferty TD, Berkowitz RL. Hemodynamics in patients with severe toxemia during labor and delivery. AM J OBSTET GYNECOL 1980;138:263-70.
6. Phelan JP, Yurth DA. Severe preeclampsia. I. Peripartum hemodynamic observations. AM J OBSTET GYNECOL 1982;144:17-22.
7. Ihle BU, Long P, Oats J. Early onset preeclampsia: recognition of underlying renal disease. Br Med J 1987; 294:79-81.
8. Bader RA, Bader ME, Rose DJ, Braunwald E. Hemodynamics at rest and during exercise in normal pregnancy as studied by cardiac catheterization. J Clin Invest 1955; 34:1524-36.
9. Ueland K, Hansen JM. Maternal cardiovascular dynamics. AM J OBSTET GYNECOL 1969;103:8-18.
10. Kerr MG. Cardiovascular dynamics in pregnancy and labor. Br Med Bull 1968;24:19-24.
11. Cotton DB, Lee W, Huhta JC, Dorman KF. Hemodynamic profile of severe pregnancy-induced hypertension. AM J OBSTET GYNECOL 1988;158:523-9.
12. Wallenburg HCS. Hemodynamics in hypertensive pregnancy. In: Rubin PC, ed. Hypertension in pregnancy. 1st ed. Amsterdam: Elsevier, 1988:66-101.
13. Grossman W, Barry WH. Cardiac catheterization. In: Braunwald E, ed. Heart disease. 3rd ed. Philadelphia: WB Saunders, 1988:242-72.
14. Colditz RB, Josey WE. Central venous pressure in supine position during normal pregnancy. Obstet Gynecol 1970; 36:769-72.
15. Frohlich ED. Hemodynamic considerations in clinical hypertension. Med Clin North Am 1987;71:803-12.
16. Tarazi RC. The hemodynamics of hypertension. In: Genest J, Kuchel O, Hamet P, Cantin M, eds. Hypertension. 2nd ed. New York: McGraw-Hill, 1983:15-42.
17. Chesley LC. Hypertensive disorders in pregnancy. 1st ed. New York: Appleton-Century-Crofts, 1978:203.
18. Tarazi RC. Hemodynamic role of extracellular fluid in hypertension. Circ Res 1976;38(suppl 2):73-83.

Major Advances in Human Female Reproduction.

May 10-12, 1990 Universita' Cattolica Rome, Italy

The aim of the symposium is to discuss the physiopathological mechanisms in most important aspects of human female reproduction and provide the recent achievements in this field. The perspectives of new clinical approaches will be discussed.

MAIN TOPICS

Puberty - The follicle - The corpus luteum - Hyperandrogenism

SCIENTIFIC COMMITTEE

S. Mancuso
E. Y. Adashi (USA)
D. T. Baird (UK)
G. Benagiano (I)
H. G. Burger (AUS)
K. Catt (USA)
C. J. Hodgen (USA)
R. Lauro (I)
L. Martini (I)
F. Naftolin (USA)

SCIENTIFIC SECRETARIES

A. Caruso - A. Lanzone - A. M. Fulghesu
Universita' Cattolica
Clinica Ostetrica e Ginecologica
Largo A. Gemelli, 8
00168 Rome, Italy
Tel. (6) 33054979
FAX (6) 3388066

Registration fee: 180 $

A limited number of free communications will be accepted for poster presentation. Abstracts must be sent to A. Caruso before February 10th, 1990 (postmarked).

PRACTICE IN THE PERFECT PLACE

Excellent opportunity for BC/BE OB/GYN to join well-established, highly respected practice or to establish solo practice in Saratoga Springs, NY area. Competitive salary guarantee offered. Strong medical staff support and call sharing available.

Located three hours from NYC, Montreal and Boston, and a half-hour from Albany, Saratoga is an historic, close-knit community with lovely neighborhoods, fine restaurants, specialty shops and entertainment including thoroughbred racing and the Saratoga Performing Arts Center. Its proximity to Lake Placid and the Green Mountains of Vermont is ideal for skiers and outdoor enthusiasts.

Forward CV to:
Denise Romand, Personnel Recruiter
Saratoga Hospital, 211 Church Street,
Saratoga Springs, New York 12866
(518) 583-8465 days, (518) 583-3120 evenings

Saratoga Hospital

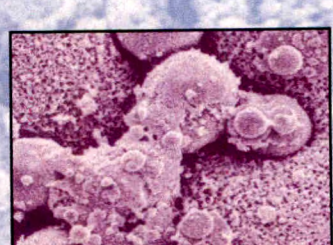
211 Church Street, Saratoga Springs, NY 12866



BEFORE
Scanning electron micrograph (×3300) of *Candida albicans* grown for 48 hours on agar.

Femstat® Prefill destroys the integrity of the yeast cell.

Culture



AFTER
Scanning electron micrograph (×3300) of *Candida albicans* on agar plate with butoconazole nitrate.

1. Droegemueller W, Adamson DG, Brown D et al: Three-day treatment with butoconazole nitrate for vulvovaginal candidiasis. *Obstet Gynecol* 1984;64(4):530-534.
2. Prescribing information for Femstat® Prefill vaginal cream.

*Microbiological cure rates in 95% of patients eight days following Femstat three-day therapy.[1]

**In pregnant patients (second and third trimesters only), therapy should be extended to six days.

Please see brief summary of prescribing information on page following this advertisement.

© 1989 Syntex Laboratories, Inc.

Shock.

Cure[1]* with 3-day therapy**

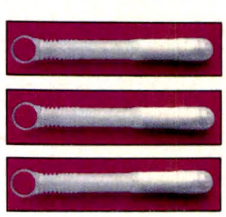
- Microbiological cure rates in 95% of patients eight days following Femstat three-day therapy.[1]

Broad spectrum of activity

- Clinically effective against *Candida albicans* and other *Candida* species, including *Candida tropicalis*.

Broad margin of safety

- A low incidence of reported adverse reactions.[2]
- NOT a triazole/NOT associated with flu-like symptoms.
- Proven safe and effective for use in pregnant patients** (second and third trimesters only).



3 prefilled disposable applicators

- Reduces risk of reinfection and ensures unit-dose accuracy.
- Eliminates messy filling/cleaning procedures.

FEMSTAT® PREFILL

(butoconazole nitrate) VAGINAL CREAM 2%

3-day victory over vulvovaginal candidiasis**

SYNTEX

SYNTEX LABORATORIES, INC.
PALO ALTO, CA 94304

FEMSTAT PREFILL

(butoconazole nitrate) VAGINAL CREAM 2%



BRIEF SUMMARY

INDICATIONS: FEMSTAT® (butoconazole nitrate) vaginal cream 2.0% is indicated for local treatment of vulvovaginal mycotic infections caused by Candida species. Confirm the diagnosis by KOH smears and/or cultures. FEMSTAT can be used with oral contraceptives and antibiotics. It is effective in non-pregnant women and during the second and third trimesters of pregnancy.

CONTRAINDICATIONS: FEMSTAT is contraindicated in patients hypersensitive to any of the ingredients.

PRECAUTIONS: General: If clinical symptoms persist, repeat microbiological tests to rule out other pathogens and confirm the diagnosis. Discontinue drug if sensitization or irritation occurs.

Information for the Patient: Do not discontinue prematurely during menstruation or because of symptomatic relief.

Carcinogenesis: Animal studies have not been done.

Mutagenesis: Mutagenicity studies were negative.

Impairment of Fertility: Animal studies showed no impairment of fertility.

Pregnancy Category C: Adverse effects were noted in animals treated with high oral doses. No studies were done in women during first trimester. Patients in the second or third trimester have shown no adverse effects attributable to the drug.

Nursing Mothers: Use with caution.

Pediatric Use: Safety and efficacy have not been established.

ADVERSE REACTIONS: Vulvar/vaginal burning in 2.3% of patients, vulvar itching in 0.9%, discharge, soreness, swelling, itching of fingers each in 0.2%. Complaints caused 1.6% to discontinue drug.

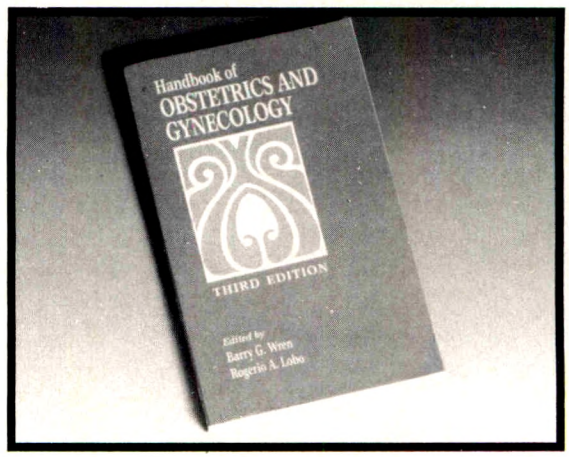
DOSAGE AND ADMINISTRATION: Non-pregnant Patients: The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for three days. Treatment can be extended for an additional three days if necessary.

Pregnant Patients (second and third trimesters only): The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for six days.

CAUTION:
Federal law prohibits dispensing without prescription.

SYNTEX
SYNTEX LABORATORIES, INC.
PALO ALTO, CA 94304

Usable, Accessible Facts For Busy OB/GYNS



New 3rd Edition!

HANDBOOK OF OBSTETRICS AND GYNECOLOGY

Edited by **Barry G. Wren**, M.D., M.B., B.S., M.H.P.Ed., F.R.A.C.O.G., F.R.C.O.G. and **Rogerio A. Lobo**, M.D.; with 12 contributors

Clinically oriented and up-to-date, this concise handbook provides a core of obstetrical and gynecological information. It's a quick guide to what to do, when to do it and how to do it.

- history taking and physical exam
- normal and abnormal pregnancy and labor
- abnormal labor
- infertility and contraception
- prolapse and urinary incontinence and much more

1989. 396 pages, illustrated. **(05844) $27.95** (U.S.); $36.50 (Can.) A Chapman & Hall publication.

TO ORDER, CALL TOLL FREE: 800-221-7700, ext. 15A 24 hours a day, 7 days a week. Please mention this number when calling: FLR-431-031. FAX Orders: 314-432-1380, Attn: Direct Marketing. For Customer Service: 800-633-6699, Monday-Friday, 7AM-6PM, Central Time. In Canada, call collect: 416-298-2465

The C.V. Mosby Company
11830 Westline Industrial Drive, St. Louis, MO 63146
The C.V. Mosby Company, Ltd.
5240 Finch Avenue East, Scarborough, Ontario, Canada, M1S 5A2

YES! Send _____ copies of **WREN (05844)** priced at **$27.95** (U.S.); $36.50 (Can.)
SAVE MONEY! Send a check with your order or charge it to your credit card. Mosby pays the shipping and handling on all prepaid orders.

( ) Payment enclosed ( ) MasterCard ( ) VISA ( ) AMEX

Card # _____ Exp. date _____

Signature _____

Business phone (_____) _____

( ) Bill me, plus shipping and handling

( ) Add me to your mailing list. (No purchase necessary)

PRINT OR TYPE

Name _____

Address _____

City _____

State _____ Zip _____

If using a Purchase Order, please atttach this coupon. 30-day approval good in U.S. and Canada. Prices outside the U.S. slightly higher. Outside the U.S. and Canada, please pay by check or money order in dollar equivalent. **Prices subject to change without notice. Add applicable sales tax.**

FLR-431-031-03

Mosby

Cardiovascular changes in early phase of pregnancy

Eleanor L. Capeless, MD, and James F. Clapp, MD
Burlington, Vermont

To assess the magnitude and timing of cardiovascular changes in pregnancy, eight subjects were serially studied before conception and at 8, 16, and 24 weeks' gestation. With the use of M-mode echocardiography, cardiac output, ejection fraction, stroke volume, and end-diastolic volume were calculated from left ventricular dimensions with subjects in the left lateral position. Systemic vascular resistance was calculated with the use of cardiac output and simultaneously obtained measurements of arterial pressure. Cardiac output increased 1 L/min at 8 weeks' gestation, which represented >50% of the total change seen. Cardiac output increased primarily because of stroke volume rather than heart rate. By 8 weeks' gestation, systemic vascular resistance had fallen to 70% of its preconceptional value. Thus when subjects are studied before conception and during the early phase of pregnancy, the majority of the pregnancy-induced changes in these parameters occur during the embryonic period. (AM J OBSTET GYNECOL 1989;161:1449-53.)

Key words: Echocardiography, pregnancy, hemodynamics, cardiac output, systemic vascular resistance

The cardiovascular system undergoes profound alterations during pregnancy. Although the changes in cardiac output, heart rate, and systemic vascular resistance have been well characterized during the latter two thirds of pregnancy,[1-7] the timing and magnitude of these changes during the first trimester remain unknown. In addition, the changes observed during pregnancy have traditionally been compared with postpartum values, but it has not been established when or whether cardiovascular function returns to its preconceptional state. Because measurements were not obtained before pregnancy, previous studies may have underestimated the magnitude of the changes seen in pregnancy.

Our previous observations that basal morning heart rate was significantly elevated at the time of missed menses in a group of recreational runners[8] led us to investigate early cardiovascular changes in the first trimester. The purpose of this study was to document the magnitude and timing of changes in cardiac output, stroke volume, heart rate, mean arterial pressure, and systemic vascular resistance. We hypothesized that the majority of the changes seen in these parameters occur in the first 8 weeks after conception (embryonic period). A longitudinal serial study design in which each subject was studied before conception was used so that comparisons could be based on each subject's preconceptional baseline.

From the Department of Obstetrics and Gynecology, University of Vermont College of Medicine.
Supported by National Institutes of Health grants ROI 21268 and P50 HD211089.
Presented in part at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.
Reprint requests: Eleanor L. Capeless, MD, Department of Obstetrics and Gynecology, Medical Center Hospital of Vermont, Burlington, VT 05401.
6/6/15664

Table I. Study population

	Mean	*Range*
Age (yr)	30.8	25-36
Parity	0.4	0-1
Maximum oxygen consumption (ml/kg)	46.9	41.7-56.8
Body fat (%)	17.5	11.7-23.4

Material and methods

Subjects were enrolled before conception and had accurately dated singleton pregnancies. All subjects had positive β–human chorionic gonadotropin determinations within 48 hours of missed menses and an ultrasound examination at 5½ weeks after conception. Pregnancy evaluations were scheduled serially 6 to 8, 14 to 16, and 22 to 24 weeks after conception. These study periods will be referred to as 8, 16, and 24 weeks' gestation. All subjects were initially evaluated within 3 months before conception. All subjects were nonsmoking physically active women with no history of heart disease. Demographic data on age, parity, fitness, and body composition before pregnancy are presented in Table I.

All studies were preceded by an initial 15-minute accommodation to the laboratory environment. At this

Table II. Cardiovascular measurements

	Before pregnancy	*8 weeks' gestation*	*16 weeks' gestation*	*24 weeks' gestation*	*Significance*	*p Value*
Heart rate (beats/min)	65 ± 3	68 ± 4	72 ± 3	73 ± 3	Before pregnancy vs. 16	< 0.05
					Before pregnancy vs. 24	< 0.05
End-diastolic volume (ml)	109 ± 9	122 ± 9	125 ± 8	126 ± 8	Before pregnancy vs. 8	< 0.05
					Before pregnancy vs. 16	< 0.01
					Before pregnancy vs. 24	< 0.001
Stroke volume (ml)	65 ± 5	79 ± 5	83 ± 6	81 ± 5	Before pregnancy vs. 8	< 0.02
					Before pregnancy vs. 16	< 0.001
					Before pregnancy vs. 24	< 0.001
Ejection fraction (%)	61 ± 2	64 ± 2	66 ± 2	64 ± 2	All time periods	NS
Cardiac output (L/min)	4.2 ± 0.4	5.2 ± 0.3	5.9 ± 0.4	5.7 ± 0.3	Before pregnancy vs. 8	< 0.01
					Before pregnancy vs. 16	< 0.01
					Before pregnancy vs. 24	< 0.001
Mean arterial pressure (mm Hg)	69 ± 3	62 ± 4	69 ± 2	67 ± 4	Before pregnancy vs. 16	< 0.05
Systemic vascular resistance (dynes · sec · cm^{-5})	1376 ± 143	969 ± 76	926 ± 68	930 ± 82	Before pregnancy 8	< 0.05
					Before pregnancy 16	< 0.01
					Before pregnancy 24	< 0.01

Data presented are mean ± SEM.

time a heart rate monitor and blood pressure cuff were placed and the subject assumed a comfortable left lateral position. Blood pressure was measured every 2 minutes with a mercury manometer that was zeroed at the level of the subject's left ventricle. M-mode echocardiography was performed with the GE 3600 ultrasound machine with a 3.5 MHz transducer. The left parasternal window was used to obtain the long axis view of the heart, which with real-time scanning visualizes the left ventricle, mitral, and aortic valves. The M-mode beam was then positioned just below the tips of the mitral valve leaflets. Measurements of the end-systolic and end-diastolic dimensions were made in accordance with the guidelines published by the American Society of Echocardiography.[9] An additional 5 minutes of imaging preceded the initial measurements in an effort to further accommodate the subject to the study. Heart rate was directly measured during the intervals in which cardiac output was determined. Three or more separate sets of measurements were obtained over 15 minutes with each set measuring three to four cardiac cycles. Thus the values obtained represent the average of 9 to 12 separate cardiac cycles interspersed throughout the respiratory cycle. Over the 15-minute study period, the variability of each set of measurements from the mean cardiac output value averaged 6% with a range from 2% to 17%. In 88% of the studies the variability of successive measurements over the 15-minute study time was <10%.

Left ventricular volumes were calculated from the end-diastolic dimension (EDD) and end-systolic dimension (ESD) in accordance with the formula of Teichholz et al.[10] for a modified prolate ellipsoid. This formula is as follows:

1. End-systolic volume [ESV (ml)] = 7x $(ESD)^3/2.4$ + ESD
2. End-diastolic volume [EDV (ml)] = 7x $(EDD)^3/2.4$ + EDD

Standard equations were used to calculate the following functional parameters:

1. Stroke volume [SV (ml)] = EDV − ESV
2. Cardiac output [CO (ml/min)] = SV × heart rate
3. Ejection fraction [EF (%)] = (SV/EDV) × 100
4. Mean arterial pressure [MAP (mm Hg)] = 2 (diastolic) + systolic/3
5. Systemic vascular resistance [SVR dyne · sec · cm^5] = 80 MAP/CO

Statistical analysis was performed with repeated measures analysis of variance with the Bonferroni correction for multiple comparisons. Significance was set at the 0.05 level.

Results

Eight subjects have completed all study periods. Cardiac output, end-diastolic volume, stroke volume, ejection fraction, heart rate, MAP and systemic vascular resistance are presented in Table II. Stroke volume and heart rate as a function of weeks gestation are presented graphically in Fig. 1. Cardiac output and MAP as a function of gestational age are graphically shown in Fig. 2.

Cardiac output increased 1 L/min by 8 weeks' gestation. This represented a 22% change over preconception values and 57% of the total increase in cardiac output achieved by 24 weeks' gestation. After 24 weeks' gestation, the pattern seen in the subjects was variable and the group mean did not change significantly for the remainder of the pregnancy. Cardiac output is a function of both heart rate and stroke volume (Fig. 1). The increase in stroke volume at 8 weeks' gestation represented a 22% change over preconception values and 78% of the total increase observed by 24 weeks'

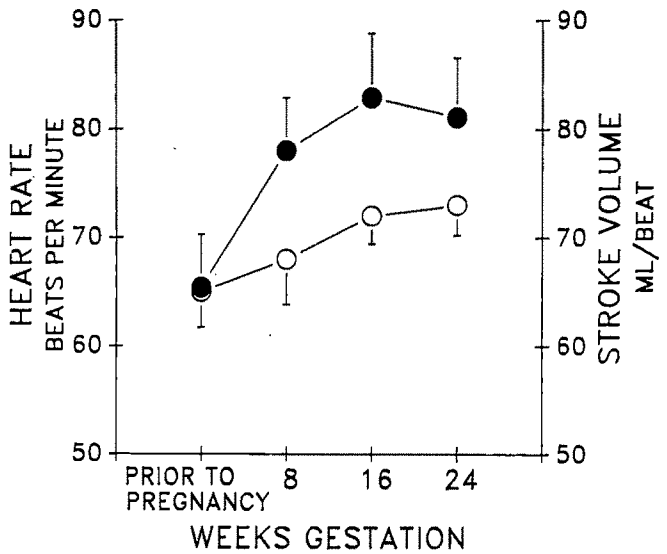


Fig. 1. Stroke volume *(filled circles)* and heart rate *(open circles)* components of cardiac output are presented for four study periods.

gestation. A statistically significant increase in heart rate did not occur until 16 weeks' gestation. This increase represented a 11% change over preconception values and 87% of the total increase observed by 24 weeks' gestation. End-diastolic volume had increased significantly by 8 weeks' gestation and 65% of the total change had occurred by this time. There was no significant change in ejection fraction in any of the study periods.

Systemic vascular resistance fell by 8 weeks' gestation to 30% below preconception values. This early decrease represented 90% of the total change in systemic vascular resistance achieved by 24 weeks' gestation. The components of systemic vascular resistance are presented in Fig. 2. Although mean arterial pressure fell at 8 weeks' gestation, the decrease was not statistically significant until 16 weeks' gestation.

Comment

These data clearly support our original hypothesis that a majority of the pregnancy-induced changes in cardiac output and systemic vascular resistance occur during the embryonic period. The increase in cardiac output can be primarily attributed to an increase in stroke volume rather than in heart rate. The increase in stroke volume was related to an increase in end-diastolic volume as ejection fraction did not change over the study periods. Although we previously had shown that basal morning heart rate increased early in pregnancy,[8] under the conditions of this experiment there was no significant change in heart rate documented.

This is the first report of serial measurements of cardiac output that began before conception. Although the changes in heart rate, blood pressure, and cardiac output during pregnancy have been well documented both by dye dilution[3, 5, 6] and echocardiography,[4, 7] all previous longitudinal studies have started late in the first trimester and have used postpartum values (6 weeks after delivery) to represent preconceptional values. It has been assumed that the women's body returns to its preconceptional state post partum. However, evidence is accumulating that this assumption may be incorrect. Hart et al.[11] have found that multiparous women have larger aortic diameters than those of primiparous women, which suggests that there are alterations that occur during pregnancy that do not revert to the prepregnant baseline. Breast-feeding because of its potential effects on plasma volume,[12] may also influence the rate at which women's cardiovascular systems recover from the metabolic demands of pregnancy. Thus the effects of parity and breast-feeding may have significantly altered the nonpregnancy value. Likewise, our study represents a mixed populace of five nulliparous and three multiparous women. Whereas significant differences were not seen in our study, a larger study sample would be necessary to determine the long-term effects of parity and breast-feeding on the cardiovascular system.

Our data are in agreement and compliment the work of others who characterized cardiovascular changes throughout the second and third trimester of pregnancy. Although all previous reports documented the increase in cardiac output seen during pregnancy, there is disagreement with regard to the relative importance of stroke volume and heart rate in the initiation of this increase. Early serial studies by Walters et al.,[7] Lees et al.,[4] and Ueland et al.[6] that used dye dilution found that cardiac output was already elevated at the time of the first study. They found minimal or no increase in

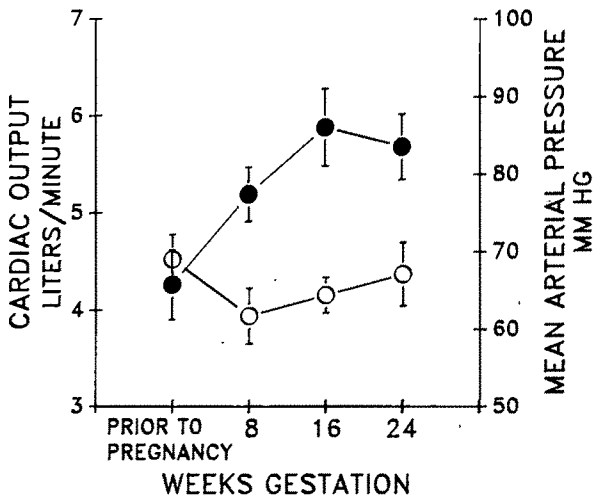


Fig. 2. Cardiac output *(filled circles)* and mean arterial pressure *(open circles)* components of systemic vascular resistance are presented for four study periods.

cardiac output after 20 to 24 weeks' gestation. They demonstrated that the increase in cardiac output resulted from an increase in stroke volume rather than an increase in heart rate. Two echocardiographic studies have serially evaluated cardiac function starting in the first trimester. Katz et al.[5] serially studied 19 women throughout pregnancy. The first evaluation was at the end of the first trimester and a postpartum evaluation was obtained 6 to 12 weeks after delivery. Our findings agree with that of Katz, who found an increase in cardiac output that was a result of an increase in stroke volume, end-diastolic volume, and an increase in heart rate. Mashimi et al.[8] studied seven patients beginning at 11.4 weeks' gestation and compared these values with those obtained 6 to 12 weeks post partum. In contrast with previous investigators, Mashimi concluded that the increases in cardiac output were attributable to changes in heart rate rather than in stroke volume. He failed to observe any change in stroke volume during pregnancy, compared to the postpartum period. In our series, approximately 75% of the increase in stroke volume occurred by the end of the first trimester. Therefore, the relative contribution of stroke volume to the increase in cardiac output could be obscured by the use of the postpartum control value and the timing of the first study period. In addition, there may be different mechanisms operative early in pregnancy to increase cardiac output (end-diastolic volume), which are then augmented by others (heart rate) later in gestation.

M-mode echocardiography is a noninvasive technique to serially follow changes in cardiovascular parameters.[13] The calculation of stroke volume with the Teichholz equation has been validated against angiography-derived stroke volume with a correlation coefficient of $r = 0.97$.[10] When the technique was compared with thermodilution cardiac output, the correlation coefficient was $r = 0.86$.[14] Thus with a normally shaped ventricle and uniform contraction of the ventricular wall, the measurements obtained should accurately reflect left ventricular volumes.[1, 15] During pregnancy the heart is known to change in shape and size and the M-mode technique has not been independently validated. Although the serial nature of this study minimizes the effects of individual variation, the absolute values for cardiac output obtained in this study should not be directly compared with other studies. However, these observations are valid to describe the relative magnitude and timing of cardiovascular changes during early pregnancy.

The magnitude of the changes in cardiac output and systemic vascular resistance that occur in the first trimester are tremendous. The mediators of these changes are unknown. Longo[16] has postulated that maternal blood volume and cardiac output are hormonally controlled with steroidogenesis in the fetal compartment, which influences the maternal environment. The early onset of these changes favor a hormonal mechanism but this may be primarily maternal in origin. The influence of the timing and magnitude of these changes on pregnancy outcome is unknown but represents an area of future study.

REFERENCES

1. Metcalfe J, Stock MK, Barron DH. Maternal physiology during gestation. In: Knobil K, Neill T, Ewing L, et al., eds. The physiology of reproduction. New York: Raven Press, 1988:2145-76.
2. Kerr MG. Cardiovascular dynamics in pregnancy and labour. Br Med Bull 1968;24:19-23.
3. Lees MM, Taylor SH, Scott DB. A study of cardiac output at rest throughout pregnancy. J Obstet Gynaecol Br Commonw 1967;74:319-28.
4. Katz R, Karliner JS, Resnik R. Effects of a natural volume overload state (pregnancy) on left ventricular performance in normal human subjects. Circulation 1978; 58:434-41.
5. Ueland K, Novy MJ, Peterson EN, et al. Maternal cardiovascular dynamics. IV. The influence of gestational age on the maternal cardiovascular response to posture and exercise. AM J OBSTET GYNECOL 1969;104:856-64.
6. Walters WAW, MacGregor WG, Hills M. Cardiac output at rest during pregnancy and the puerperium. Clin Sci 1966;30:1-11.
7. Mashini IS, Albazzaz SJ, Fadel HE, et al. Serial noninvasive evaluation of cardiovascular hemodynamics during pregnancy. AM J OBSTET GYNECOL 1987;156:1208-13.
8. Clapp JF. Maternal physiological adaptations to early human pregnancy. AM J OBSTET GYNECOL 1988;159:1456-60.
9. Sabn DJ, DeMaria A, Weyman A. Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. Circulation 1978;58:1072-83.
10. Teichholz LE, Kreulen T, Herman MV, et al. Problems in echocardiographic volume determinations: echocardiographic-angiographic correlations in the presence or absence of asynergy. Am J Cardiol 1976;37:7-11.
11. Hart MV, Morton MJ, Hosenpud JD, Metcalfe J. Aortic

function during normal human pregnancy. AM J OBSTET GYNECOL 1986;154:887-91.
12. Bond CF. Blood volume changes in the lactating rat. Endocrinology 1958;63:285.
13. Feigenbaum H, Popp RL, Wolfe SB, et al. Ultrasound measurements of the left ventricle: a correlative study with angiocardiography. Arch Intern Med 1972;129: 461-7.
14. Kronik G, Slany J, Mosslacker H. Comparative value of eight M-mode echocardiographic formulas for determining left ventricular stroke volume. Circulation 1979; 60:1308-16.
15. Weyman AE. Cross-sectional echocardiography. Philadelphia: Lea and Febiger, 1982:283.
16. Longo L. Maternal blood volume and cardiac output during pregnancy: a hypothesis of endocrinologic control. Am J Physiol 1983;245:720-8.

The effects of maternal exercise on early pregnancy outcome

James F. Clapp III, MD
Burlington, Vermont

This study was designated to test the hypothesis that vigorous aerobic exercise during both the periconceptional period and early pregnancy increases the incidence of abnormal early pregnancy outcome. Exercise performance was prospectively monitored before and during pregnancy in 47 recreational runners, 40 aerobic dancers, and 28 physically active, fit controls. Pregnancy was diagnosed by an early test for β-subunit human chorionic gonadotropin and viability was confirmed by ultrasonography at 40 days' conceptional age. Spontaneous abortion occurred in 19% of the pregnancies. The incidence was 17% in the runners, 18% in the aerobic dancers, and 25% in the controls. At term, one congenital abnormality was detected in each of the three groups. Late pregnancy events, potentially related to abnormalities of placentation, were limited to two cases of mild pregnancy-induced hypertension. We conclude that, in physically fit women, continuation of these types of aerobic activity at intensities between 50% and 85% of maximum during the periconceptional period and early pregnancy does not appreciably alter early pregnancy outcome. (AM J OBSTET GYNECOL 1989;161:1453-7.)

Key words: Pregnancy, exercise, abortion, congenital abnormality

Both retrospective[1, 2] and prospective[3-6] studies of abortion have documented that the incidence of spontaneous loss is high. Reported frequencies range from as low as 7% to as high as 40% to 60%, depending on the timing and method of pregnancy detection. In studies using standard clinical parameters alone, the overall incidence averages about 15% with distinct increases noted with advancing maternal age and previous early loss. Inclusion of β-subunit human chorionic gonadotropin (β-hCG) testing produces a variable increase in the rate by identifying subclinical losses. In general, the earlier the testing is done, the higher the detection rate and the reported incidence of spontaneous loss.

From the Department of Obstetrics and Gynecology, University of Vermont College of Medicine.
Supported in part by National Institutes of Health grants HD 21268 and 11089 and March of Dimes Birth Defects Foundation grant 6-434.
Presented in part at the Thirty-sixth Annual Meeting of the Society For Gynecologic Investigation, San Diego, California, March 15-18, 1989.
Reprint requests: James F. Clapp III, MD, University of Vermont College of Medicine, Department of Obstetrics and Gynecology, Given Medical Building, Burlington, VT 05405-0058.
6/6/16516

Approximately one third of these losses are associated with a chromosomal anomaly,[7] and in selected cases specific environmental factors such as subchorionic hemorrhage[8] and defective luteal function[9] have been shown to play a role. The etiologic factors involved in the remainder are poorly understood but are felt to be primarily related to anomalous environmental circumstance. The current study was designed to explore theoretical[10] and epidemiologic[11] concerns that suggested that early wastage should be increased by the cardiovascular, thermal, and hormonal perturbations associated with intense physical activity in early pregnancy.[10-12] Its specific goal was to prospectively test the hypothesis that continuing endurance types of recreational exercise in either the periconceptional period or early pregnancy, or both, increase the incidence of abnormal early pregnancy outcome.

Material and methods

A total of 119 women were studied prospectively for several cycles before conception and then for six additional cycles or throughout subsequent pregnancy. Written informed consent was obtained from each subject before enrollment. All subjects worked outside the

Table I. Group characteristics

Parameter	*Runners*	*Aerobic dancers*	*Controls*
No.	49	41	29
Age (yr)	31 ± 3	31 ± 3	31 ± 3
Nulligravid (%)	51	51	48
Primigravid (%)	31	34	28
Secundigravid (%)	18	15	24
Prior abortion (%)	16	0	14
Weight (kg)	58 ± 7	58 ± 6	59 ± 7
Body fat (%)	17 ± 4	18 ± 4	20 ± 5
$\dot{V}O_2$ max (ml/kg/min)	54 ± 7	53 ± 8	51 ± 8
Duration-intensity index before pregnancy	14,416 ± 357	12,341 ± 313	3,306 ± 986

Numeric data presented as mean ± SD.

home, had physically active life-styles, and, on the basis of education and income, were either upper-middle or upper socioeconomic class. All obtained routine obstetric care through private obstetricians not directly involved in the study. The mean age (± SD) was 31 ± 3 years with a range of 25 to 38 years. All were white and in excellent general health. The only historic obstetric risk factor identified was a history of previous spontaneous abortion in 12 (10%) of the subjects. Sixty (50%) were nulligravid, 37 (31%) were primigravid, and 22 (19%) were secundigravid at the time of enrollment.

Ninety women had been either running or aerobic dancing for at least 2 years on a regular basis (three times per week or more) for a minimum of 20 minutes each session at a documented intensity that exceeded 50% of their measured maximum capacity. Forty-nine were recreational runners and 41 were aerobic dancers. Twenty-nine physically active, intermittently athletic women matched for age, weight, percent body fat, and multiple life-style factors served as a control populace. Each control stopped all forms of endurance exercise before attempting conception and did not resume these types of physical activity until after the pregnancy.

In the two exercise groups daily exercise performance was recorded in the field for a minimum of 2 months before conception and throughout the subsequent pregnancy or for an additional six menstrual cycles. The method used to quantitate performance was based on that used to assess both training effect and energy expenditure in the field.[12-14] Briefly, each subject used a portable electrocardiogram telemetry system with memory capability to measure heart rate and the duration of exercise that was recorded at the end of each exercise session in an exercise log. In addition, the relationship between heart rate and exercise intensity (percent of the predetermined maximum oxygen consumption) was determined during graded exercise before conception and every 8 weeks during pregnancy. This information was used to convert the average heart rate during each run to exercise intensity. The product of exercise intensity and the duration of the exercise was averaged weekly and expressed as a duration-intensity index, which was used as the objective measure of each individual's time-specific exercise performance.[14] To allow valid comparison between subjects, the duration-intensity index was normalized and expressed as a percentage of each individual's average exercise performance over the initial 6 weeks of participation.

Maximal oxygen consumption was determined twice before conception in the initial 20 subjects using a constant-speed, progressive-grade, modified Astrand treadmill protocol.[15] There was no evidence of directionality with repeated testing and all second test values were within ±5% of the initial result. Therefore maximal oxygen consumption was determined only once in those that followed. Percent body fat was determined by both hydrodensitometry[16] and six-site skinfold thicknesses[17] in the first 50 subjects. As the mean difference between the two methods was +1.3% with a standard error of ±0.9%, only six-site skinfolds were used in the remainder.

Each subject had conception confirmed by β-hCG assay within 48 hours of missed menses and its viability was documented ultrasonographically between the fortieth and forty-fourth postconceptional day. Bleeding was followed by serial β-hCG assay and one or more ultrasonographic evaluations. At the time of abortion all tissue was examined histologically and, if viability had been recently confirmed, chromosomal analysis was performed. The course of pregnancy, labor, and delivery was independently monitored by study personnel, and all placentas and offspring were carefully examined for evidence of anomalies at the time of delivery. A subject was judged "infertile" if conception could not be documented during the initial six cycles of unprotected intercourse.

The three groups were divided into those who experienced spontaneous abortion and those who were

delivered at term. Within each group, differences in age, history of abortion, gravidity, and exercise performance were sought between those who aborted and those who did not with an unpaired Student t test. In addition, exercise performance, quantitated as the normalized duration-intensity index over various time intervals (the 2 weeks before conception, the first and second lunar months of pregnancy), was examined to see if there was a specific time in the periconceptional period when exercise performance differed in those who did and did not experience early wastage. Then, as the data were virtually identical in the two exercise groups, they were combined and compared with those of the control group, using the χ^2 statistic with Yates' correction, to detect any differences in the incidence of four objective indices of abnormal early pregnancy outcome. They included failure to conceive within 6 months, spontaneous abortion, gross congenital abnormality, and a clinical outcome suggesting anomalous placentation (specifically ectopic pregnancy, placenta previa, abruptio placentae, preterm labor, and pregnancy-induced hypertension). As no real differences were found, a similar comparison was done with the accepted incidence of these outcomes in an overall obstetric populace in an attempt to avoid the possibility of missing a small but significant difference as a result of our sample size (type II interpretive error).

Results

As detailed in Table I, there were no significant differences between the three groups in age, gravidity, preconceptional weight, percent body fat, or maximal oxygen consumption. The relatively low mean percent body fat (populace norms generally are considered to have a normal range of 22% to 28%) and high mean maximum oxygen consumption (populace 90th percentile in the range of 45 ml/kg/min) in the three groups indicate that all subjects were quite fit and in that respect are probably not representative of the obstetric populace at large.

The only significant between-group differences were a history of previous spontaneous abortion and exercise performance before pregnancy. Approximately 15% of the recreational runners and controls had experienced a prior early loss while none of the aerobic dancers had done so.

The level of preconceptional exercise performance, as assessed by the duration-intensity index, was quite varied both between and within groups. Both exercise groups had a mean level of exercise performance that was approximately fourfold that noted in the control group with the runners performing at a level some 17% above that observed in the aerobic dancers. The recreational runners ran an average of 22 miles a week (range 12 to 55), at an average pace of 8 min/mile (range 6 minutes 30 seconds to 9 minutes 50 seconds) and at a mean intensity equal to 68% of maximum capacity range (52% to 83%). The average time devoted by any individual to exercise each week ranged from a low of 1 hour 33 minutes to a high of 6 hours 37 minutes. Participation in aerobic dance averaged slightly more than four sessions a week (range 3 to 11). During each session, subjects worked at an intensity ranging between 20% and 40% of maximum during 25 to 35 minutes of warm-up and cool-down and at 55% to 88% of maximum capacity during the 25- to 30-minute high-intensity, so-called aerobic phase of the workout. This latter phase comprised approximately 70% of most individual's overall performance. In the control group exercise performance was quite erratic with individual frequencies ranging from once every other week to twice weekly. Twenty-one of these individuals only participated in weekend racquet sports in the spring, summer, and fall with a switch to cross-country or downhill skiing in winter. Their exercise performance was estimated from their average pulse rates in the field to have an average weekly duration intensity range between 1400 and 6200. The remaining eight erratically ran or danced aerobic once or twice a week and, on the basis of individual concerns about the impact of exercise on pregnancy, they stopped these activities before attempting conception. Their average duration intensity index was 2104 with a range from 1018 to 3245.

Table II. Spontaneous abortions

Parameter	*Runners*	*Aerobic dancers*	*Controls*
No.	8/49	7/39	7/28
Percentage	17	18	25
Pos. β-hCG only	1	2	1
Blighted ovum	6	3	4
Viable embryo	1 (?)	2	2

There was no within-group relationship between age, gravidity, prior loss, or overall exercise performance level and the occurrence of either early loss or other abnormal early pregnancy outcome.

Conception occurred within six cycles in 97% (115 of 119) of the subjects. The four cases of "infertility" were equally distributed between the groups and not clustered in women who had either extremely high- or low-duration intensity indices before or at the time of conception. One control subject and one recreational runner (24 miles per week in 3 hours) failed to conceive for no apparent reason. One aerobic dancer's husband had a subnormal semen analysis and she had irregular cycles, while another aerobic dancer simply decided to delay conception after a change in life situation.

As shown in Table II, there were no differences in

Table III. Within-group exercise performance*

Group	*Follicular phase*	*Weeks 1-4*	*Weeks 5-8*	*Overall*
Runners (n = 48)	93 ± 15	86 ± 19	80 ± 19	87 ± 19
Aborted (n = 8)	88 ± 13	88 ± 16	83 ± 17	86 ± 16
Term delivery (n = 40)	94 ± 15	86 ± 20	80 ± 19	87 ± 19
Dancers (n = 39)	86 ± 29	83 ± 27	83 ± 26	83 ± 27
Aborted (n = 7)	78 ± 20	78 ± 22	78 ± 24	78 ± 23
Term delivery (n = 32)	88 ± 30	84 ± 28	84 ± 26	84 ± 27

*All data presented as the percent of the preconceptional duration-intensity index (mean ± SD).

the incidence of spontaneous abortion in the three groups and histologic findings were similar. With the sample size examined, assuming a conservative spontaneous abortion rate in the populace at large of 15%, the probability that an exercise-associated, significant increase in the rate of spontaneous abortion was missed is <1%.

Likewise, histologic, ultrasonic, and chromosomal findings did not suggest that any specific etiologic differences were present between the groups. In the recreational runners there was one early loss, with conception confirmed only by β-hCG assay because no tissue other than decidua was recovered, six blighted ova, and one case in which a fetus was not found but the placental villi were vascularized. In the aerobic dancers there were two early losses with β-hCG confirmation only, three blighted ova, and two cases in which viability was confirmed, followed by embryonic death and abortion. One embryo was a trisomy 15, and in the other case there was ultrasonographic evidence of a subchorionic bleeding that was confirmed histologically. In the control group there was one very early loss with β-hCG confirmation, four blighted ova, and two cases where fetal viability was demonstrated before embryonic death and abortion. In one instance, placentation probably occurred on the septum of a previously undiagnosed subseptate uterus, and in the other, villous vascularity was markedly reduced along with pathologic findings consistent with hemorrhagic endovasculitis.

Over three specific time intervals, there were no significant differences in normalized exercise performance (percent preconceptional duration-intensity index) by the exercising women who aborted versus those who carried pregnancies to term, as shown in Table III. In the follicular phase of the cycle of conception, exercise performance in the runners who eventually aborted averaged (mean ± SD) 88% ± 13% of preconceptional duration-intensity index versus 94% ± 15% in those who carried to term. In the aerobic dancers performance averaged 78% ± 20% of preconceptional duration intensity index in those who eventually aborted versus 88% ± 30% in those who carried to term. In the initial 4 weeks after conception these values were 88% ± 16% and 86% ± 20% in the recreational runners and 78% ± 22% and 84% ± 28% in the aerobic dancers. In the ensuing 4 weeks similar values were 83% ± 17% and 80% ± 19% in the runners and 78% ± 24% and 84% ± 26% in the aerobic dancers.

The three congenital abnormalities diagnosed at birth were not clustered in either group. The single case of trisomy 21 occurred in a control subject. A case of subcoronal hypospadias was noted in the offspring of an aerobic dancer who, with the exception of postconceptional weeks 4 to 8, danced regularly three times each week throughout pregnancy, thereby maintaining her overall performance within 20% of her preconceptional duration-intensity index. A case of digital webbing or partial syndactyly between the second and third toes was noted in the offspring of a runner whose husband and two other relatives have similar anatomic findings.

The incidence of pregnancy events suggesting anomalous placentation was equally sparse. Despite careful monitoring no cases of ectopic pregnancy, placenta previa, abruptio placentae, or clinically diagnosed uteroplacental insufficiency have been observed in either group. There have been two cases of pregnancy-induced hypertension without proteinuria at term. One occurred in a control, the other in an aerobic dancer. The onset of labor occurred before the beginning of the 38th week in 5 cases with an incidence of approximately 5% in both groups.

Comment

These data do not support the initial hypothesis. Rather, they indicate that the continuation of several types of commonly performed antigravitational exercise through the periconceptional period and first trimester is not associated with an increased incidence of spontaneous abortion. Furthermore, although the data are limited, there is no suggestion that the incidence of other abnormal first-trimester events is increased by continuing exercise.

Given the fact that this type of exercise is associated with significant increases in body temperature and regional flow redistribution away from the viscera,[10-12] the interesting question is why. Limited data suggest that

the answer may lie in the extremely early timing of pregnancy-associated changes in maternal physiologic function.[17] Theoretically, early cardiovasular and thermoregulatory changes could have a protective effect by decreasing the flow redistribution requirements or exercise and diminishing the magnitude of its thermal effect. We are currently testing this hypothesis.

Given the large number of women who exercise regularly and plan to continue exercise during pregnancy,[11] these findings are reassuring. However, for several reasons, they may not be generally applicable. First, the fact that all subjects were uncompensated volunteers who had free access to information regarding some of the physiologic changes exercise induced in their bodies, both before and during pregnancy, may have introduced bias into the results as they may indeed not be representative of a more general exercise populace. Second, although the types of exercise were picked for initial study because they should have induced the greatest changes in the maternal internal environment,[10-12] other types of exercise in early pregnancy may be associated with entirely different effects. Third, the study populace was limited to women in excellent overall health and physical condition who had been exercising regularly for several years and their bodies and organ systems were undoubtedly well adapted to the recurrent physiologic stress imposed by exercise. Thus the impact of exercise on early pregnancy outcome might be very different in a group of women who, for example, began an exercise program in the periconceptional period. Fourth, with the exception of spontaneous abortion, the incidence of abnormal outcomes was extremely low. While this is reassuring, the chance of interpretive error is high and further study is warranted.

REFERENCES

1. Warburton D, Frazer FC. Spontaneous abortion risks in man: data from reproductive histories collected in a medical genetics unit. Am J Hum Genet 1964;16:1-25.
2. Rich HA, Weiss NS, Clarke EA, Miller AB. Risk factors for spontaneous abortion and its recurrence. Am J Epidemiol 1988;128:420-30.
3. Hertig AT, Rock J, Adams EC, Menkin MC. Thirty-four fertilized human ova, good, bad and indifferent, recovered from 210 women of known fertility. Pediatrics 1952;23:202-11.
4. Miller JF, Williamson E, Glue J, Gordon YB, Grudzinskas JG, Sykes A. Fetal loss after implantation. Lancet 1980; 1:554-6.
5. Edmonds DK, Lindsay KS, Miller JF, Williamson E, Wood PJ. Early embryonic mortality in women. Fertil Steril 1982;38:447-53.
6. Cashner KA, Christopher CR, Dysert GA. Spontaneous fetal loss after demonstration of a live fetus in the first trimester. Obstet Gynecol 1987;70:827-30.
7. Carr DH. The significance of chromosomal abnormalities in spontaneous abortion. In: Behrman SJ, Kistner RW, eds. Progress in infertility. Boston: Little Brown, 1975: 465-75.
8. Goldstein SR, Subramanyam BR, Raghavendra BN, Horii SC, Hilton S. Subchorionic bleeding in threatened abortion. Am J Radiol 1983;141:975-8.
9. Jouppila P, Huhtaniemi I, Tapanainen J. Early pregnancy failure: study by ultrasonic and hormonal methods. Obstet Gynecol 1980;55:42-7.
10. Clapp JF. The effects of maternal exercise during pregnancy on uterine blood flow and pregnancy outcome. In: Moawad AH, Lindheimer MD, eds. Uterine and placental blood flow. New York: Masson, 1982:171-6.
11. Clapp JF, Dickstein S. Endurance exercise and pregnancy outcome. Med Sci Sports Exerc 1984;16:556-62.
12. Clapp JF, Wesley M, Sleamaker RH. Thermoregulatory and metabolic responses to jogging prior to and during pregnancy. Med Sci Sports Exerc 1987;19:124-30.
13. Bradfield RB. A technique for determination of usual daily energy expenditure in the field. Am J Clin Nutr 1971;24:1148-54.
14. Pollock ML. The quantification of endurance training programs. In: Wilmore J, ed. Exercise and sports science reviews. New York: Academic Press, 1973:155-8.
15. Pollock ML, Wilmore JH, Fox SM. Health and fitness through physical activity. New York: John Wiley, 1978: 281-5.
16. Goldman ER, Busnick ER. A method for underwater weighing and the determination of body density. In: Brozeck J, Herschel A, eds. Techniques for measuring body composition. Washington, DC: US Government Printing Office, 1961:78-108.
17. Clapp JF, Seaward BL, Sleamaker RM, Hiser J. Maternal physiological adaptations to early human pregnancy. Am J Obstet Gynecol 1988;159:1456-60.

Oxygen consumption during treadmill exercise before, during, and after pregnancy

James F. Clapp III, MD
Burlington, Vermont

This study was designed to test the hypothesis that the amount of oxygen required to complete a specific treadmill exercise protocol changes during pregnancy as a function of gestational age, weight gain, and an individual's exercise regimen. The amount of oxygen required to complete a three-step, graded workload treadmill protocol was monitored before, every 6 to 8 weeks during, and after a clinically normal, accurately dated, singleton pregnancy in 18 fit, recreational athletes. Nine subjects continued a moderate- to high-intensity exercise regimen and nine did not. The amount of oxygen required to complete each stage of the protocol decreased significantly (6% to 15%) in both groups in early pregnancy. In the women who continued exercise, the amount of oxygen required remained at or significantly below that required before conception for the remainder of the pregnancy and the initial postpartum period. Conversely, in the women who discontinued regular exercise, the amount of oxygen required for each stage rose progressively throughout the remainder of pregnancy at an average rate of 2% per lunar month. It peaked in the thirty-seventh week and remained significantly elevated 7 weeks post partum. However, when the data were corrected for the additional requirement imposed by pregnancy weight gain, net efficiency was increased in both groups throughout pregnancy. These data support three conclusions. First, the efficacy of low- to moderate-intensity treadmill exercise in physically active women is improved in early pregnancy. Second, this increased efficiency is masked later in pregnancy by the effects of weight gain. Third, the degree of efficiency is enhanced throughout pregnancy in women who continue a regular exercise regimen at or above a basic conditioning level. (Am J Obstet Gynecol 1989;161:1458-64.)

Key words: Pregnancy, exercise, oxygen consumption, efficiency

The interaction between the physiologic adaptations to pregnancy and endurance exercise are complex and to date poorly understood.[1, 2] The impact that one may have on the other is unclear since a specific adaptation to one may either oppose or complement the overall physiologic readjustment required by the other.[3, 4] For example, the cardiovascular and metabolic adaptations to pregnancy that occur by the seventh postconceptional week[5, 6] appear to compliment some of the physiologic adaptations produced by endurance training. These pregnancy-induced changes provide a potential physiologic explanation for the anecdotal observation that the competitive performance of world-class athletes may be improved in early pregnancy. This is also supported by one case report that documented an increased exercise efficiency in an individual who maintained her jogging regimen throughout pregnancy.[7] Longitudinal studies, however, of healthy pregnant women, begun after the first trimester, conclude that the efficiency of non-weight-bearing ergometer exercise is either unchanged or decreased[8-10] and that the efficiency of weight-bearing treadmill exercise decreases in rough proportion to the increase in body weight.[8, 10-12]

The current study was designed to examine the impact of pregnancy on the efficiency of weight-bearing exercise in greater detail by serially monitoring the oxygen required to complete a standardized, progressive workload, exercise task before, during, and after pregnancy, controlling for the confounding effects of initial fitness, exercise performance during pregnancy, and weight gain. It was specifically designed to test the hypothesis that the amount of oxygen required to complete a specific weight-bearing exercise task during pregnancy changes as a function of gestational age, maternal weight gain, and an individual's concurrent exercise regimen.

Material and methods

Eighteen women were studied serially before, every 6 to 8 weeks during, and 6 to 8 weeks after a clinically normal, accurately dated, singleton pregnancy. All subjects were breast-feeding without supplementation at

From the Department of Obstetrics and Gynecology, University of Vermont College of Medicine.
Supported in part by National Institutes of Health grant HD-21268 and grant 6-464 from the March of Dimes Birth Defects Foundation.
Presented in part at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.
Reprint requests: James F. Clapp III, MD, Department of Obstetrics and Gynecology, Given Medical Building, Burlington, VT 05405-0068.
6/6/15970

Table I. Study group characteristics

Parameter	*Exercise-continued subgroup*	*Exercise-stopped subgroup*	*All subjects*
Age (yr)	30.8 ± 0.7	31.0 ± 0.9	30.9 ± 0.6
Nulliparous/primiparous	5/4	6/3	11/7
Runners/aerobics	5/4	5/4	10/8
Duration-intensity index (before pregnancy)	11,760 ± 588	11,390 ± 602	11,580 ± 596
AM pulse (beats/min)	51 ± 2	52 ± 2	51 ± 2
Weight (kg)	58.8 ± 1.5	57.6 ± 1.0	58.2 ± 0.9
Body fat (%)	16.5 ± 1.0	20.4 ± 1.9*	18.4 ± 1.4
$\dot{V}O_2$ max (ml/kg/min)	56.3 ± 2.4	52.5 ± 1.4	54.5 ± 1.5

All data presented as mean ± SEM.
*Significant between-subgroup difference at the 0.05 level.

the time of the postpartum study. Written informed consent was obtained from each subject before enrollment. All subjects worked outside the home, had physically active life-styles, and were upper-middle or upper socioeconomic class. All were in excellent health and had maintained a regular antigravitational exercise regimen (running, cross-country skiing, aerobics), consisting of at least three moderate- to high-intensity training sessions (50% to 85% of maximum intensity for a minimum of 30 minutes) per week for ≥2 years before study entry.

In all subjects reported exercise performance was confirmed and quantitated by monitoring individual daily exercise performance for a minimum of 6 weeks before conception and throughout pregnancy by means of standard field techniques described elsewhere.[13] Briefly, maximal oxygen consumption was measured before pregnancy, and the linear relationship between heart rate and oxygen consumption was determined serially before and during pregnancy. This relationship was used to convert the pulse rate during exercise, which was continuously recorded during each field exercise session, to intensity (percent of maximum intensity). The product of this and duration (the duration-intensity index) was used as the quantitative measure of exercise performance.

Nine women maintained their exercise regimens throughout pregnancy at an overall performance level well above 50% of their preconceptional level and form the exercise-continued subgroup. The remaining nine subjects form the exercise-stopped subgroup. On the basis of individual concerns or preference, they either stopped their training regimens completely at the time of conception ($n = 5$) or continued to exercise only intermittently and at low intensity with an overall performance level well below 25% of the preconceptional level ($n = 4$). It should be stressed that subgroup assignment was based on subject preference and was not randomized.

The characteristics of the overall study group and two subgroups are detailed in Table I. The subjects ranged in age from 26 to 35 and approximately two thirds were nulligravid at the time of study entry. Running or jogging was the primary exercise for approximately 60%, while 40% preferred aerobics. Before pregnancy, weekly exercise performance, quantitiated as the duration-intensity index, was similar in the two subgroups. Preconceptional body weights ranged between 53.0 and 64.7 kg.

Fitness was objectively assessed before conception according to AM heart rate, fat mass, and maximum oxygen consumption. The first was recorded with a telemetry device[13] on awaking and before arising. Values ranged between 42 and 59 beats/min in both groups. Body fat was estimated with the sum of six-site skinfolds[5] and ranged between 11.5% and 29.2%. Maximum oxygen consumption was measured directly by means of automated respiratory calorimetry and a constant speed, progressive slope, treadmill protocol.[13] All subjects registered values in the upper 5th percentile. The only significant difference between subgroups was a greater percent of body fat in the controls.

The treadmill protocol always began 90 to 150 minutes after a normal meal and was the first exercise of the day. On arrival the subjects changed into lightweight exercise apparel and then were weighed and fitted with electrocardiogram electrodes and respiratory headgear. The laboratory temperature and relative humidity were maintained between 19° and 21° C and between 30% and 50%, respectively. Each individual's attire was the same for each of her testing sessions.

On each testing occasions, the subjects initially remained at standing rest for 8 minutes while they adapted to the respiratory apparatus and laboratory environment. During the next 7 minutes, measurements of oxygen consumption at standing rest were obtained. Then they began a 36-minute, progressive workload, exercise protocol that was divided into three 12-minute stages. Throughout each progressive stage, the treadmill speed and grade were kept constant, and the initial 5 minutes of data was discarded to be sure

that a new steady state of oxygen consumption had been reached (variability $< \pm 10\%$). The stable oxygen consumption over the last 7 minutes was measured, averaged, and, after subtraction of the oxygen consumption at standing rest, used as the oxygen cost of that workload in later data analysis.

To allow meaningful comparison between individuals, the intensity of the three progressive workloads was standardized at the time of the preconceptional test to require an oxygen consumption equal to approximately 20%, 30%, and 40% of each individual's preconceptional maximum oxygen consumption. Once determined, the same treadmill speed and grades were used for testing the subject during each subsequent session.

Minute ventilation was measured with a pneumotachometer that was calibrated monthly and had a precision of $\pm 3\%$ when compared under actual test conditions with a Tissot apparatus. The oxygen and carbon dioxide content of room air and expired air were measured with applied electrochemistry analyzers, which were calibrated with standard gasses before and after each use. The precision of the entire system for the measurement of oxygen consumption under test conditions was $\pm 5\%$ when compared with concomitant use of a Douglas bag, dry gas meter, and oxygen analyzer.

The initial 10 subjects completed the same protocol twice before pregnancy to assess the biologic variability of the protocol and to determine if a training effect existed. The results of the second testing session ranged from 95% to 107% of the first with a group average of $101\% \pm 3\%$. Thus the biologic variability was no greater than the reproducibility of the measurement and there was no evidence of a training effect.

The oxygen consumption data were analyzed in several ways to detect pregnancy- and exercise-associated changes in efficiency. First, the raw data were analyzed longitudinally without correction for weight gain. Second, the amount of oxygen required to climb vertically at a rate of 5 m/min was calculated from the increase in oxygen consumption produced by a change in treadmill grade at a constant speed. Finally, these data were corrected for the effect of weight gain by use of the longitudinal data gathered by Hanson[14] in subjects who exprienced similar weight gains and completed similar protocols in studies of experimental obesity. Within-group significant changes over time were detected by analysis of variance, repeated measures analysis of variance, and Duncan's multiple range test. Between-group differences at a single time point were detected with Student's unpaired t test. A p value <0.05 was set as the level of significance.

Results

Table II details the longitudinal changes in the amount of oxygen required to perform the progressive treadmill workloads before, during, and after pregnancy in the two subgroups and the overall study populace. The subjects who continued exercise during pregnancy experienced a significant decrease in the oxygen required to complete all three workloads in the seventh and fifteenth weeks of pregnancy, and in the seventh week post partum. At the 20% workload the oxygen requirement remained significantly lower through the thirty-first week. At the various time points and workloads, the group mean was between 6% and 16% below the preconceptional value. The women who discontinued a regular exercise regimen at the beginning of pregnancy experienced a similar decrease in the oxygen requirement in the seventh week of pregnancy that reached significance at the two highest workloads. Thereafter their oxygen requirements rose significantly over time at all workloads at an approximate rate of 3% per lunar month through the thirty-seventh week and reached values that peaked 15% to 18% above those observed preconceptionally. The mean values were significantly higher than the preconceptional ones from the twenty-fifth week onward at the 30% workload, from the thirty-first week onward at the 40% workload, and only during the thirty-seventh week at the 20% workload. At all workloads the postpartum requirement remained significantly higher than the preconceptional one by 6% to 10%. The divergent patterns in the two subgroups offset one another so that the only consistent significant change in the overall study populace was a fall in the oxygen requirement at 7 weeks' gestation. However, when the two subgroups were compared with each other at each time point, significant differences in the absolute change from preconceptional values were present from the fifteenth week onward at all workloads. For the 40% workload, this difference peaked at 170 ml/min in the thirty-first and thirty-seventh weeks and was maintained at that level in the initial postpartum period. Overall, the relative oxygen cost in the women who continued exercise remained 15% to 18% below that of those who did not.

The first portion of Table III presents the longitudinal changes in the calculated oxygen cost of vertical work in the two subgroups. In this calculation, the difference in the oxygen consumption produced by an increase in treadmill grade at a constant speed was simply normalized for each subject to a climbing rate of 5 m/min. Throughout pregnancy and the initial postpartum period, the oxygen requirement of the subjects who continued the exercise regimen remained at or below the preconceptional level. The maximum decrease of 15% was observed in the seventh week, but the values were also significantly lower in the fifteenth and thirty-first weeks despite increases in body weight of 8% and 18%, respectively. The subjects who stopped

Table II. Oxygen cost of the three treadmill workloads

Group	Before pregnancy	Weeks' gestation 7	15	23	31	37	Post partum
20% Workload							
EX	405 ± 31	341 ± 29*	365 ± 26*	361 ± 35*	372 ± 37*	399 ± 39	369 ± 27*
NO	361 ± 19	339 ± 30	361 ± 27	372 ± 28	381 ± 33	422 ± 28*	387 ± 24*
ALL	383 ± 18	341 ± 20*	363 ± 18*	366 ± 22	376 ± 24	410 ± 23	378 ± 18
30% Workload							
EX	687 ± 46	622 ± 41*	647 ± 47*	684 ± 61	685 ± 66	691 ± 63	632 ± 61*
NO	586 ± 28	557 ± 34*	590 ± 39	628 ± 39*	653 ± 44*	673 ± 40*	626 ± 35*
ALL	636 ± 36	589 ± 27*	618 ± 30	656 ± 36	669 ± 39*	682 ± 36*	638 ± 35
40% Workload							
EX	1055 ± 64	957 ± 56*	978 ± 55*	1024 ± 59	1018 ± 80	1065 ± 59	956 ± 56*
NO	895 ± 25	829 ± 51*	921 ± 41	988 ± 59	1027 ± 57*	1055 ± 72*	964 ± 53*
ALL	980 ± 40	897 ± 40*	952 ± 35	1007 ± 41	1022 ± 49	1061 ± 45	960 ± 38

All data presented in milliliters of oxygen per minute as mean ± SEM. *EX*, Exercise-continued subgroup. *NO*, exercise-stopped subgroup. *ALL*, Overall study populace.

*Significantly ($p < 0.05$) higher or lower than preconceptional value.

their regular exercise regimens also experienced a significant 8% decrease in the oxygen requirement in the seventh week, but this was followed by a significant increase over time in the oxygen requirement through the thirty-seventh week at an approximate rate of 29 ml per lunar month. As a result, the oxygen requirement was significantly higher by 18% to 19% in the thirty-first and thirty-seventh weeks. In both subgroups the value returned to preconceptional levels in the initial pospartum period. Again, the divergent patterns in the two subgroups offset one another so that the only significant longitudinal difference in the overall study populace was a 12% reduction in the oxygen requirement in the seventh week. However, as noted, earlier in the raw workload data, significant between-subgroup differences in the absolute changes from preconceptional values were present at the individual time points from the fifteenth week onward. At the various time points, the relative oxygen cost of climbing at a rate of 5 m/min ranged between 15% and 31% less in the women who continued their training regimens throughout pregnancy.

Significant progressive weight gain was noted throughout pregnancy. This was roughly linear for each subject (R^2 between 0.87 and 0.99). In the exercise-continued and exercise-stopped subgroups, the average rate of increase approximated 1.3 and 1.7 kg per lunar month, respectively. This represented a total weight gain approximating 21% and 27% of preconceptional weight in the two subgroups, which clearly exceeded the percentage increase in oxygen consumption, indicating that the oxygen requirement per kilogram actually decreased. Indeed, the calculated increase in the workload of climbing at the rate of 5 m/min because of weight gain was 61 and 79 kpm/min in the two respective subgroups. To offset this increase in workload, the data were adjusted for the increased oxygen cost attributable to individual weight gain at each time point by a correction factor of −2.6 ml oxygen per kilopond meter per minute. This was calculated directly from data obtained in a study of experimental obesity in which a nearly identical treadmill workload was used before and after similar weight gains and losses.[14] The weight-corrected data are presented in the second portion of Table III. Note that when the effect of pregnancy weight gain is considered the initial significant decrease in the oxygen requirement is maintained in both subgroups throughout pregnancy and the initial postpartum period. The greatest decreases were observed in the third trimester when the values were 37% to 42% lower in the exercise subgroup and 23% to 35% lower in the control subgroup. When the relative change from preconceptional levels in the weight-corrected oxygen requirements of the two subgroups was compared at the various time points of study, the decrease was significantly greater in the exercise subgroup in the seventh, fifteenth, twenty-fourth, and thirty-first weeks. At these time points, the relative decrease in the execise-stopped subgroup was between 33% and 54% of that observed in the exercise-continued subgroup with a peak difference of 104 ml/min in the thirty-first week. No significant difference between subgroups was observed at either 37 weeks or in the seventh week post partum.

Comment

These data support the basic hypothesis in an unambiguous fashion. First, they demonstrate that, in physically fit and active women, there is a consistent (16 of 18 subjects) decrease in the oxygen required to complete a series of progressive, low- to medium-load, antigravitational exercise tasks in the middle of the first

trimester despite a 3% increase in body weight. Furthermore, work efficiency, measured by the decrease in oxygen requirement for the vertical component of the exercise, was clearly increased at this point in pregnancy in both subgroups. Second, they indicate that the continuation of an endurance exercise training regimen during pregnancy significantly modifies the longitudinal change in oxygen required to complete the three workloads throughout pregnancy, as well as the longitudinal change in antigravitational work efficiency. Without adjustment for the effects of pregnancy weight gain, the changes in oxygen requirement were at least 15% less in the exercise-continued subgroup and the longitudinal changes in efficiency were at least 15% less in the exercise-stopped subgroup from the fifteenth week onward. Finally, the data indicate that the magnitude of the decrease in oxygen requirement in the exercise-continued subgroup and the increase in the exercise efficiency in both subgroups is obscured after the fifteenth week by the pregnancy-associated increases in maternal weight. For example, in the thirty-seventh week maternal weight gain should have increased the oxygen requirement for antigravitational work to a level approximately 40% above that observed before pregnancy. The fact that an increase of this magnitude was not observed clearly suggests a marked increase in efficiency that is documented by the weight-corrected data. Thus, when the data are adjusted for weight gain, the increased efficiency seen in the seventh week is maintained throughout the remainder of pregnancy in both subgroups with the improvement being greater in the women who maintained their preconceptional exercise regimens.

These findings and conclusions differ from those reported by others. Hutchinson et al.[11] reported that the oxygen required to run on the flat at 6 mph rose at a rate of 50 ml/month from the third month of pregnancy onward with a value 4 months post partum equivalent to that recorded in the third month. Unfortunately, measurements were not made either before conception or at the time we noted the initial decrease in the oxygen requirement, nor could these data be corrected for the impact of pregnancy weight gain and postpartum weight loss as no vertical work was done. Thus comparison with the present data is difficult. It is interesting, however, to note that the percentage change in weight exceeded the percentage increase in oxygen consumption during pregnancy and that the postpartum oxygen requirement exceeded that recorded in the third month of pregnancy, despite the fact that the subject weighed 1.6 kg less post partum. Both suggest that the weight-corrected oxygen requirement may well have been less during pregnancy, especially early pregnancy.

Knuttegen and Emerson[8] studied 13 pregnant women during treadmill exercise that was roughly equivalent to our 30% workload. The data reported clearly show that the mean oxygen requirement for the group fell post partum in rough proportion to the decrease in weight. In this regard the data from the current study are similar. On the basis of this single finding, however, the authors concluded that the increased stress of treadmill exercise during pregnancy is due to weight gain. In contrast, they noted that only two of the women showed a progressive increase in oxygen requirement for the protocol during pregnancy. This was not discussed further and the longitudinal trends observed in the other 11 women were not described, nor was the relationship between serial weight gain and oxygen requirement addressed. Clearly, it is possible that the weight-corrected oxygen requirement might have decreased over the latter half of pregnancy in 11 of the 13 subjects studied. Blackburn and Calloway[10] used a graded treadmill exercise protocol to study work efficiency serially throughout the latter half of pregnancy and post partum. They concluded that the caloric expenditure for the different workloads increased progressively during the latter half of pregnancy and fell post partum and that the cost per unit weight was unchanged over the time period of study. They also noted that efficiency might actually be increased if one subtracted the resting caloric expenditure, which also rose progressively. Unfortunately, although their data allowed it, they did not pursue the issue of efficiency further by calculating the longitudinal change in the caloric requirement for vertical work. When this is calculated without a correction for weight gain, the caloric requirement to climb at a rate of 8 m/min was decreased by 12% between the twentieth and thirty-sixth weeks of pregnancy when compared with the cost post partum and was no different from the postpartum value between the thirty-seventh and fortieth weeks. Thus their data indicate that work efficiency is improved in pregnancy and are actually in substantial agreement with those obtained in the current study.

Despite these findings, however, it is clear that the oxygen cost of vertical work increases in direct proportion to weight gain and decreases in direct proportion to weight loss in nonpregnant subjects studied serially during changes in either fat mass[14] or weighted load.[15] Thus it appears that there is a fundamental change in the efficiency of uphill walking associated with pregnancy, but the mechanism for this change is unclear. While pregnancy-associated factors such as the change in the center of gravity and stride length could potentially influence efficiency, the magnitude of the change suggests either a fundamental improvement in contractile efficiency at a cellular level or an unappreciated systematic error in data accrual. Factors favoring the former explanation are speculative at this time but include the increases in metabolic efficiency associated with training and with nutritional and work stress,[16-18]

Table III. Oxygen required for vertical work at a rate of 5 m/min

Group	Before pregnancy	Weeks' gestation					Post partum
		7	15	23	31	37	
Absolute values							
EX	467 ± 30	396 ± 10*	419 ± 18*	442 ± 19	406 ± 20*	472 ± 15	464 ± 19
NO	417 ± 23	382 ± 21*	439 ± 17	479 ± 23	491 ± 26*	497 ± 45*	429 ± 23
ALL	442 ± 19	389 ± 11*	430 ± 12	466 ± 16	448 ± 19	485 ± 23	446 ± 15
Corrected values							
EX	467 ± 30	369 ± 8*	362 ± 15*	331 ± 17*	269 ± 19*	313 ± 14*	422 ± 18*
NO	417 ± 23	364 ± 20*	383 ± 16*	355 ± 21*	323 ± 24*	293 ± 40*	359 ± 21*
ALL	442 ± 19	366 ± 10*	373 ± 10*	349 ± 14*	296 ± 16*	300 ± 20*	393 ± 14*

Data presented in milliliters of oxygen per minute as mean ± SEM. *EX*, Exercise-continued subgroup. *No*, Exercise-stopped subgroup. *ALL*, Overall study populace.

*Significant difference from preconceptional values.

particularly when heavy physical activity and nutritional stress are combined in pregnancy.[18] The most likely factors to cause a systematic error would be the presence of a training effect, an anaerobic metabolic component, or a marked oxygen debt. The first is quite unlikely because we found no evidence to support a training effect in our initial replicate studies nor have others seen it at similar intensities.[8, 10, 14] The second, although possible, is unlikely for several reasons. First, these were serial studies in very fit subjects and the workloads were light to moderate. Second, in earlier studies in similar women, lactate levels actually fell with exercise of slightly greater intensity than this during mid and late pregnancy.[13] Finally, a pregnancy-associated increase in oxygen debt is a real possibility noted or considered by others.[9, 10] This, however, was not measured in the current study for several reasons. First, the study design, with three progressive workloads without separate recoveries, did not allow us to measure it for each workload independently. Second, there is no evidence that the oxygen debt accrued at the very beginning of muscular activity is increased further by a change in exercise intensity of this magnitude. Third, in the current study we assessed changes in efficiency from the change in the difference in oxygen consumption produced by a change in grade. Any potential oxygen debt should not influence this measurement. Thus an increase in oxygen debt also appears to be an unlikely explanation.

As we only determined maximum oxygen consumption before pregnancy (human subject safety concerns), one must consider whether a pregnancy-associated increase or decrease in an individual's maximum oxygen consumption would in and of itself influence the oxygen required to perform a set, progressive load, treadmill exercise task. Training and detraining data in pregnancy[19] suggest that the associated increases and decreases in maximum oxygen consumption are reflected by changes in the relative oxygen requirement for a standard task (percent of maximum oxygen consumption). There is no evidence, however, that a change in maximum oxygen consumption significantly influences the absolute oxygen requirement for a standard task (milliliters per minute).[20] Thus exercise performance should influence perceived exertion, heart rate, etc., but should not affect the absolute oxygen requirement. In the current study we assumed that changes in maximum oxygen consumption would not change the absolute oxygen requirement and used maximum oxygen consumption only as a means to standardize the relative preconceptional oxygen requirements (intensity) for the progressive workload task between individuals. Because of differences in weight and efficiency, the actual individual absolute oxygen requirements and tasks were quite different. Once set, each individual's progressive workload task did not change in the subsequent testing periods. While it is likely that maximum oxygen consumption and therefore the relative oxygen requirement (percent of maximum) changed during pregnancy as a function of exercise performance, in and of itself, this should not have influenced the absolute oxygen requirement for performing the identical progressive workload task.[20] The latter would be influenced by weight[15, 16] and the other efficiency-related factors discussed above.

In summary, the data obtained in this prospective, serial study of a fairly uniform, physically fit populace support the initial hypothesis. They indicate that in this populace the efficiency of low- to moderate-intensity exercise is increased throughout pregnancy and that the increase is enhanced in women who choose to continue their training regimens throughout pregnancy. Review of several earlier studies suggests that these findings also may apply to less physically active groups of women. Currently, the mechanism responsible for the pregnancy-associated change in efficiency is not understood.

REFERENCES

1. Artal R, Wiswell RA. Exercise in pregnancy. Baltimore: Williams & Wilkins, 1986.

2. Clapp JF. Pregnancy. In: Franklin BA, Gordon S, Timmis GC, eds. Exercise in modern medicine. Baltimore: Williams & Wilkins, 189:268-79.
3. Clapp JF, Dickstein S. Endurance exercise and pregnancy outcome. Med Sci Sports Exerc 1984;16:556-62.
4. Clapp JF. Physical exercise and sport during pregnancy. Gynakologe 1987;20:144-50.
5. Clapp JF, Seaward BL, Sleamaker RH, Hiser J. Maternal physiologic adaptations to early human pregnancy. Am J Obstet Gynecol 1988;159:1456-60.
6. Capeless EL, Clapp JF. Cardiovascular changes in early phase of pregnancy. Am J Obstet Gynecol 1989;161: 1449-53.
7. Ruhling RO, Cameron J, Sibley L, Christensen CL, Bolen T. Maintaining aerobic fitness while jogging through a pregnancy! A case study. Med Sci Sports Exerc 1981; 13:93.
8. Knuttengen HG, Emerson K. Physiological response to pregnancy at rest and during exercise. J Appl Physiol 1974;36:549-53.
9. Pernoll ML, Metcalfe J, Schlenker TL, Welck JE, Matsumoto JA. Oxygen consumption at rest and during exercise in pregnancy. Respir Physiol 1975;25:285-93.
10. Blackburn MW, Calloway DH. Basal metabolic rate and work energy expenditure of mature, pregnant women. J Am Diet Assoc 1976;69:24-8.
11. Hutchinson PL, Cureton KJ, Sparling PB. Metabolic and circulatory responses to running during pregnancy. Phys Sportsmed 1981;9:55-61.
12. Diblee L, Graham TE. A longitudinal study of changes in aerobic fitness, body composition, and energy intake in primigravid patients. Am J Obstet Gynecol 1983; 147:908-14.
13. Clapp JF, Wesley M, Sleamaker RH. Thermoregulatory and metabolic responses to jogging prior to and during pregnancy. Med Sci Sports Exerc 1987;19:124-30.
14. Hanson JS. Exercise responses following production of experimental obesity. J Appl Physiol 1973;35:587-91.
15. Goldman RF, Iampietro PF. Energy cost of load carriage. J Appl Phyiol 1962;17:675-6.
16. Westerterp KR, Saris WHM, van Es M, ten Hoor F. Use of the doubly labled water technique in humans during heavy sustained exercise. J Appl Physiol 1986;61:2162-7.
17. de Boer JO, van Es AJH, Roovers LA, van Raaij JMA, Hautvast JGAJ. Adaptation of energy metabolism of overweight women to low-energy intake, studied with whole body calorimeters. Am J Clin Nutr 1986;44:585-95.
18. Tafari N, Naeye RL, Gobezie A. Effects of maternal undernutrition and heavy physical work during pregnancy on birth weight. Br J Obstet Gynaecol 1980;87:222-6.
19. Collings CA, Curet LB, Mullin LB. Maternal and fetal responses to a maternal aerobic exercise program. Am J Obstet Gynecol 1983;145:702-7.
20. Astrand PO, Rodal K. Textbook of work physiology: physiological basis of exercise. 3rd ed. New York: McGraw Hill, 1986:436, 470.

Exercise prescription in pregnancy: Weight-bearing versus non-weight-bearing exercise

Raul Artal, MD, Damon I. Masaki, MD, Nazareth Khodiguian, PhD, Yitzhak Romem, MD, Susan E. Rutherford, MD, and Robert A. Wiswell, PhD

Los Angeles, California

Bicycle ergometry (non-weight-bearing exercise) and treadmill (weight-bearing exercise) were compared to assess physiologic responses to similar work loads. A total of 22 subjects at 29.3 ± 1.6 (±SEM) weeks' gestation who performed non-weight-bearing exercise were compared with 15 similarly fit subjects at 26.1 ± 2.3 weeks' gestation who performed weight-bearing exercise at three submaximal levels. Measurements by indirect calorimetry indicate preferential carbohydrate use during non-weight-bearing exercise at submaximal levels. (Am J Obstet Gynecol 1989;161:1464-9.)

Key words: Exercise prescription in pregnancy

Exercise is routinely prescribed because of various clinical conditions in nonpregnant patients. Recently, exercise has been proposed as adjunctive therapy for gestational diabetes.[1] It appears that exercise facilitates the use of glucose uptake by postreceptor mechanisms that regulate glucose transport and intracellular glucose metabolism.[2, 3] Because gestational diabetes is a disease of glucose intolerance, therapeutic modalities to overcome this resistance and improve glucose metabolism may be preferred rather than insulin administration. If exercise can successfully maintain normoglycemia and avert the use of insulin in patients with gestational diabetes, this could reduce maternal and infant morbidity and avoid complications related with insulin therapy.

Exercise prescription requires knowledge of poten-

From the Departments of Obstetrics and Gynecology and Exercise Sciences, University of Southern California School of Medicine.
Presented at the Thirty-fifth Annual Meeting of the Society for Gynecologic Investigation, Baltimore, Maryland, March 17-20, 1988.
Reprint requests: Raul Artal, MD, University of Southern California School of Medicine, Women's Hospital, Department of Obstetrics and Gynecology, Room 5K-40 1240 N. Mission Road, Los Angeles, CA 90033.
6/6/15657

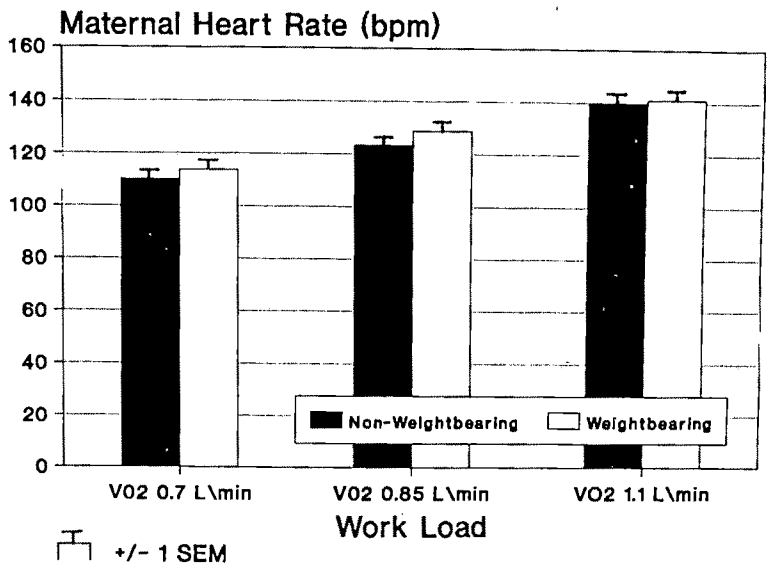


Fig. 1. Maternal heart rate responses during non-weight-bearing (bicycle ergometry) and weight-bearing (treadmill) exercise at incremental work loads.

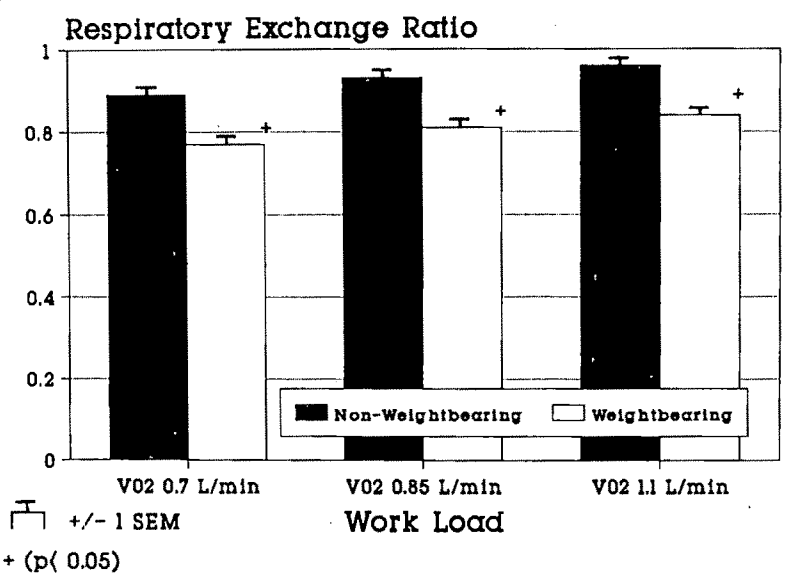


Fig. 2. Respiratory exchange ratio during non-weight-bearing (bicycle ergometry) and weight-bearing (treadmill) exercise at incremental work loads.

Table I. Clinical data of study subjects

Characteristics	*Non-weight-bearing exercise*	*Weight-bearing exercise*	*p Value*
Number of subjects	22	15	NS
Age (yr)	29.8 ± 0.5	30.3 ± 1.9	NS
Gestational age (wk)	28.3 ± 1.8	25.9 ± 2.5	NS
Weight (kg)	70.8 ± 2.7	65.1 ± 2.9	NS
VO_2max/kg (ml/kg/min)	28.6 ± 1.8	26.5 ± 1.7	NS
Tidal volume at rest (L)	0.69 ± 0.04	0.68 ± 0.06	NS
Tidal volume at VO_2max (L)	1.96 ± 0.08	1.67 ± 0.10	0.03

Mean ± SEM.
NS = Not significant ($p > 0.05$).

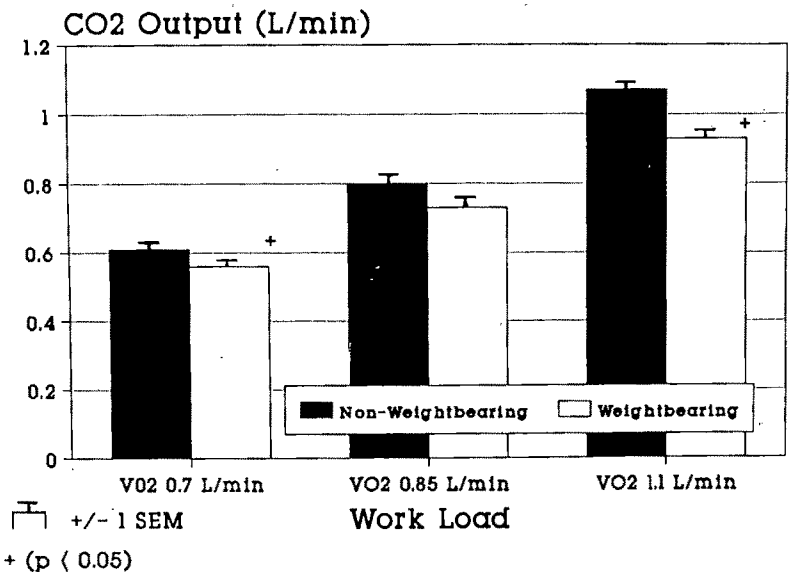


Fig. 3. CO_2 output (L/min) during non-weight-bearing (bicycle ergometry) and weight-bearing (treadmill) exercise at incremental work loads.

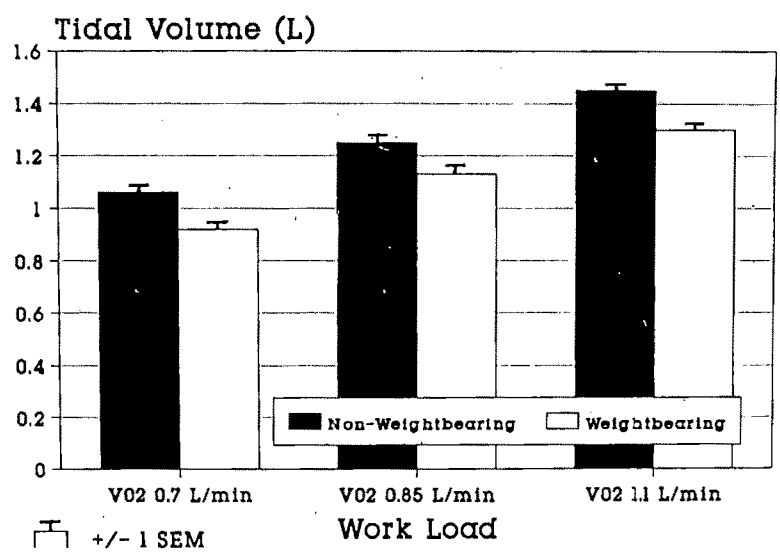


Fig. 4. Tidal volume (L) during non-weight-bearing (bicycle ergometry) and weight-bearing (treadmill) exercise at incremental work loads.

tial risks, general physical condition, and work capacity to estimate the training intensity necessary to induce conditioning or desired effects. To establish the safety of exercise therapy in gestational diabetes a pilot study was performed.[4] We investigated the effects of mild treadmill exercise on normal mothers and their infants as well as pregnancies complicated by gestational diabetes and reported no immediate adverse effects.

Exercise prescription in the treatment of gestational diabetes is investigational. Therefore exercise studies on substrate use during pregnancy are needed. One method to assess substrate use is indirect calorimetry through determinations of the respiratory exchange ratio (R). R is a respiratory variable that reflects the ratio between carbon dioxide output (VCO_2) and oxygen uptake (VO_2) and provides information on the proportion of substrate derived from various foodstuffs.[5,6] If carbohydrates are completely oxidized to CO_2 and water (H_2O), one volume of CO_2 is produced for each volume of O_2 consumed. Hence an R value of 1.0 would indicate that only carbohydrates are being used ($C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$; $R = VCO_2/VO_2 = 6/6 = 1.0$). An R value of 0.7 would indicate that fats are being burned. Any value between 0.7 and 1.0 would reflect the relative proportion of carbohydrate and fat being used.

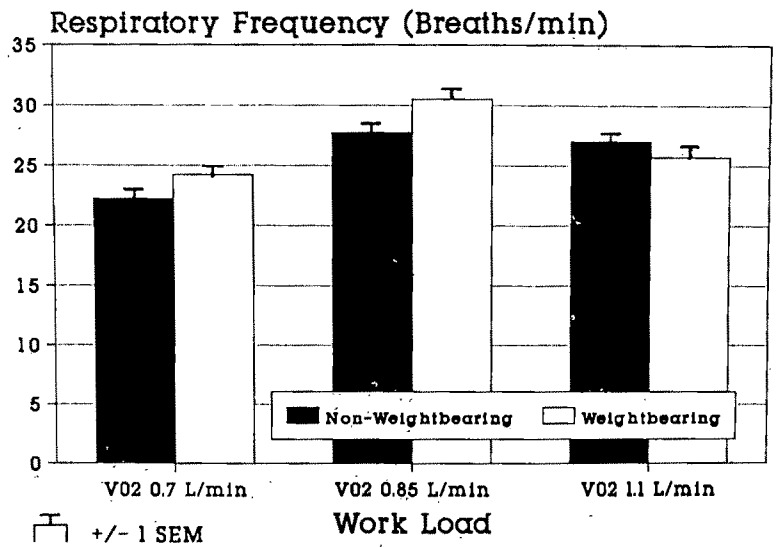


Fig. 5. Respiratory frequency (breaths/min) during non-weight-bearing (bicycle ergometry) and weight-bearing (treadmill) exercise at incremental work loads.

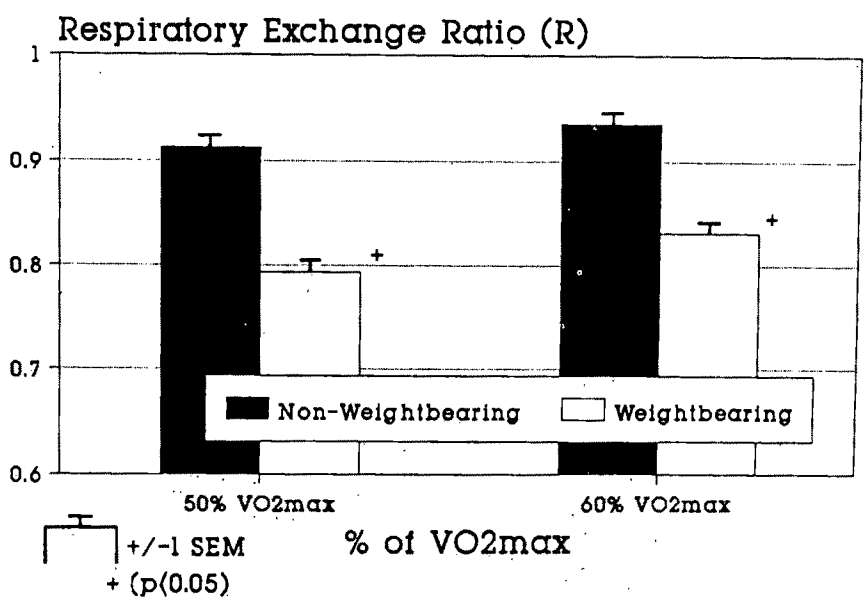


Fig. 6. Respiratory exchange ratio at 50% and 60% of the individual VO_2max.

The objective of this study was to compare different exercise regimens and responses between non-weight-bearing exercise such as bicycle ergometry and weight-bearing exercise such as the treadmill in persons with uncomplicated pregnancies.

Material and methods

A total of 37 healthy pregnant subjects were studied after informed consent was obtained. The subject characteristics are summarized in Table I. On the basis of an intake questionnaire, both groups were found to be of similar low-to-average levels of fitness judged by their regular physical activities and later confirmed by similar maximal O_2 uptake (VO_2max) stress test. A total of 22 subjects performed bicycle ergometry exercise and 15 subjects performed treadmill exercise. The participants were not randomly assigned in the study; group assignment was not stratified but rather was based on date of entry into the study. Bicycle ergometry exercise involved work loads of 25, 50, and 75 W with steady state achieved in 7 minutes with 5-minute rest periods between stages. To achieve VO_2max an initial work load of 75 W with increasing increments of 25 W every 2 minutes was performed. A modified Balke protocol was used in the treadmill group.[7] The intensity of exercise was started at 2.5 mph at 0% eleva-

tion and was increased 2% every minute until exhaustion. Similar submaximal levels of exercise were performed by both groups. For the purpose of analysis, the levels of exercise were quantitated by the level of VO_2 by arbitrarily chosen increasing work loads. The data were grouped by incremental work loads of $VO_2 = 0.7 \pm 0.05$ L/min, $VO_2 = 0.85 \pm 0.05$ L/min, and $VO_2 = 1.1 \pm 0.05$ L/min. In addition, to obtain uniform standardized data independent of weight and level of fitness, the R data were then grouped and analyzed at points that corresponded with the 50% and 60% of the individual VO_2max (see Fig. 6).

VCO_2, VO_2, ventilatory parameters, and maternal heart rate were measured at each work load. The Beckman Metabolic Measurement Cart (model MMC) with open circuit spirometry was used for all measurements. The equipment was calibrated daily before the experiments were started. Indirect calorimetry was used to derive R.[5]

Fetal heart rate (FHR) and uterine activity were continuously monitored before and after exercise with a Corometrics fetal monitor (model 140). FHR and uterine activity were also monitored during exercise when technically feasible. Unpaired Student *t* test analysis with the use of MR-TOD (Medical Research–Time Oriented Data base, Seattle, Wash.) for statistical significance were performed with α equal to 0.05.

This investigation was approved by the University of Southern California School of Medicine Human Research Committee.

Results

Table I presents the subject characteristics of both the non-weight-bearing and weight-bearing exercise groups. No statistical differences were found between the subject characteristics at rest.

Figs. 1 to 5 summarize maternal heart rate response, R values, VCO_2, and ventilatory parameters (tidal volume and respiratory frequency). Maternal heart rate responses were similar in both groups. The R values were significantly higher during non-weight-bearing exercise than during weight-bearing exercise. This difference was consistent at all preset submaximal levels of exercise (Fig. 2) and at 50% and 60% of the individual VO_2max (Fig. 6). VO_2 levels were similar at each submaximal work load by study design. Inasmuch as R is derived from the ratio of the VCO_2 to VO_2, the higher R values reflect elevated VCO_2 as seen in Figs. 2 and 3. Ventilatory parameters were similar in both groups at each submaximal work load.

FHR monitoring showed good reactivity and rare uterine contractions before, during, and after exercise.

Comment

Comparative physiologic studies between weight-bearing and non-weight-bearing exercise have not been previously conducted in pregnancy. A similar respiratory tidal volume while pedaling the bicycle ergometer at the same VO_2 and while walking on the treadmill were reported in prior studies in nonpregnant subjects.[8,9] Similarly, in our study at submaximal work loads the tidal volumes were not statistically different. In our study, bicycle ergometry induced hyperventilation only at VO_2max, as compared with walking.

The use of the R value to assess energy use is valid if no ventilatory abnormalities exist such as hyperventilation, which would increase VCO_2 and therefore increase the R value. Such differences in ventilation were noticed in our study only at VO_2max.

The significantly higher R values in the non-weight-bearing exercise group during exercise may reflect preferential carbohydrate use over lipids during non-weight-bearing mild exercise in comparison with weight-bearing mild exercise in pregnancy. This observation in our study was independent of the fitness level and weight as reflected by similar VO_2max/kg values in the two groups as summarized in Table I and Fig. 6.

Theoretically, during prolonged exercise higher lactate values could be associated with an elevation in R. The lactate is buffered by sodium bicarbonate and results in excess CO_2 formation that is independent of fuel metabolism.[10] Elevated lactate production during bicycle ergometry that leads to metabolic acidosis could explain the higher R values. This is unlikely to occur in brief exercise sessions as performed by our subjects.

One potential clinical application of this finding is its use as an alternative therapeutic modality to gestational diabetes. Because gestational diabetes appears to be a state of impaired glucose use, noninsulin intervention would be advantageous. The preferential carbohydrate use during brief non-weight-bearing exercise in comparison with weight-bearing exercise during pregnancy requires further investigation. This preferential use of carbohydrates may be a result of the anaerobic component of this type of non-weight-bearing exercise.

Recognition of potential risks is essential when exercise is prescribed. Comparative studies such as this one allow an assessment of physiologic responses. The risks involved with exercise in pregnancy in previously sedentary obese individuals include soft tissue musculoskeletal injuries.[11] The anatomic changes of pregnancy that include laxity of joints and ligaments, coupled with a continuous shift in the center of gravity and increasing weight, place sedentary persons at higher risk for injuries (primarily in weight-bearing activities) during exercise. Thus certain types of exertion such as weight-bearing exercise may not be suitable for this type of subject. This body weight and the change in the point of gravity have a much smaller effect on bicycle ergometer performance and therefore less risk of injury is involved.

Should exercise become a therapeutic modality for women with gestational diabetes or in the otherwise normal sedentary person, it appears from these preliminary studies that non-weight-bearing exercise such as bicycle ergometry may be the preferred form of exercise to be prescribed.

REFERENCES

1. Summary and recommendations of the second international workshop-conference on gestational diabetes mellitus: therapeutic strategies. Diabetes 1985;34(suppl 2): 125.
2. Wallberg-Henriksson H, Holloszy JD. Contractive activity increases glucose uptake by muscle in severely diabetic rats. J Appl Physiol 1984;57:1045-9.
3. Vinten J, Norgaard PL, Sonne B, Galbo H. Effect of physical training on glucose transporters in fat cell fractions. Biochem Biophys Acta 1985;841:223-7.
4. Artal R, Wiswell RA, Romem Y. Hormonal responses to exercise in diabetic and non-diabetic pregnant patients. Diabetes 1985;39(suppl 2):78-80.
5. Consolazio CF, Johnson RE, Pecora LJ. Physiological measurements of metabolic functions in man. New York: McGraw-Hill, 1963.
6. de Vries HA. Physiology of exercise. 3rd ed. Dubuque: William C. Brown, 1980:218.
7. Pollock ML, Bohannon RL, Cooper KH, et al. A comparative analysis of 4 protocols for maximal treadmill stress testing. Am Heart J 1976;92:39-46.
8. Bobbert AC. Physiological comparison of three types of ergometry. J Appl Physiol 1960;15:1007-14.
9. McKay GA, Banister EW. A comparison of maximum oxygen uptake determination by bicycle ergometry at various pedaling frequencies and by treadmill running at various speeds. Eur J Appl Physiol 1976;35:191-200.
10. McArdle WD, Katch FI, Katch VL. Exercise physiology, energy, nutrition, and human performance. Philadelphia: Lea and Febiger, 1982:102.
11. Leon AS. Patients with diabetes mellitus. In: Franklin BA, Seymur G, Timmis GO, eds. Exercise in modern medicine. Baltimore: Williams and Wilkins, 1989:140.

Make researching easier with the Ten-Year Cumulative Index (1978-1987) to the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY

The Ten-Year Cumulative Index is a complete guide to more than 30,000 pages of original articles, case reports, letters, and editorials published in the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY from 1978 through 1987 (volumes 130 to 157). Numbering more than 800 pages, the Cumulative Index includes both a subject and an author index. This hard-cover volume is $64.50 ($69.00 international). Prices include shipping. *Payment must accompany all orders and must be in U.S. funds, drawn on a U.S. bank.*

Contact The C.V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318 USA; phone (314) 872-8370, ext. 394. In the U.S., call toll-free: 1-800-325-4177, ext. 394. Please allow six weeks for delivery.

Human fetal behavioral states after vibratory stimulation

Robert Gagnon, MD, Cora Hunse, RN, and John Foreman, PEng
London, Ontario, Canada

A total of 14 healthy pregnant women between 37 and 41 weeks' gestational age were studied to determine the effects of a 5-second external vibratory stimulus (100 Hz, square wave) on human fetal behavioral states. Fetal behavioral state observations were made with simultaneous recordings of fetal heart rate, gross fetal body movements, and fetal eye movement between 8:30 AM and 4:00 PM. During the first episode of behavioral state 1F (quiet sleep state) ≥10 minutes duration on two consecutive half days, the vibratory stimulus was placed on the surface of the maternal abdomen over the fetal head. Each fetus received one 5-second vibratory stimulus during either the first or second half day of observation. The time at which the instrument was applied but no vibratory stimulus was administered was considered as the control period. All women were then observed for 2 hours after the control period and after stimulation. A transition period from a quiet sleep state to a stable active sleep state (state 2F, rapid eye movement sleep) averaged ≤3 minutes after stimulation, as compared with 23 minutes after the control period. These changes in fetal behavioral states persisted for 20 minutes after vibratory stimulation. There also was an immediate and sustained increase in long-term fetal heart rate variability, the number of fetal heart rate accelerations and gross fetal body movement during the first 20 minutes after stimulus, without changes in basal fetal heart rate. We hypothesize that changes in fetal behavior seen after application of low frequency vibratory stimulus are more physiologic than the profound changes in fetal heart rate and gross fetal body movement previously described after vibratory acoustic stimulation with an electronic artificial larynx. (AM J OBSTET GYNECOL 1989;161:1470-6.)

Key words: Human fetal behavior, sound and vibration, fetal heart rate

Healthy human fetuses undergo rest-activity cycles that are usually related to different behavioral states.[1] It is believed that human fetuses may change from a state of sleep to a state of wakefulness as a result of vibratory acoustic stimulation.[2, 3] Recently, low-frequency vibration as a source of external stimulation has been suggested as the cause of the increase in fetal heart rate (FHR) reactivity[4] and as a possible means to decrease the length of nonstress tests.

The aim of this study was to investigate the effect of an external vibratory stimulus on fetal behavioral states in a group of healthy human fetuses at term.

Patients and methods

Patients. Informed consent was obtained from 16 pregnant women between 37 and 41 weeks' gestational age. All remained in good health and had normal term deliveries that confirmed gestational age and the good health of the fetuses studied. The average gestational age at delivery was 40.5 ± 0.4 weeks (SEM). The average weight of the fetuses at birth was 3664 ± 141 gm (SEM). No Apgar score was <8 at 5 minutes. Umbilical cord blood arterial samples were obtained at birth in all fetuses. The mean umbilical arterial P_{O_2} was 17.1 ± 1.2 mm Hg, the P_{CO_2} was 54.1 ± 2.0 mm Hg, and the pH was 7.28 ± 0.01. Two (12.5%) women were delivered of infants by cesarean section because of cephalopelvic disproportion. All fetuses were in vertex presentation at the time of delivery.

Experimental design. The women received a standardized 800 kcal breakfast or lunch at 8:00 AM or 12:00 PM, respectively. All studies began 30 minutes later at 8:30 AM or 12:30 PM and were conducted in a quiet room with the patients either sitting upright in bed or resting in a lateral recumbent position. Women were asked not to smoke for ≥4 hours before the studies. Smoking or food intake by patients or observers was not permitted during the studies.

The external vibratory stimulus used in this study was generated with a Model 4810 minishaker (Bruel and Kjaer, Copenhagen, Denmark) matched with a minishaker power amplifier (Department of Mechnical Engineering, University of Western Ontario, London, Ontario, Canada), firmly applied on the maternal abdomen. The signal was generated with the output of a Fast Fourier Transform analyzer (Model SP-20, Sig-

From the Department of Obstetrics and Gynecology and the Faculty of Engineering Science, Lawson Research Institute, St. Joseph's Hospital, University of Western Ontario.
Supported by the Physician's Services Incorporated Foundation, the Academic Development Fund of the University of Western Ontario, and the Canadian Medical Research Council.
Presented at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.
Reprint requests: Robert Gagnon, MD, Department of Obstetrics and Gynecology, St. Joseph's Health Centre, 268 Grosvenor St., London, Ontario, N6A 4V2 Canada.
6/6/15661

nology, Campbell, Calif.) and adjusted to generate a square wave of 100 Hz during 5 seconds. During a pilot study in our laboratories (Gagnon et al. Unpublished observations), this type of stimulus was associated with a more consistent fetal response than a sine wave of the same frequency or higher frequencies. The surface of the minishaker in contact with the maternal abdomen was 9.6 cm^2, which was approximately twice the moving surface of the Model 5C electronic artificial larynx (4.9 cm^2; Western Electric, New York, N. Y.). The surface of the instrument vibrated at a fundamental frequency of 100 Hz with multiple harmonics up to at least 5000 Hz.[4] The maximum force of vibration was 48 dB at 100 Hz, equivalent to an absolute force of 4.35×10^{-3} N.[4] A similar spectrum of vibration (maximum force of 45 dB at 100 Hz) is produced by the electronic artificial larynx.[5]

All patients were acclimatized by touching the surface of the activated minishaker with their hand before the studies began. Women were studied continuously for 3½ hours on each of 2 half days. Fetal behavioral state observations were made with simultaneous recordings of FHR, gross fetal body movement, and fetal eye movement between 8:30 AM and 4:00 PM. During the first episode of behavioral state 1F (FHR, pattern A; gross fetal body movement, incidental; fetal eye movement, absent)[1] on each consecutive half day, the minishaker was placed on the surface of the maternal abdomen over the fetal head. The device was applied only after the fetus has been in state 1F for 10 minutes. This technique allowed us to synchronize all fetuses in the same behavioral state (quiet sleep state) for the same length of observation (10 minutes) just before both the stimulus and the control were applied. Each fetus received one 5-second vibratory stimulus during either the first or second half day of observation. Patients were informed that the stimulus might or might not be activated during the two times that the instrument was placed on the abdomen. The choice of whether to stimulate the abdomen during the first or second episode of state 1F was made from random number tables. The time at which the instrument was applied but no vibratory stimulus was administered was considered as the control period. All women were observed for 2 hours after the control period and stimulation.

One healthy fetus did not show evidence of any episode of state 1F after 3 hours of continuous observation, and fetal eye movements could not be adequately visualized in another fetus. These two study participants were excluded from further analysis.

FHR measurement. FHR was recorded with a Hewlett Packard Model 8040 A external monitor (Hewlett Packard GMBH, Boeblinger, Federal Republic of Germany) with a compact transducer and was analyzed on-line by a Sage II microprocessor (Sage Technology, Reno, Nev.) as previously described.[2,6] During analysis, a baseline was fitted to the heart rate record and FHR accelerations ≥10 beats/min for ≥15 seconds above the baseline were automatically detected. For each 10-minute interval, the basal FHR (beats/min), the number of FHR accelerations, the total gained beats (total area of FHR accelerations above the baseline), and the mean minute range (milliseconds) were calculated on-line.

Measurement of fetal activity. Fetal breathing movement and gross fetal body movement were measured with a real-time ADR ultrasound scanner (Model 2130, Advanced Diagnostic Research Corp., Tempe, Ariz.) and were analyzed on-line in 10-minute intervals with a PDP 11/34 minicomputer as previously described.[7] Individual gross fetal body movement and fetal breathing movement were identified on a video monitor and coded with an event marker. With the use of this technique, the agreement between observers to detect fetal breathing movement and gross fetal body movement is 94%.[7] The 3.5 MHz transducer was positioned over the maternal abdomen to permit continuous observation of a longitudinal cross-section of fetal chest and abdominal wall echoes.[7]

Measurement of fetal eye movement. A second real-time ultrasound scanner (Model SAL-30A, Toshiba Corp., Shimoishi, Japan) was used to identify fetal eye movement. The 3.5 MHz transducer was positioned on the maternal abdomen so that an oblique cross-section of the fetal face was obtained.[8] In such a section one of the fetal eyes can be seen as a sonolucent globe, behind which lies a wedge-shaped echo-rich area. Fetal eye movement can be detected easily in the real-time image as small movements in the periphery of the sonolucent area and in the retroocular echo-rich area.[8] Individual fetal eye movements were identified on a second video monitor adjusted to magnify the size of the real-time image approximately eight times and the movements were coded with an event marker. The interobserver agreement to detect fetal eye movement with real-time image is reported to be 90%.[8]

Measurement of fetal behavioral states. FHR tracing, gross fetal body movement, fetal breathing movement, fetal eye movement, and failed time were recorded on an eight-channel chart recorder (Model 7, Grass Instrument Co., Quincy, Mass.). Electrical signals from the analog output of the HP 8040A external monitor and the two event markers were processed by means of a common mode rejection preamplifier (Model 7, P51 IJ, Grass Instrument Co.). The paper speed was adjusted at 25 mm/min for off-line analysis of fetal behavioral states.

Data analysis. The basal FHR, the number of FHR accelerations ≥10 beats/min for ≥15 seconds above the baseline, the total gained beats, and the mean minute range were determined for successive 10-minute intervals. Portions of records that could not be analyzed

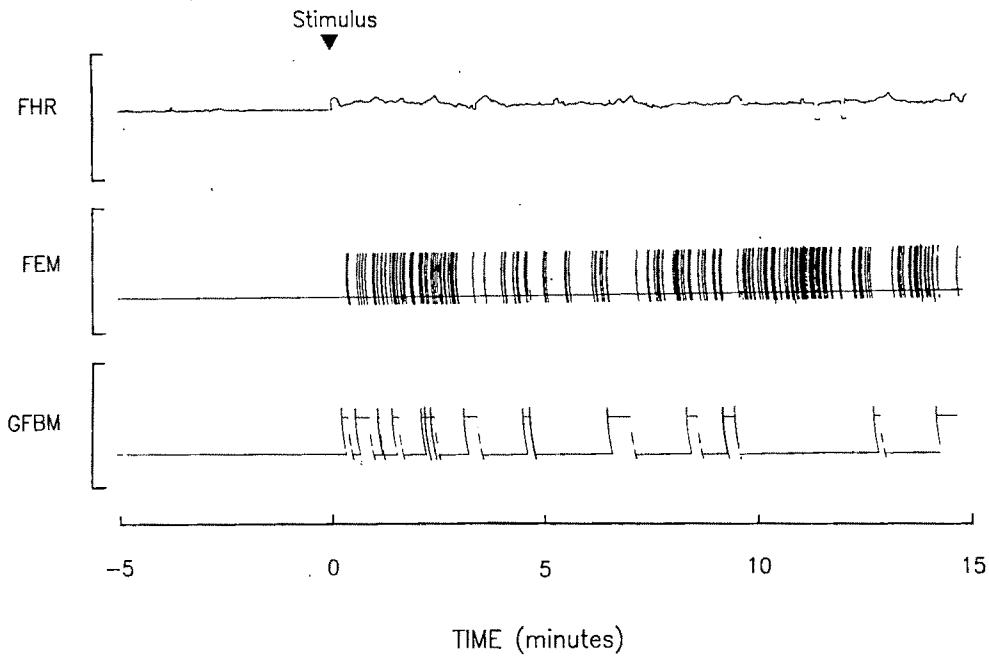


Fig. 1. Typical example of transition from state 1F (quiet sleep state) to state 2F (rapid eye movement sleep state) after 5-second external vibratory stimulation *(arrow)* in healthy term human fetus. (*FHR*, fetal heart rate; *FEM*, fetal eye movements; *GFBM*, gross fetal body movements).

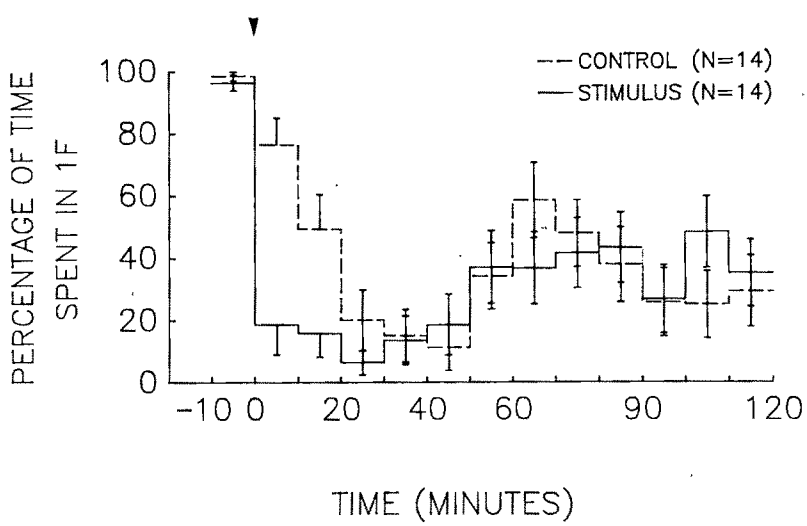


Fig. 2. Mean incidence of coincidence 1F (±SEM) was plotted in 10-minute intervals before and after stimulation (——) or control (- - - -).

because of FHR signal loss of >30% or patient interruption were not included. These portions made up 5.0% of the total study time.

Measurements of the percentage of time spent making gross fetal body movements, the percentage of time spent making breathing movements, and the frequency distribution of breath-to-breath intervals were calculated for each 10-minute interval of the 61 hours of total observation time. Portions of movement records that could not be analyzed because of technical problems or patient interruption made up 5.1% of the total study time.

Each of the three fetal behavioral state variables (body movement, eye movement, and FHR pattern) were analyzed separately for condition (parameter for presence or absence of body and eye movement; for heart rate patterns A through D) with the use of a 3-minute moving window advanced in half-minute steps.[1] The result of this procedure was a profile that shows the changes of each variable between its possible con-

ditions (parameters). The three behavioral state variables were then analyzed simultaneously to identify epochs in which the parameters of all three were present in the previously defined combinations.[1] Periods during which the parameters of the behavioral state variables were present in the previously defined combinations were called periods of coincidence 1F to 4F, respectively. To accept the presence of behavioral states, the defined combinations of the behavioral state variables had to persist ≥3 minutes without interruption and transitions from one of the specified combinations to another had to be completed within a maximum of 3 minutes. Portions of fetal eye movement records that could not be analyzed because of technical problems made up 6.0% of the total study time.

Results are presented as means and SE (±SEM). Statistical significance between groups for the 10 minutes before and the 2 hours after the stimulation or the control period was determined by a two-way analysis of variance that compared overall means and each of the 10-minute intervals.[9]

Results

Fetal behavioral states. An example of the changes in fetal behavioral states after external vibratory stimulus is shown in Fig. 1. All fetuses were in state 1F (quiet sleep state) before application of the stimulus or the control period. The mean incidence of coincidence 1F (quiet sleep state) decreased to 18.6% ± 7.5% (SEM) during the first 10 minutes after stimulation, which was significantly lower than 76.4% ± 8.9% after the control period (analysis of variance, $p < 0.01$) (Fig. 2). The percentage of time spent in the quiet sleep state remained below control values up to 20 minutes after the 5-second vibratory stimulus (Fig. 2). The mean incidence of fetal eye movement was 71.4% ± 10.9% during the first 10 minutes after vibratory stimulation and remained above control values (5.0% ± 2.0%) for 20 minutes (analysis of variance, $p < 0.01$) (Fig. 3). Similarly, the mean incidence of coincidence 2F (rapid-eye movement sleep state) increased up to 65.0% ± 11.1% immediately after the stimulus was applied and remained significantly above control values for the first 20 minutes after vibratory stimulation (analysis of variance, $p < 0.01$) (Fig. 3).

In 12 (85.7%) fetuses, fetal behavioral states changed from a quiet (1F) to an active (2F) sleep state immediately after stimulus, two (14.3%) fetuses had isolated FHR accelerations without a shift in behavioral state, and one fetus changed to an indeterminate state characterized by the presence of continuous gross fetal body movement and small-amplitude FHR accelerations. The mean transition time from the time the minishaker was applied on the maternal abdomen to the onset of state 2F (FHR, pattern B; gross fetal body movement, present; fetal eye movement, present) was 3.1 ± 1.5 minutes after stimulation, which was significantly lower than after the control period (23.9 ± 4.0 minutes) ($p < 0.001$) (Fig. 4).

FHR and fetal activity patterns. The mean 10-minute mean minute range was 29.4 ± 4.1 msec before stimulation. After vibratory stimulation, the mean 10-minute mean minute range increased to 54.7 ± 7.0 msec during the first 10 minutes after application of the stimulus and remained above the control value (34.0 ± 4.6 msec) for 20 minutes (analysis of variance, $p < 0.01$). No significant change in the mean basal FHR was found after square wave vibratory stimulation (control value, 135 ± 2 beats/min). Fig. 5 shows that there was an immediate and sustained increase in both the mean number of FHR accelerations and the mean number of gross fetal body movements during the first 20 minutes after vibratory stimulation, as compared with the control period (analysis of variance, both $p < 0.01$).

It is important to note that all biophysical variables including FHR, FHR variability, gross fetal body movement, fetal eye movement, and the time spent in the different behavioral states followed similar patterns in both stimulus and control groups between 20 and 120 minutes after either the control period or application of the stimulus (Figs. 2, 3, and 5). Also, the maximum values in these variables after stimulation was not excessively high, as compared to the maximum values after the control period (Figs. 2, 3, and 5).

Comment

The 5-second external vibratory stimulation (100 Hz, square wave) used in this study was associated with significant changes in human fetal behavioral states at term. There was a transition from a stable quiet sleep state (1F) to a stable active sleep state (rapid eye movement sleep, 2F), within 3 minutes after the application of this stimulus on the surface of the maternal abdomen. These changes in fetal behavioral states persisted for 20 minutes and did not exceed the maximum time spent in an active sleep state after spontaneous transition from state 1F to state 2F after the control period. There also was an immediate and sustained increase in long-term FHR variability and in the number of FHR accelerations and gross fetal body movements during the first 20 minutes after stimulation, without changes in basal FHR, which confirms previous observations.[4]

Behavioral states in healthy term newborns alternate between state 1 (quiet sleep) and state 2 (rapid eye movement sleep).[10, 11] At the time of feedings, there is a shift from state 2 to state 3 (quiet wakefulness) and ultimately to state 4 and state 5 (wakefulness and crying, respectively).[11] The well-organized pattern of these spontaneous and progressive changes in newborns is

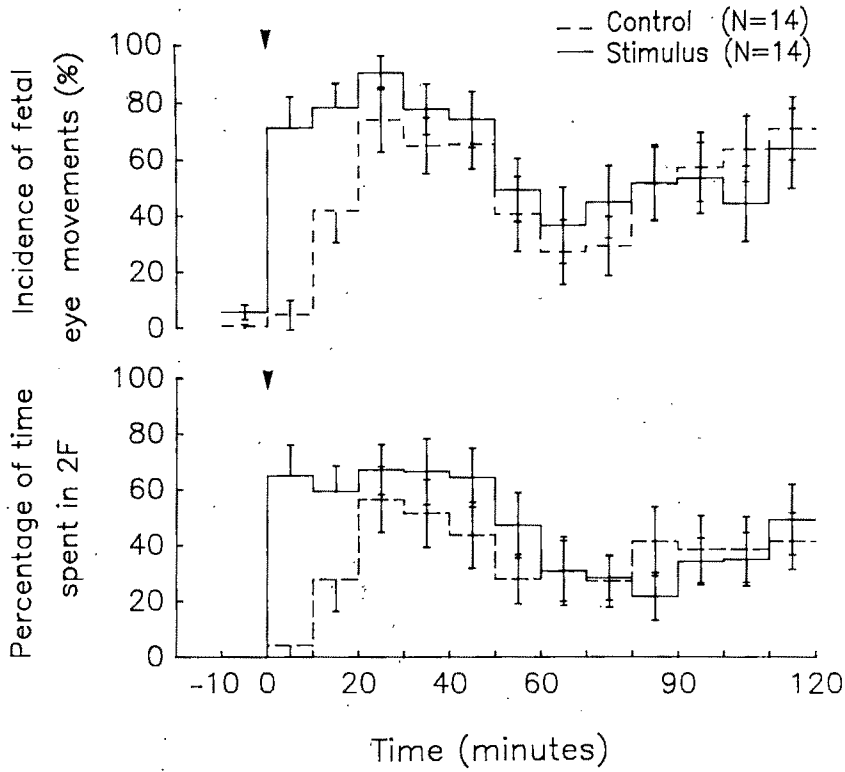


Fig. 3. Mean incidence of fetal eye movements and mean incidence of coincidence 2F were plotted in 10-minute intervals before and after stimulation (——) or control (- - - -).

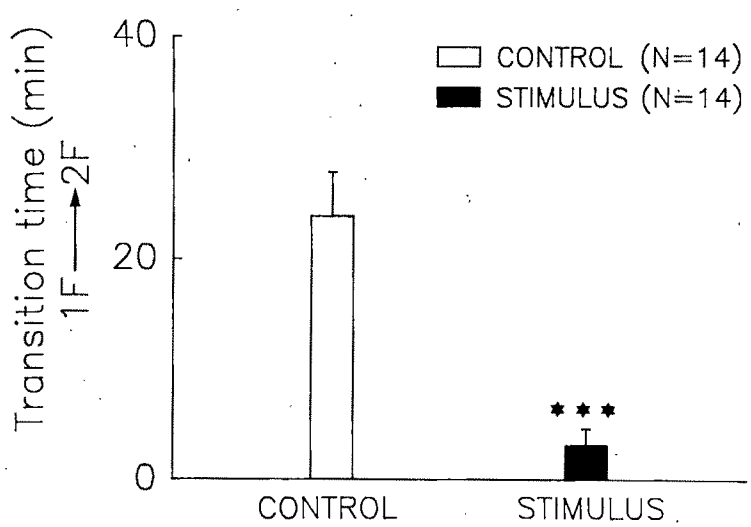


Fig. 4. Mean transition time (±SEM) from time vibratory stimulus *(closed bar)* or control *(open bar)* was applied to onset of state 2F is shown.

believed to reflect the integrity of the central nervous system.[11] Nijhuis et al.,[1] with use of real-time ultrasonography, observed that fetuses of 36 week's gestation had developed states that were, in their organization, fully comparable to the states originally described by Prechtl et al.[10] in neonates. In contrast with the neonates, FHR pattern has been used as a state criteria because it can be easily recorded and four specific patterns can be clearly defined related to the four different fetal behavioral states. FHR pattern B, which is usually seen after vibratory stimulation (Fig. 1), has been defined as a wide oscillation band width with frequent accelerations during fetal movements that occurs during state 2F (rapid eye movement sleep).[1] Clinicians have recognized that the presence of FHR accelerations associated with fetal movements (FHR pattern B, Fig. 1) is a reliable indicator of fetal health.[12]

Hutt et al.,[13] in a series of carefully designed exper-

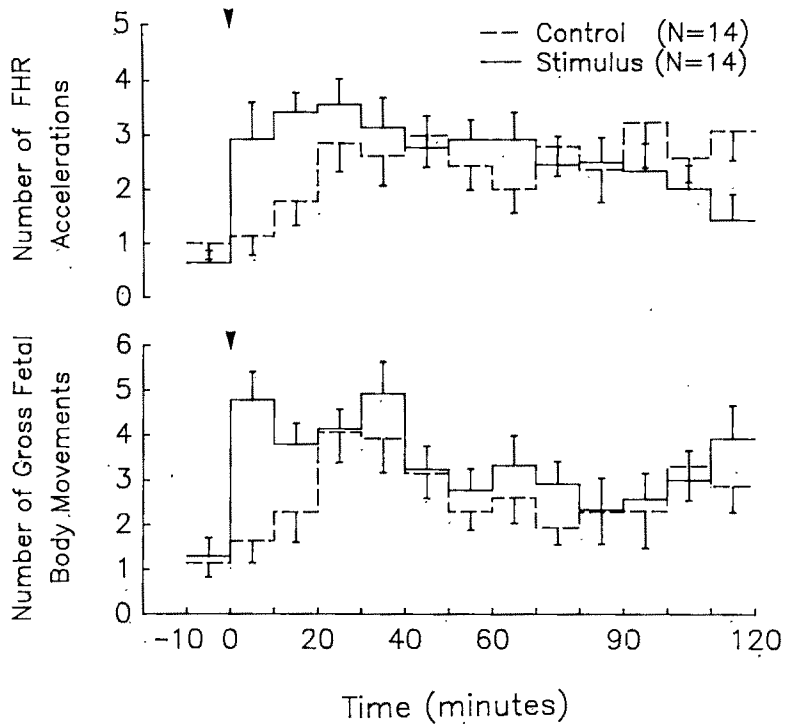


Fig. 5. Mean number of FHR accelerations and mean number of gross fetal body movements were plotted in 10-minute intervals before and after stimulation (——) or control period (- - - -).

iments, reported that acoustic broad-band signals (such as square wave pulses), at an intensity >90 dB sound pressure level could alter behavioral states in the 2- to 3-day-old healthy newborn. Such stimulation applied 10 cm from the newborn's ear during quiet sleep state generally produced a "startle reflex" followed by a period of rapid eye movement sleep state or by waking.[13] In our study, although the stimulus used was vibratory rather than acoustic, similar changes in fetal behavioral states were seen (Figs. 1 and 3). Whether or not such stimulus, after being transmitted through maternal soft tissues, could be perceived as an acoustic stimulation by the fetus remains to be determined. Although not measured in the current study, it is also possible that the pressure applied against the skin by the minishaker is a clinically significant variable that will need to be addressed.

Healthy human fetuses may take as long as 80 minutes to demonstrate activity.[14] To decrease the length of nonstress tests, external vibratory acoustic stimulation with an electronic artificial larynx has been suggested to increase FHR reactivity.[15] However, it has been shown in the pregnant ewe that the fetus might be exposed to extremely high sound pressure levels (135 dB) during stimulation with the electronic artificial larynx.[16] This might explain the excessive FHR and movement responses after stimulation with the electronic artificial larynx, which is believed to reflect a state of fetal wakefulness.[2, 3]

Human term fetuses that were exposed to vibratory stimulation underwent changes in behavioral states from a state of quiet sleep to an active sleep state that were similar to spontaneous changes seen in healthy newborns[11] and were typical of a reactive nonstress test.[12] This contrasted with the frequent occurrence of FHR pattern D (seen in wakefulness) characterized by large FHR accelerations often fused into sustained tachycardia after stimulation with the electronic artificial larynx.[2] Wakefulness state usually does not occur spontaneously after quiet sleep state in healthy term neonates.[11] Therefore it is possible to hypothesize that the changes in fetal behavior seen after low-frequency vibratory stimulation are more physiologic than the profound changes in FHR and fetal movements that occur after stimulation with the electronic artificial larynx.[2, 3]

The mechanisms by which an external source of sound or vibration can alter human fetal behavior are unknown. The clinical efficacy of such stimuli with regard to the prediction of fetal outcome and a decrease in the length of nonstress tests remains to be determined.

We thank Dr. J. Patrick for his interest in this work and Mrs. Maria de Sousa for her excellent technical assistance.

REFERENCES

1. Nijhuis JC, Prechtl HFR, Martin CB Jr, Bots RSGM. Are there behavioral states in the human fetus? Early Hum Dev 1982;6:177-95.

2. Gagnon R, Hunse C, Carmichael L, et al. External vibratory acoustic stimulation near term: fetal heart rate and heart rate variability responses. Am J Obstet Gynecol 1987;156:323-7.
3. Gagnon R, Hunse C, Carmichael L, et al. Human fetal responses to vibratory acoustic stimulation from twenty-six weeks to term. Am J Obstet Gynecol 1987;157:1375-81.
4. Gagnon R, Foreman J, Hunse C, Patrick J. Effects of low-frequency vibration on human term fetuses. Am J Obstet Gynecol 1989;161:1479-85.
5. Gagnon R, Patrick J, Foreman J, West R. Stimulation of human fetuses with sound and vibration. Am J Obstet Gynecol 1986;155:848-51.
6. Dawes GS, Redman CWG, Smith JH. Improvements in the registration and analysis of fetal heart rate records at the bedside. Br J Obstet Gynaecol 1985;92:317-25.
7. Patrick J, Natale R, Richardson B. Patterns of human fetal breathing activity at 34 to 35 weeks' gestational age. Am J Obstet Gynecol 1978;132:507-13.
8. Bots RSGM, Nijhuis JG, Martin CB Jr, Prechtl HFR. Human fetal eye movements: detection in utero by ultrasonography. Early Hum Dev 1981;5:87-94.
9. Hicks CR. Fundamental concepts in the design of experiments. 2nd ed. New York: Holt, Rinehart and Winston, 1973:100.
10. Prechtl HFR, Akiyama Y, Zinkin P, Kerr Grant D. Polygraphic and qualitative analysis. In: Box MCO, Mackeith RC, eds. Studies in infancy. London: Heinemann, 1968:1-25.
11. Prechtl HFR. The behavioral states of the newborn infant (a review). Brain Res 1974;76:185-212.
12. Brown R, Patrick J. The nonstress test: how long is enough. Am J Obstet Gynecol 1981;141:646-51.
13. Hutt SJ, Lenard HG, Prechtl HFR. Psychophysiological studies in newborn infants. Adv Child Dev Behav 1969;4:127-55.
14. Patrick J, Campbell K, Carmichael L, et al. Patterns of gross fetal body movements over 24-hour observation intervals during the last 10 weeks of pregnancy. Am J Obstet Gynecol 1982;142:363-71.
15. Smith CV, Phelan JP, Platt LD, et al. Fetal acoustic stimulation testing. II. A randomized clinical comparison with the nonstress test. Am J Obstet Gynecol 1986;155:131-4.
16. Gerhardt KJ, Abrams RM, Kovaz BM, et al. Intrauterine noise levels produced in pregnant ewes by sound applied to the abdomen. Am J Obstet Gynecol 1988;159:228-33.

The effect of vibroacoustic stimulation on the nonstress test at gestational ages of thirty-two weeks or less

Maurice L. Druzin, MD, Terri G. Edersheim, MD, J. M. Hutson, MD, and Annette L. Bond, MD

New York, New York

The effect of vibroacoustic stimulation on the nonstress test at gestational ages of ≤32 weeks was studied in 15 patients who underwent a total of 316 nonstress tests starting at 20 to 25 weeks' gestation. There were 168 nonreactive nonstress tests that were followed by 3 seconds of vibroacoustic stimulation. The incidence of reactive nonstress tests after vibroacoustic stimulation was significantly increased after 26 weeks' gestation. This may have clinical applicability and may be related to functional maturation of the fetal auditory system. (Am J Obstet Gynecol 1989;161:1476-8.)

Key words: Vibroacoustic stimulation, nonstress test, preterm delivery

Fetal heart rate (FHR) accelerations have long been known to reflect fetal well-being. Vibratory acoustic stimulation has been used over the years to elicit FHR accelerations in an attempt to decrease the false-abnormal (nonreactive) nonstress test.[1, 2] Studies have shown that FHR accelerations in response to sound may reflect fetal well-being.[3, 4] This increase in heart rate may be related to a change in fetal state. This has recently been described as a startle response of the fetus.[5, 6] Fetal scalp stimulation resulting in FHR acceleration has been correlated with normal acid-base status.[7] Recently, FHR accelerations in response to vibratory acoustic stimulation have been shown to be predictive of fetal pH in labor.[8]

A recent study evaluated fetal accelerations in response to vibratory acoustic stimulation from 26 weeks to term and demonstrated what may be the normal neurodevelopmental status of the fetus.[9] It has been shown that the response to vibratory acoustic stimulation is active as early as 24 weeks' gestation when studied serially from 12 to 32 weeks.[10]

This study was undertaken to determine the effect

From the Department of Obstetrics and Gynecology, The New York Hospital-Cornell Medical Center.
Presented in part at the Thirty-sixth Annual Meeting of the Society For Gynecologic Investigation, San Diego, California, March 15-18, 1989.
Reprint requests: Maurice L. Druzin, MD, 525 East 68th St., New York, NY 10021.
6/6/16239

Table I. Results of nonstress tests followed by vibratory acoustic stimulation

Estimated gestational age (wk)	*Total NSTs*	*Nonreactive NST*	*Reactive NST after VAS*
20-22	16	15 (94%)	1 (7%)
22-24	21	18 (86%)	3 (17%)
24-26	26	23 (88%)	5 (22%)
26-28	30	19 (63%)	17 (89%)
28-30	38	18 (47%)	17 (94%)
30-32	37	18 (49%)	18 (100%)
32-34	34	23 (68%))	22 (96%)
34-36	38	14 (37%)	14 (100%)
>36	76	20 (26%)	19 (95%)
TOTAL	316	168	116

NST, Nonstress test; *VAS,* vibratory acoustic stimulation. The incidence of reactive nonstress tests after vibratory acoustic stimulation was significantly increased after 26 weeks' gestation ($p < 0.001$).

of vibratory acoustic stimulation on the fetus in terms of the nonstress test at gestational ages from 20 to 32 weeks.

Material and methods

Fifteen patients underwent 316 nonstress tests starting at 20 to 25 weeks of gestation. Six patients were volunteers with no obstetric risk factors. Nine patients underwent tests for preterm labor ($n = 4$), diabetes ($n = 2$), preeclampsia ($n = 1$), incompetent cervix ($n = 1$), or third-trimester bleeding ($n = 1$). The nonstress test is performed with an external system to monitor fetal movement, uterine contractions, and FHR. Uterine activity and fetal movements are obtained with a tokodynamometer strapped to the abdomen in conjunction with manual palpation of the uterus by the examiner. This method will register the frequency and relative duration but not the actual strength of the contractions. The patient is given an "event marker," with which she can register perceived fetal movements on the strip chart. Thus both the patient and the examiner may record fetal movements. The FHR can be derived from ultrasonographic phonocardiogram, or abdominal wall electrocardiogram signals. Ultrasonography will provide an adequate FHR tracing in up to 95% of the cases, while the success rate of the other two methods has been reported as 40% to 60% of cases.

A nonstress test is considered reactive (normal) if there are two accelerations of the FHR, ≥15 beats/min above the baseline, lasting at least 15 seconds, associated with fetal movement in a 20-minute period.

A nonstress test is considered nonreactive (abnormal) if the above criteria are (1) not met within an initial 20-minute period and (2) not met after vibratory acoustic stimulation is performed in a consecutive 20-minute period.

Vibratory acoustic stimulation was performed by using a single 3-second vibratory acoustic stimulus over the fetal vertex with the use of a Western Electric model 5C electronic artificial larynx. Sound pressure levels of this device measured at 1 m in air averaged 82 dB. Spectral analysis of the artificial larynx shows a fundamental frequency of 80 Hz; harmonics ranging from 20 to 9000 Hz are also observed. The fetal vertex was identified by Leopold maneuvers. In instances where the fetal position was unclear, ultrasonography was used to verify the location of the fetal vertex. FHR response was then observed after vibratory acoustic stimulation and the classification of the nonstress test was then determined.

Results

There were 168 nonreactive nonstress tests that were followed by vibratory acoustic stimulation applied for 3 seconds. The incidence of reactive nonstress tests after vibratory acoustic stimulation was significantly increased after 26 weeks of gestation (Table I). All neonates were appropriate for gestational age (>2800 gm) and all had 5-minute Apgar scores >8. One neonatal death was due to neonatal group B β-hemolytic streptococcal sepsis. There were no congenital anomalies.

Comment

A variety of sound frequencies and levels have been used in fetal vibratory acoustic stimulation to obtain a FHR response. It is thought that lower frequencies may evoke fetal tactile vibrational responses, as well as auditory responses. It is also thought that high frequencies (above 1500 Hz) have minimal vibratory influence and that a response is probably auditory in origin.[11] The study of two anencephalic fetuses revealed that there was no fetal response to vibratory acoustic stimulation in the anencephalic fetuses.[12] These authors therefore concluded that the fetal response to vibratory acoustic stimulation is mediated by auditory mecha-

nisms. This position is supported by other investigators.[10] Our study supports the impression that fetal response to vibratory acoustic stimulation occurs significantly more frequently after 26 weeks' gestation than before that gestational age. Recent studies suggest that there may be a threshold response to vibroacoustic stimulation.[13] Further studies are warranted to confirm this at various gestational ages. This finding may have clinical applicability and may be related to functional maturation of the fetal auditory system.

We acknowledge Audrey Waltner for her assistance.

REFERENCES

1. Smith CV, Phelan JP, Paul RH, Broussard P. Fetal acoustic stimulation testing: a retrospective experience with the fetal acoustic stimulation test. Am J Obstet Gynecol 1985;153:567-8.
2. Smith CV, Phelan JP, Platt LD, Broussard P, Paul RH. Fetal acoustic stimulation testing. II. A randomized clinical comparison with the nonstress test. Am J Obstet Gynecol 1986;155:131-4.
3. Read JA, Miller FC. Fetal heart rate accleration in response to acoustic stimulation as a measure of fetal well-being. Am J Obstet Gynecol 1977;129:512-7.
4. Goodlin RC, Schmidt W. Human fetal arousal levels as indicated by heart rate recordings. Am J Obstet Gynecol 1972;114:613-21.
5. Divon MY, Platt LD, Cantrell CJ, Smith CV, Yeh S-Y, Paul RH. Evoked fetal startle response: a possible intrauterine neurological examination. Am J Obstet Gynecol 1985;153:454-6.
6. Gagnon R, Hunse C, Carmichael L, Fellows F, Patrick J. Effects of vibratory acoustic stimulation on human fetal breathing and gross fetal body movements near term. Am J Obstet Gynecol 1986;155:1227-30.
7. Clark SL, Gimovsky ML, Miller FC. The scalp stimulation test: a clinical alternative to fetal scalp blood sampling. Am J Obstet Gynecol 1984;148:274-7.
8. Edershelm TG, Hutson JM, Druzin ML, Kogut EA. Fetal heart rate response to vibratory acoustic stimulation predicts fetal pH in labor. Am J Obstet Gynecol 1987;157:1557-60.
9. Gagnon R, Hunse C, Carmichael L, Fellows F, Patrick J. Human fetal responses to vibratory acoustic stimulation from twenty-six weeks to term. Am J Obstet Gynecol 1987;157:1375-81.
10. Birnholz JC, Benacerraf BR. The development of human fetal hearing. Science 1983;222:516-8.
11. Woods JR, Plessinger MA, Barden TP. Stimulating the fetus with sound. Contemp Ob Gyn 1984;23:213-26.
12. Ohel G, Simon A, Linder N, Mor-Yosef S. Anencephaly and the nature of fetal response to vibroacoustic stimulation. Am J Perinatol 1986;3:345-6.
13. Kisilevsky B, Muir DW, Low JA. Human fetal responses to sound as a function of stimulus intensity. Obstet Gynecol 1989;73:971-6.

Effects of low-frequency vibration on human term fetuses

Robert Gagnon, MD, John Foreman, PEng, Cora Hunse, RN, and John Patrick, MD
London, Ontario, Canada

Thirteen healthy pregnant women between 37 and 41 weeks' gestational age were studied to examine effects of a 5-second external vibratory stimulus (100 Hz, square wave) on fetal heart rate, fetal breathing, and gross fetal body movement patterns. All fetuses were stimulated during an episode of low fetal heart rate variability (mean minute range ≤32 milliseconds for ≥5 minutes) lasting at least 5 minutes. There was an immediate and sustained increase in long-term FHR variability, number of FHR accelerations, and gross fetal body movements after stimulation. Fetuses made breathing movements more irregularly after vibratory stimulus. We hypothesize that external low-frequency vibratory stimulus, applied during episodes of low fetal heart rate variability, causes a change from a state of quiet sleep to a state of rapid-eye-movement sleep in healthy term fetuses. (Am J Obstet Gynecol 1989;161:1479-85.)

Key words: Effects of vibration on human fetuses; human fetal behavioral states; proprioceptive fetal sensory receptors

External stimulation of human fetal sensory receptors with sound[1] or vibratory acoustic stimulation[2,3] has been suggested as a method of assessment of human fetal health. Fetal heart rate (FHR) responses to a pure-tone sound stimulus have been reported to be unpredictable and possibly dependent on the behavioral state at the time of the stimulus.[4] Although vibratory acoustic stimulation with an electronic artificial larynx can reproducibly alter FHR and fetal movement patterns during third trimester,[2,5] it is not yet clear whether the stimulus influences human fetuses by auditory or vibrational pathways.

The purpose of this study was to define, in a group of healthy fetuses at term, changes in fetal heart rate, fetal breathing, and gross body movements after a pure external vibratory stimulus applied during an episode of low FHR variability.

Patients and methods

Patients. Informed consent was obtained from 14 pregnant women between 37 and 41 weeks' gestational age. All remained in good health and had normal term deliveries that confirmed gestational age and the good health of fetuses studied. The average gestational age at delivery was 40.6 ± 0.2 weeks (SEM). The average weight of the fetuses at birth was 3765 ± 118 gm. The average 5-minute Apgar score was 9.2 ± 0.1. No Apgar score was <9 at 5 minutes. Umbilical cord blood arterial samples were obtained at birth in 12 of the 14 fetuses. The mean umbilical arterial Po_2 was 16.1 ± 1.2 mm Hg, the Pco_2 was 54.7 ± 3.2 mm Hg, and the pH was 7.27 ± 0.02. Three (21%) women were delivered by cesarean section (two because of failure to progress and one because of a footling breech presentation at the onset of labor). Thirteen fetuses were in vertex presentation and one was in breech position at the time of delivery.

Experimental design. The women received a standardized 800 kcal breakfast or lunch at 8 AM or 12 noon. All studies began 30 minutes later and were conducted in a quiet room with the patients either sitting upright in bed or resting in a lateral recumbent position. Women were asked not to smoke for at least 4 hours before the studies. Smoking or food intake by patients or observers was not permitted during the studies.

The external vibratory stimulus used in this study was generated with a model 4810 minishaker (Bruel and Kjaer, Copenhagen) matched with a minishaker power amplifier (Department of Mechanical Engineering, University of Western Ontario). A real-time, two-channel Fast Fourier Transform analyzer (model SP-20, Signology, Campbell, Calif.) having two input and one output channels was used. The digital output of the built-in signal generator was adjusted to generate a square wave of 100 Hz during 5 seconds. The quality and the amplitude of the signal generated by the Fast Fourier Transform analyzer and received by the minishaker were simultaneously recorded on-line with the two input channels. This procedure allowed us to

From the Department of Obstetrics and Gynecology and the Faculty of Engineering Science, Lawson Research Institute, St. Joseph's Hospital, University of Western Ontario.
This work was supported by the Physician's Services Incorporated Foundation, the Academic Development Fund, the University of Western Ontario, and the Canadian Medical Research Council. J. P. is an investigator in the Canadian Medical Research Council Group in Reproductive Biology.
Presented at the Thirty-fifth Annual Meeting of the Society for Gynecologic Investigation, Baltimore, Maryland, March 17-20, 1988.
Reprint requests: Dr. Robert Gagnon, Department of Obstetrics and Gynecology, St. Joseph's Hospital, 268 Grosvenor St., London, Ontario, Canada N6A 4V2.
6/6/15956

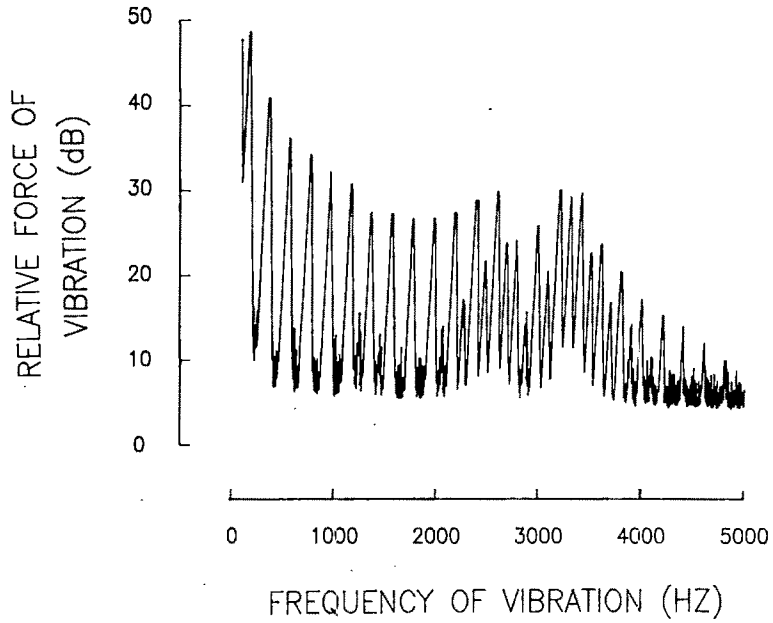


Fig. 1. Relationship between amplitude of vibration (decibels) and frequency of vibration (hertz) produced by minishaker (square wave, 100 Hz).

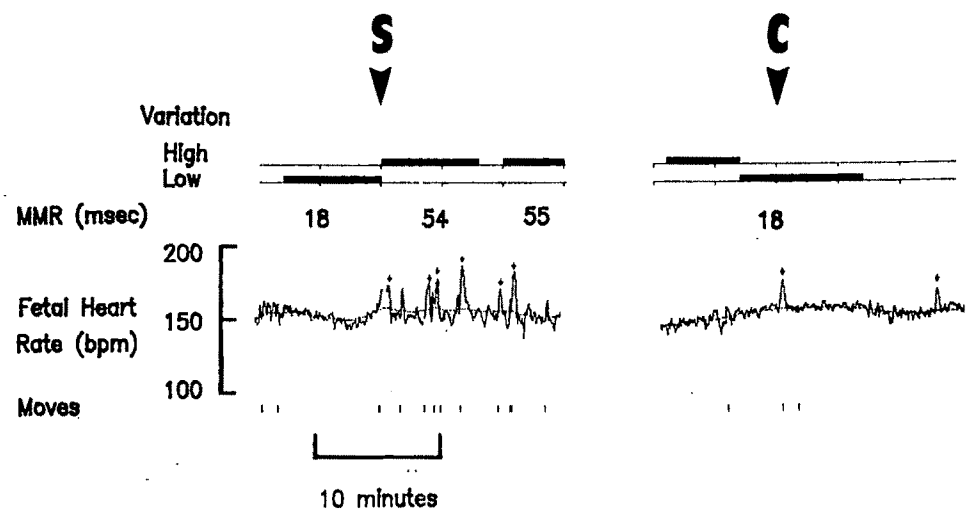


Fig. 2. Example of increase in FHR accelerations and fetal movements after application of a 5-second vibratory stimulus *(S)* during episode of low FHR variability.

apply a reproducible stimulus on the surface of the maternal abdomen.

The surface of the minishaker in contact with the maternal abdomen was 9.6 cm^2, which was approximately twice the moving surface of the electronic artificial larynx (4.9 cm^2). The surface of the instrument vibrated at a fundamental frequency of 100 Hz with multiple harmonics up to 4000 Hz, which are, by definition, the frequency components of a square wave (Fig. 1). The maximum force of vibration was 48 dB at 100 Hz (equivalent to an absolute force of 4.35×10^{-3} N; measurements made on the surface of the source with a model 086B80 impedance hammer, PCB Electronics, Boston; Fig. 1). A similar spectrum of vibration (maximum force of 45 dB at 100 Hz) is produced by the electronic artificial larynx.[6]

All patients had been acclimatized by touching the surface of the activated minishaker with a hand before the beginning of studies. Women were studied continuously for 4 hours. Episodes of low FHR variability (mean minute range of ≤32 milliseconds for ≥5 minutes) were automatically detected on-line by a Sage II microprocessor (Sage Technology, Reno) as previously described.[7,8]

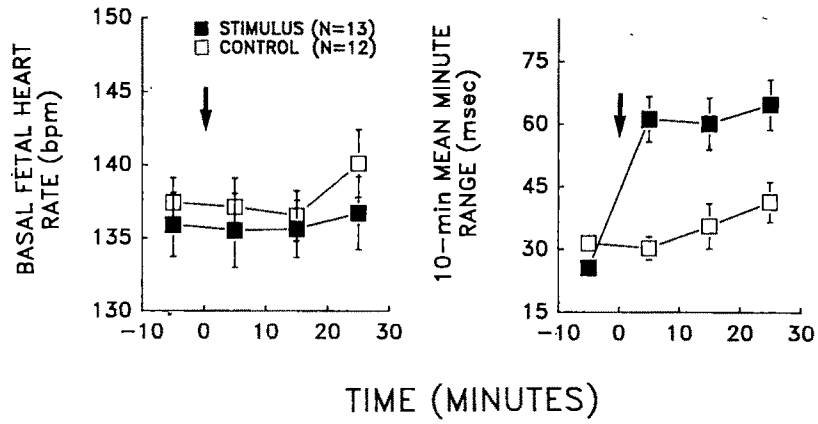


Fig. 3. Mean basal FHR (±SEM) and mean 10-minute mean minute range were plotted in 10-minute intervals before and after stimulus *(solid squares)* or control *(open squares)*.

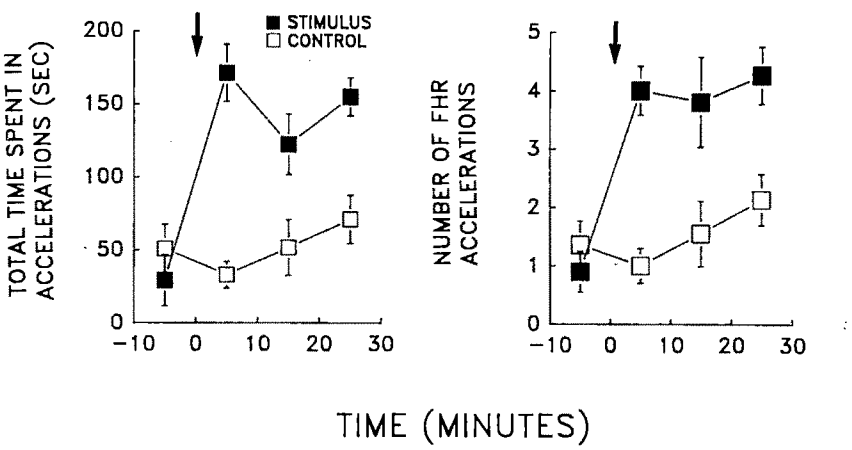


Fig. 4. Mean time spent in accelerations and mean number of FHR accelerations were plotted in 10-minute intervals before and after stimulus *(solid squares)* or control *(open squares)*.

During the first and second episodes of low FHR variability, the minishaker was placed on the surface of the maternal abdomen over the fetal head. Each fetus received one 5-second vibratory stimulus during either the first or second episode of low FHR variability of at least 5 minutes' duration. Patients were informed that the stimulus might or might not be activated during the two times that the instrument was placed on the abdomen. The choice of whether to stimulate the abdomen during the first or second episode of low FHR variability was made from random number tables. The time at which the instrument was applied but no vibratory stimulus was administered was considered the control period. All women received only one stimulus to the abdomen during individual studies and were observed for at least 30 minutes after control and stimulus.

One healthy fetus did not demonstrate any episode of low FHR variability after 4 hours of continuous observation and was excluded from further analysis.

FHR measurements. The FHR was recorded with a Hewlett Packard model 8040A external monitor (Hewlett Packard GmBH, Boeblinger, Federal Republic of Germany) with a compact transducer and analyzed on-line by a Sage II microprocessor (Sage Technology) as previously described.[7,8] During analysis, a baseline was fitted to the heart rate record and episodes of low (mean minute range ≤32 milliseconds for ≥5 minutes) and high (mean minute range >32 milliseconds for ≥5 minutes) FHR variability were recognized and updated automatically every 5 minutes.[8] FHR accelerations of ≥10 beats/min for ≥15 seconds above the baseline were automatically detected.

For each 10-minute interval the basal FHR (beats per minute) and the mean minute range (milliseconds) were calculated.

Measurements of fetal activity. Fetal breathing movements and gross fetal body movements were measured with a real-time ADR ultrasonographic scanner (model 2130, Advanced Diagnostic Research Corp., Tempe, Ariz.) and analyzed on-line in 10-minute intervals with a PDP 11/34 minicomputer (Digital Corp.,

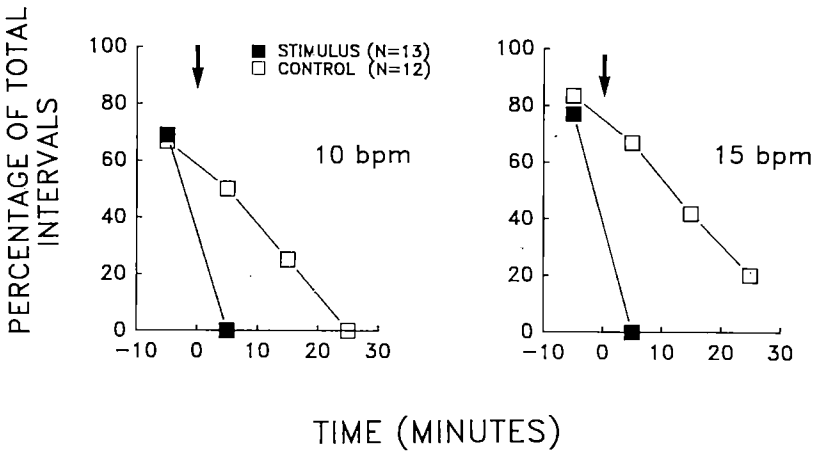


Fig. 5. Percentage of time intervals during which fewer than two FHR accelerations of ≥10 beats/min *(10 bpm)* and of ≥15 beats/min *(15 bpm)* for ≥15 seconds occurred was plotted for all fetuses before and after stimulus *(solid squares)* or control *(open squares)*.

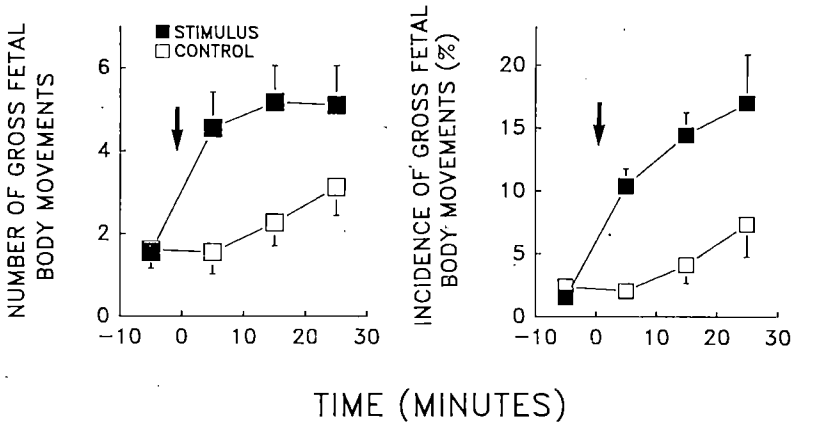


Fig. 6. Mean number and mean incidence of gross fetal body movements were plotted in 10-minute intervals before and after stimulus *(solid squares)* or control *(open squares)*.

Toronto) as previously described.[9] The transducer operated at a frequency of 3.5 MHz with an average intensity of 0.045 mW/cm². Individual gross fetal body movements and fetal breathing movements were identified on a video monitor and coded with an event marker. The transducer was positioned over the maternal abdomen to permit continuous observation of a longitudinal cross section of fetal chest and abdominal wall echoes.[9]

Data analysis. The basal FHR, the number of FHR accelerations of ≥10 beats/min for ≥15 seconds above the baseline, the duration and amplitude of individual FHR accelerations, the total time spent in accelerations, and the mean minute range were determined for successive 10-minute intervals. All results are presented as means ± SEM for successive 10-minute intervals during the 10 minutes before and the 30 minutes after stimulus or control. Portions of records that could not be analyzed because of FHR signal loss of >30% or patient interruption were not included. These portions made up 4.0% of total study time.

Measurements of the percentage of time spent making gross fetal body movements, the percentage of time spent making breathing movements, and the frequency distribution of breath-to-breath intervals were calculated for each 10-minute interval of the 25 hours of total observation time. Portions of movement records that could not be analyzed because of technical problems or patient interruption made up 5.0% of total time.

Statistical significance between groups before and after stimulus or control was determined by using a mixed, nested analysis of variance comparing overall means and each of the 10-minute intervals[10] with an HP 9845B microprocessor (Hewlett Packard, Computer Division, Fort Collins, Colo.).

Results

Basal FHR and FHR variability. An example of the FHR response after a pure vibratory stimulus, during an episode of low FHR variability (mean minute range = 18 milliseconds) at 39 weeks, is shown in Fig.

2. The mean 10-minute mean minute range was 25.6 ± 2.3 milliseconds before stimulus. After vibratory stimulation, the mean 10-minute mean minute range increased to 61.2 ± 5.4 milliseconds during the first 10 minutes after stimulus and remained above control for 30 minutes (analysis of variance, $p < 0.001$) (Fig. 3). No significant change in the mean basal FHR was found after square-wave vibratory stimulation (Fig. 3).

Characteristics of FHR accelerations. The mean total time spent in accelerations per 10 minutes was 29 ± 14 seconds before stimulus and increased to 149 ± 14 seconds during the 30 minutes after stimulation compared with 52 ± 11 seconds after control (analysis of variance, $p < 0.001$) (Fig. 4). Similarly, there was an immediate and sustained increase in the number of FHR accelerations from 0.9 ± 0.4 during the 10 minutes before stimulus to 4.0 ± 0.4 after vibratory stimulus (analysis of variance, $p < 0.001$) (Fig. 4). No significant change was found in the mean amplitude or the mean duration of FHR accelerations after either stimulus or control (34.4 ± 5.1 beats/min and 37 ± 5 seconds, respectively). It was important that all fetuses (100%) had more than two FHR accelerations during the first 10 minutes after vibratory stimulation with either 10 or 15 beats/min for acceleration amplitudes compared with 50% and 35%, respectively, after control (Fig. 5). This suggested that at least 50% of the fetal responses were due to vibratory stimulus compared with control.

Gross fetal body movements. Fig. 6 demonstrates that there was an immediate and sustained increase in both number and incidence of gross fetal body movements from 1.6 ± 0.3 and 1.5% ± 0.8% before stimulus to 5.0 ± 1.5 and 13.9% ± 1.9% during the 30 minutes after vibratory stimulus (analysis of variance, both $p < 0.001$).

Fetal breathing movements. Fetuses made breathing movements 26.9% ± 8.0% of the time during the 10 minutes before vibratory stimulation, and the incidence of breathing movements was not significantly altered by stimulus compared with control.

Fig. 7 demonstrates a histogram plot of the mean percentage of total breath-to-breath intervals in successive 0.5-second increments in 12 fetuses during the 10 minutes before control ($n = 11$) or stimulus ($n = 12$) (Fig. 7, *A*) and in the same fetuses during the 30 minutes after control or stimulus (Fig. 7, *B*). There was a skewed distribution of breath-to-breath intervals with a peak at 1.0 to 1.5 seconds before and after control (Fig. 7, *A* and *B*). However, during the 30 minutes after stimulus, there was a broad distribution of breath-to-breath intervals at 0.5 to 1.5 seconds without a well-defined peak (Fig. 7, *B*). This suggested that fetuses were breathing more irregularly after square-wave vibratory stimulation.

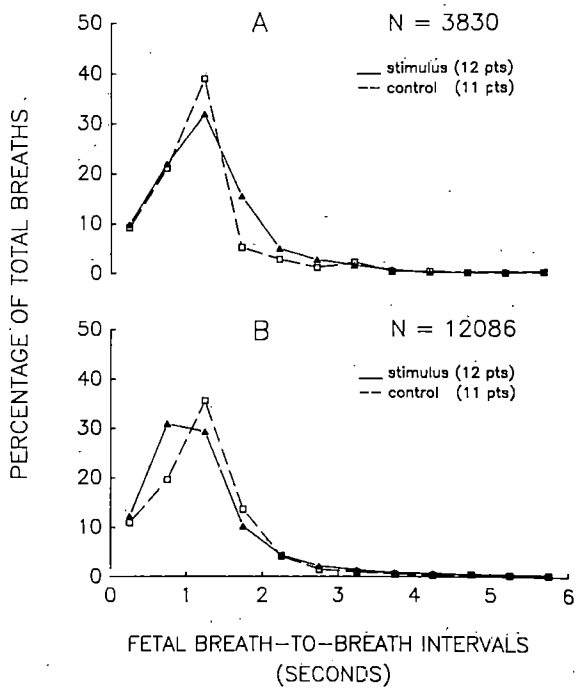


Fig. 7. Fetal breath-to-breath intervals were measured, and histograms of mean percentage of total breath-to-breath intervals in 0.5-second increments were plotted for 12 fetuses during 10 minutes before **(A)** and 30 minutes after **(B)** stimulus (——) or control (- - - -). There was a broad distribution of breath-to-breath intervals at 0.5 to 1.5 seconds without a well-defined peak after stimulus.

Comment

The results indicated that, in a group of healthy term fetuses, the patterns in the FHR and fetal movement were significantly altered after a 5-second external vibratory stimulus (100 Hz, square wave) applied during an episode of low FHR variability. There was an immediate and sustained increase in long-term FHR variability, number of FHR accelerations, and gross fetal body movements. Analysis of fetal breathing intervals demonstrated a broader distribution of breath-to-breath intervals after stimulus. No significant change was found in the basal FHR or the characteristics of FHR accelerations.

In humans, Meissner's and pacinian corpuscles (fundamental frequency response beween 80 and 150 Hz) are thought to be the primary vibration-sensitive receptors and are related to proprioceptive sensation. In healthy term newborns, proprioceptive reflexes can be elicited mainly in behavioral state 1 (quiet sleep)[11]; auditory response is best obtained in state 2 (rapid-eye-movement sleep) and state 3 (quiet wakefulness).[12] Nociceptive reflexes, elicited after stimuli such as tickling, scratching of the skin, and Babinski reflex, are state independent.[11] Finally, auditory responses at high intensity (above 95 dB) are state independent.[13]

During a pilot study in our laboratories (Gagnon R,

et al., unpublished observations), no fetal response was found with the same vibratory stimulus during episodes of high FHR variability associated with FHR accelerations. Also, no response was found at frequencies above 150 Hz. This suggested that vibratory (best stimulated in quiet sleep) rather than auditory (best stimulated in active sleep, fundamental frequency response 2000 Hz) sensory receptors were stimulated in the present study. Proprioceptive reflexes, which are best obtained in quiet sleep state in newborns,[11] might therefore also be present in healthy term fetuses.

There is evidence that the nature of the stimulus may be important in obtaining a response in the human fetus and newborn. The spectral component of a square wave is the superposition of multiple odd harmonics of a pure tone sine wave[14] (Fig. 1). Hutt et al.[13] measured instantaneous heart rate and electromyographic responses in upper and lower limbs to acoustic stimulation in term newborns. The best heart rate and electromyographic responses occurred after a square-wave stimulus of 125 Hz, 75 dB. No consistent response was seen with a sine wave or at frequencies above 500 Hz.[13] Bernstein et al.[15] measured vibrotactile sensitivity thresholds from two profoundly deaf children, to sine-wave and square-wave vibratory stimuli, at frequencies between 20 and 160 Hz. The deaf children had the same sensitivity thresholds only to square-wave vibratory stimulus, when compared with a group of children with normal hearing.[16] The authors suggested that square-wave vibrotactile stimulation may be potentially useful to assess neurologic function in profoundly deaf children.[15] The lack of any correlation between congenital deafness and the FHR response to vibrotactile stimulation[16] also supports this suggestion.

Similarly, we recently reported more consistent FHR and fetal body movement responses after square-wave vibratory stimulus (100 Hz, 48 dB) than after sine-wave stimulus of the same intensity (Gagnon R., et al., unpublished observations). This indicated that, as in newborns[13] and children,[15] the type of stimulus (square wave vs. sine wave) used might be important to predict human fetal sensory responses to low-frequency vibration.

The lack of any increase in basal FHR and duration of FHR accelerations after vibratory stimulus contrasted with the sustained increase in basal FHR and large FHR accelerations (which we believe to be wakefulness) after vibratory acoustic stimulation with the electronic artificial larynx. This suggested that the acoustic and vibratory components[6] of the electronic artificial larynx are important to elicit fetal responses to a mixed vibratory acoustic stimulus.[5-7]

Current data demonstrated an immediate increase in FHR variability, FHR accelerations, and fetal movements after vibratory stimulus. Fetuses also breathed more irregularly. Both FHR and fetal activity patterns were similar to rapid-eye-movement sleep in newborns[17, 18] and to state 2F in healthy term fetuses.[19] These observations would be compatible with the hypothesis that fetuses have been changed from a state of quiet sleep to a state of rapid-eye-movement sleep as a result of low-frequency, square-wave vibratory stimulation. It is not possible to determine whether the higher frequencies (>1500 Hz) of the square-wave vibratory stimulus could be transmitted as an "auditory stimulus" available to the fetus. In the near future, using a miniaturized hydrophone to record intrauterine sound pressure level at the time of vibratory stimulation, we hope to be able to answer that question.

Although human fetal responses to sound and vibration are potentially useful to assess fetal health[2, 3] and, perhaps, fetal neurologic function,[5] it remains to be determined if human fetuses, during such stimuli, are exposed to higher intensities of sound or vibration than measured in air.[6]

If clinicians wish to shorten nonstress testing times to be certain that FHR accelerations and fetal movements are occurring, pure vibration applied during quiet periods might be preferable to the electronic artificial larynx, which causes a prolonged fetal tachycardia[7] and shortens nonstress testing by only 4½ minutes.[2] Determination of long-term neurobehavioral development after repeated stimuli with the electronic artificial larynx also is important before the electronic artificial larynx is suggested for use in clinical practice.

We thank Mrs. Maria de Sousa for her excellent technical assistance.

REFERENCES

1. Read JA, Miller FC. Fetal heart rate acceleration in response to acoustic stimulation as a measure of fetal well-being. Am J Obstet Gynecol 1977;129:512.
2. Smith CV, Phelan JP, Platt LD, et al. Fetal acoustic stimulation testing. II. A randomized clinical comparison with the nonstress test. Am J Obstet Gynecol 1986;155:131.
3. Leader LR, Baillie P, Martin B, Vermeulen E. The assessment and significance of habituation to a repeated stimulus by the human fetus. Early Hum Dev 1982;7:211.
4. Schmidt W, Boos R, Gnirs LA, Schulze S. Fetal behavioural states and controlled sound stimulation. Early Hum Dev 1985;12:145.
5. Gagnon R, Hunse C, Carmichael L, et al. Human fetal responses to vibratory acoustic stimulation from twenty-six weeks to term. Am J Obstet Gynecol 1987;157:1375.
6. Gagnon R, Patrick J, Foreman J, West R. Stimulation of human fetuses with sound and vibration. Am J Obstet Gynecol 1986;155:848.
7. Gagnon R, Hunse C, Carmichael L, et al. External vibratory acoustic stimulation near term: fetal heart rate and heart rate variability responses. Am J Obstet Gynecol 1987;156:323.
8. Dawes GS, Redman CWG, Smith JH. Improvements in the registration and analysis of fetal heart rate records at the bedside. Br J Obstet Gynaecol 1985;92:317.

9. Patrick J, Campbell K, Carmichael L, et al. A definition of human fetal apnea and the distribution of fetal apneic intervals during the last ten weeks of pregnancy. AM J OBSTET GYNECOL 1980;136:471.
10. Hicks CR. Fundamental concepts in the design of experiments. 2nd ed. New York: Holt, Rinehart and Winston, 1973:100.
11. Lenard HG, Bernuth H, Prechtl HFR. Reflexes and their relationships to behavioural state in the newborn. Acta Paediatr Scand 1968;3:177.
12. Hutt SJ, Hutt C, Lenard HG, et al. Auditory responsivity in the human neonate. Nature (Lond) 1968;218:888.
13. Hutt SJ, Lenard HG, Prechtl HFR. Psychophysiological studies in newborn infants. Adv Child Dev Behav 1969;4:127.
14. Malvino AP. Electronic principles. New York: McGraw-Hill, 1973:603.
15. Bernstein LE, Schechter MB, Goldstein MH Jr. Child and adult vibrotactile thresholds for sinusoidal and pulsatile stimuli. J Acoust Soc Am 1986;80:118.
16. Querleu D, Boutteville C, Renard X, Crepin G. Evaluation diagnostique de la souffrance foetale pendant la grossesse au moyen d'un test de stimulation sonore. J Gynecol Obstet Biol Reprod 1984;13:789.
17. Dehaan R, Patrick J, Chess GF, Jaco NT. Definition of sleep state in the newborn infant by heart rate analysis. AM J OBSTET GYNECOL 1977;127:753.
18. Prechtl HFR, Akiyama Y, Zinkin P, Kerr Grant D. Polygraphic studies of the fullterm newborn. I. Technical aspects and qualitative analysis. In: Bax MCO, Mackeith RC, eds. London: Heinemann, 1968:1.
19. Nijhuis JG, Prechtl HFR, Martin CB Jr, Bots RSGM. Are there behavioural states in the human fetus? Early Hum Dev 1982;6:177.

Nifedipine pharmacokinetics during preterm labor tocolysis

James E. Ferguson II, MD,[a] Thomas Schutz, BA,[a] Robert Pershe, BS,[c] David K. Stevenson, MD,[b] and Terrence Blaschke, MD[c]
Stanford, California

Nifedipine, a calcium entry blocker, has known relaxing effects on the myometrium. Thirteen women in preterm labor received nifedipine for tocolysis. Blood samples obtained serially during treatment and at the time of delivery were assayed for maternal and neonatal nifedipine concentrations. The peak concentration of nifedipine during sublingual therapy ranged from 23.4 to 197.9 ng/ml and reflected substantial interpatient variability. The mean (±SD) measurable trough value in patients who received 20 mg of nifedipine orally every 6 hours was 7.2 ± 5.5 ng/ml. The maternal mean half-life of nifedipine was 81 minutes (range 49 to 137 minutes). At delivery, neonatal nifedipine levels were nondetectable in 6 of the 11 neonates available for study; in 5, values ranged from 29.5 to 1.8 ng/ml. From these results we conclude that both sublingual and oral nifedipine treatment results in variable but usually measurable maternal plasma concentrations and that placental transfer of nifedipine occurs. (AM J OBSTET GYNECOL 1989;161:1485-90.)

Key words: Preterm labor, nifedipine, pharmacokinetics, calcium entry blockers

Preterm labor continues to complicate a significant number of pregnancies and contributes substantially to perinatal morbidity and mortality.[1] Although a variety of pharmacologic agents are currently used to treat preterm labor, only the β-adrenergic agent ritodrine has a Food and Drug Administration–labeled indication for such treatment.[2] The use of β-adrenergic agents can be associated with undesirable side effects.[2] Because of these potential side effects, we were prompted to study an alternate tocolytic agent.

Calcium entry blockers, by virtue of their relaxing effects on the myometrium, show promise. Preliminary studies with nifedipine have suggested that the drug can inhibit labor while producing few apparent side effects.[3-5]

Although the pharmacokinetics of nifedipine have been studied in healthy subjects[6,7] and in patients with various cardiovascular disorders,[8] there are no data available for pregnant women. Because of the clinical interest in nifedipine as a tocolytic agent, we sought to

From the Section of Maternal-Fetal Medicine, Department of Gynecology and Obstetrics,[a] and the Departments of Pediatrics[b] and Medicine,[c] Stanford University School of Medicine.
This research was supported in part by a Mellon Foundation Fellowship Award and National Institutes of Health grants RR-81, HD-14426, and GM22209.
Presented at the Thirty-fifth Annual Meeting of the Society for Gynecologic Investigation, Baltimore, Maryland, March 17-20, 1988.
Reprint requests: J. E. Ferguson II, MD, Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of Virginia School of Medicine, Charlottesville, VA 22908.
6/6/16237

define maternal peak values and the half-life of nifedipine and to determine if placental transfer of nifedipine occurs in patients undergoing preterm labor tocolysis.

Material and methods

Patients with preterm labor occurring between 20 and 36 completed weeks of gestation were eligible for enrollment into the study. Informed consent, as approved by the Stanford University Committee for the Protection of Human Subjects in Research, was obtained from each patient. Preterm labor was defined as regular uterine contractions with a documented change in cervical dilatation or effacement after 20 weeks but before 36 completed weeks' gestational age. Patients with these maternal factors were excluded: prior recent tocolytic exposure, diabetes, hyperthyroidism, cardiac disease, severe preeclampsia-eclampsia, abruptio placentae, chorioamnionitis, multiple gestation, hydramnios, and cervical dilatation >4 cm. Fetal distress, suspected intrauterine growth retardation, a dead fetus, or a fetal anomaly incompatible with life constituted fetal factors for exclusion.

The pharmacokinetic investigations in the present study were performed in a group of patients who were already part of an ongoing prospective, randomized clinical trial comparing nifedipine and ritodrine tocolysis. Patients selected by the randomization process to receive nifedipine gave additional informed consent for the extra phlebotomies required for the pharmacokinetic studies. An intravenous infusion of 5% dextrose in lactated Ringer's solution at 100 ml/hr was administered to all patients after an initial 200 ml fluid bolus. Nifedipine tocolysis was initiated with a 10 mg capsule given sublingually; patients were instructed to bite the capsule between their molars and, if possible, to avoid swallowing to facilitate sublingual absorption. If uterine contractions persisted after 20 minutes, a similar dose was given. This pattern was repeated, if needed, up to a maximal total dose of 40 mg administered sublingually during the first hour of treatment. If sublingual tocolysis abolished the uterine activity, then oral therapy with 20 mg of nifedipine was initiated 6 hours after the last sublingual capsule. This dose was repeated at 4- to 6-hour intervals or, if continuation of tocolysis was clinically indicated, occasionally at more frequent intervals. Tocolysis was attempted for 48 hours when glucocorticoids were administered to patients with ruptured membranes and through 36 completed weeks of gestation when the membranes were intact. If delivery was delayed beyond these intervals, tocolysis was considered "successful." Before and at 12 hours of treatment, an electrocardiogram was obtained on each patient. Throughout the initial 24-hour period of treatment, patients remained in the left lateral decubitus position and received nothing by mouth, except for sips of water with oral nifedipine.

Fluid intake was limited to 2400 ml/24 hours, and records of total intake and urine output were made. Maternal blood pressure, pulse and respiratory rate, fetal heart rate, and uterine activity were recorded at frequent intervals. Serial laboratory evaluations were performed, and the use of amniocentesis and steroid administration followed our standard practice as previously described.[3]

To facilitate blood sampling, a heparin lock was placed in each patient participating in the pharmacokinetic studies. The heparin lock was maintained with 1:1000 sodium heparin solution and flushed at regular intervals. Five milliliters of blood was obtained before the first sublingual nifedipine dose and 20 minutes after each sublingual dose given (before each subsequent sublingual dose). After the last sublingual dose, blood was obtained on an hourly basis until the first oral dose. These samples were used for a basis to determine the half-life of the drug. Patients receiving oral nifedipine had blood samples for nifedipine levels obtained at 4- to 6-hour intervals (immediately before each subsequent oral dose) during the initial 24 hours of treatment. Occasionally (for instance, at the time of discharge from the hospital or when a patient was seen on an outpatient basis), blood was drawn for trough concentrations after the initial 24-hour study interval. Additionally, maternal and neonatal samples were obtained at the time of delivery, and neonatal sampling was repeated at 12 and 24 hours of life. In one patient blood samples (maternal and neonatal) were drawn only at the time of delivery, not during initial sublingual or oral tocolysis. Blood samples were transferred to heparinized screw-top vials wrapped with aluminum foil (to protect against light degradation) and placed in a refrigerator. Within 24 hours, the samples were centrifuged and the plasma was placed in cold storage (−20° C) until assayed.

Plasma nifedipine concentrations were measured by high-performance liquid chromotography with ultraviolet detection by means of a minor modification of the method developed by Raemsch and Sommer.[7] The system consisted of a Waters model 6000A pump with an IBM 9505 autosampler and a Spectra-Physics 4290 integrator. A 15 cm Waters NovaPak C_{18} column was used and a Waters model 441 ultraviolet absorbance detector was operated at 254 nm and AUFS (absorbance units full scale) 0.005. The mobile phase was 55% methanol and 45% 0.05 mol/L monobasic potassium phosphate (pH 3.0 by addition of 85% phosphoric acid). The flow rate was 1.0 ml/min. Extraction of 1.0 ml of plasma, 100 ng of the internal standard (nitrendipine), and 500 μl of 1 mol/L sodium hydroxide was done with 7.5 ml of ethyl ether and hexane (1:1, vol/vol).

The aqueous phase was frozen in a dry ice–acetone bath, and the organic layer was separated and evaporated at 37° C under a vacuum. The residue was reconstituted with 200 μl of the mobile phase and 100 μl was injected onto the chromatograph. All extraction and chromatographic procedures were performed under yellow light to prevent the photodegradation of nifedipine.

A set of nifedipine standards was run along with each set of unknown samples. Normalized peak area ratios, derived from the standard curve, were used to calculate nifedipine concentrations in the unknowns. In general, the lower limit of the nifedipine assay was 5 ng/ml; however, in some cases, if the background noise in the chromatogram was <30% of the nifedipine signal, plasma concentrations of 1 to 2 ng/ml could be detected. The half-life of nifedipine was estimated by fitting the postpeak plasma concentration-versus-time data to a monoexponential function, by means of a weighted nonlinear regression analysis.[10]

Results

Demographic data that relate to the study patients are depicted in Table I. None of the patients had a prior history or current evidence of hepatic impairment. The mean (±SD) peak concentration of nifedipine in the 12 patients in whom blood samples were drawn during sublingual therapy was 96.7 ± 45.3 ng/ml (range 23.4 to 197.9 ng/ml). Plasma concentrations from the study subjects obtained during sublingual dosing are presented in Fig. 1. Subjects generally had increasing plasma concentrations after each successive dose of sublingual nifedipine. Several patients (Nos. 3, 5, and 7), however, demonstrated nearly equal or lower values with each additional sublingual dose. The half-life (mean ± SD) of nifedipine, calculated from plasma concentrations obtained hourly after the last sublingual dose but before the first oral dose, was 81 ± 26 minutes (range 49 to 137 minutes).

Sublingual tocolysis was effective in 11 of 13 patients and they were subsequently treated with oral nifedipine. In 10 of these 11 patients trough values were determined routinely during the initial 24-hour study period. An every-6-hour oral dosing schedule was followed in 9 patients. Blood for the determination of the 6-hour trough values during the initial 24 hours of treatment was therefore obtained in 27 instances. Measurable values were detected in 24 samples; the mean (±SD) for these values was 7.2 ± 5.5 ng/ml (n = 24). The levels ranged between 1.5 and 21 ng/ml. Some drug accumulation seemed to occur in the remaining patient, who received her first oral dose 5 hours after the last sublingual dose, then an oral dose 5 hours later, followed by three oral doses at 4-hour intervals. The oral trough plasma concentrations for blood samples obtained during the 5-hour and subsequent 4-hour intervals from this patient were 34, 44, 48, and 57 ng/ml. Additionally, four of the nine patients who were receiving 20 mg of nifedipine orally every 6 hours had a single trough plasma concentration determined between 2 and 21 days after the initial pharmacokinetic studies. The values obtained 2 to 21 days later were 5.9, 6.0, 7.0, and 5.7 ng/ml, compared with the corresponding initial average 6-hour trough values of 5.4, 3.9, 6.0, and 5.0 ng/ml.

Table I. Maternal characteristics

Characteristic	*Value*
Age (yr)*	26.4 ± 5.3
Gestational age (wk)*	29.7 ± 4.0
Parity	
0	7/13
>0	6/13
Cervical dilatation	
≤2 cm	10/13
>2, ≤4 cm	3/13
Weight (kg)*	66.5 ± 10.9
ROM†	6/13

*Values are mean ± SD.
†Ruptured chorioamniotic membranes.

Neonatal and corresponding maternal nifedipine levels at the time of delivery are shown in Table II. In patients 1, 7, and 8, tocolysis failed and delivery occurred within 2, 5, and 3 hours, respectively, of the last maternal nifedipine dose. In the remaining 10 patients, nifedipine was discontinued either because the gestation had been prolonged ≥48 hours in patients with ruptured membranes or beyond 36 weeks in patients with intact membranes or, in one instance, because a patient wished to withdraw from the study. As shown, delivery occurred at various intervals after the last nifedipine dose. The disposition of nifedipine could be determined in the neonate of patient 1 and neonatal levels at 12 and 24 hours of life were 13.6 and 10.4 ng/ml, respectively, indicating a half-life of 26.5 hours. In the remaining 10 infants available for study, neonatal nifedipine concentrations at 12 and 24 hours of life were nondetectable in six and not obtained in four neonates. The calculated fetal/maternal ratios for patients 1, 3, 4, and 13 were 0.63, 0, 1.3, and 0.75, respectively.

Comment

To our knowledge, neither the disposition of nifedipine in pregnant women nor the concentration of drug in neonates at the time of delivery has been previously reported. We have demonstrated that both sublingual and oral nifedipine administration results in variable but usually measurable maternal blood levels in patients receiving this drug for preterm labor tocolysis. Our results are consistent with previous studies in healthy

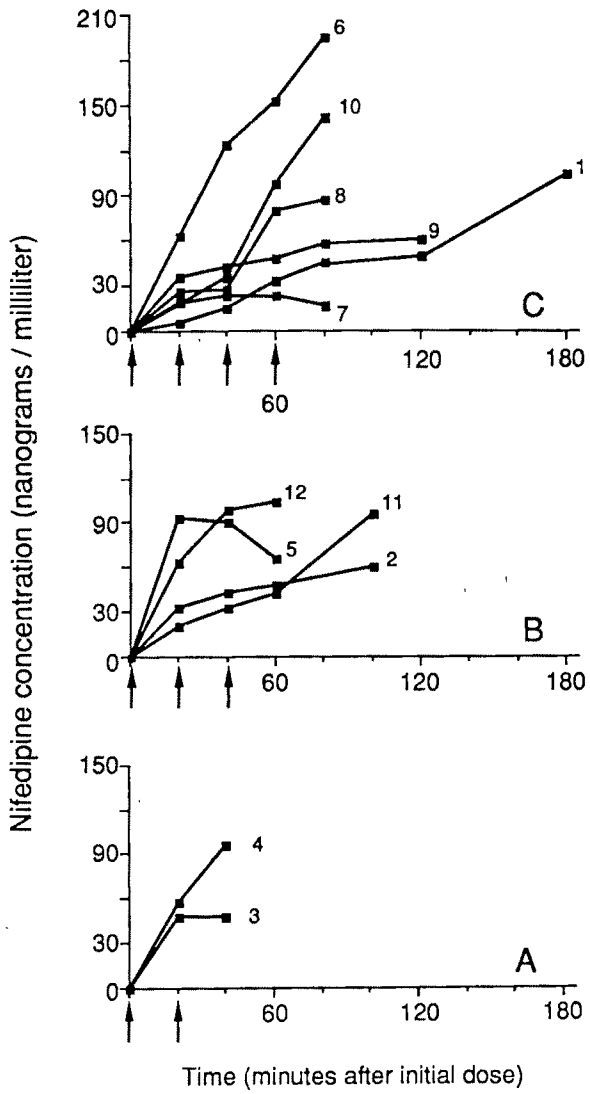


Fig. 1. Nifedipine concentrations for 12 patients receiving two, three, and four sublingual doses, respectively, as shown in panels *A*, *B*, and *C*. *Arrows* indicate approximate times of sublingual 10 mg nifedipine administration. Each curve ends at the corresponding patient's maximum measured concentration, excepting the curves labeled *3*, *5*, and *7*, which are unique in that concentrations remained the same or even fell during serial administration of the drug (these curves end at the first measurement after the final sublingual dose).

volunteers and patients with hypertension, which likewise demonstrated extreme interpatient variability in pharmacokinetic parameters including half-life, magnitude and timing of peak plasma concentrations, and trough levels.[6-8] In these studies the interpatient variability existed irrespective of whether the drug was administered intravenously, orally (capsules or tablets), sublingually, or rectally.

Although the exact cause of the large interpatient variations in measured peak plasma concentrations after sublingual administration among the patients in this study and other studies of nonpregnant patients is unknown, variations may be attributed to differences in the site and rate of absorption, extent of first-pass hepatic extraction, and rate of metabolism. It is generally accepted by investigators that sublingual nifedipine absorption occurs and is rapid[7]; nonetheless, some controversy exists concerning the efficiency of nifedipine absorption through the buccal mucosa when compared with absorption from the stomach and intestine. Indeed, McAllister[11] suggested that sublingual absorption of nifedipine may be limited and that active absorption may occur only after patients swallow and the drug reaches the stomach. If so, interpatient differences in the length of time a "sublingual" dose is held in the mouth before swallowing may, by potentially delaying absorption, also contribute to the observed variability. Although nearly all of a swallowed dose is thought to be absorbed in the stomach and small intestine, Nakashima et al.[12] suggested that patients with "fast" and "slow" absorption exist and that they differentially absorb nifedipine in the stomach and small intestine, respectively, accounting for further differences in the timing and magnitude of peak plasma concentrations. Additionally, Kleinbloesem et al.[13] have identified the existence of an oxidative polymorphism regulating the metabolism of nifedipine. The majority of patients have been phenotypically classified as "rapid metabolizers"; approximately 20% are thought to be "slow metabolizers." The differing rates of metabolism could also compound the interindividual differences reported.

Direct comparison of pharmacokinetic parameters with other studies is difficult because pregnant women have not been previously studied and because differing dosages and routes of administration have been used in prior studies. Although the physiologic changes of pregnancy could have affected the absorption, distribution, metabolism, and excretion of nifedipine, the pharmacokinetic parameters we observed were similar when compared with those of nonpregnant patients. There are few data on the pharmacokinetics of nifedipine after sublingual administration. Raemsch and Sommer[7] administered a single 10 mg dose of nifedipine sublingually to 10 fasting patients. Although this group of investigators also noted marked differences in individual plasma levels, the mean plasma concentration-versus-time curves generated are remarkably similar when compared with the profiles obtained from patients in this study. Drug half-life has been previously determined after single intravenous bolus and single oral dose exposure in healthy volunteers. Kleinbloesem et al.[6] noted a mean half-life of 1.7 ± 0.4 hours in six male volunteers who had intravenously received nifedipine, 0.015 mg/kg of body weight and a half-life of the same order for this same group of volunteers when a 20 mg capsule of nifedipine

Table II. Maternal half-life of nifedipine with simultaneous maternal (peripheral venous) and neonatal (umbilical venous) nifedipine concentrations at time of delivery

Patient no.	$t_{1/2}$*	*Maternal Cp at delivery*	*Neonatal Cp at delivery*	*Last dose (mg)*	*Hours†*
1	49	30	19	20	2
2	73	Nd	Nd	20	20
3	55	3.9	Nd	20	8
4	76	4.5	6.0	20	8
5	82	+	+	+	+
6	106	X	4.8	20	15.5
7	85	Nd	Nd	10	5
8	106	X	29.5	20	3
9	77	Nd	Nd	20	14.5
10	137	Nd	Nd	20	17
11	51	Nd	Nd	20	144
12	77	+	+	+	+
13	‡	2.4	1.8	20	14

Cp, Plasma nifedipine concentration in nanograms per milliliter. *Nd,* Nondetectable nifedipine level. *X,* Blood for nifedipine assay not obtained. *+,* Infant outborn after 36 weeks of gestation (nifedipine had been discontinued and maternal and neonatal blood for nifedipine assay was not requested).

*Maternal half-life (in minutes).

†Interval (in hours) from the last maternal nifedipine dose to delivery.

‡Maternal nifedipine concentration determined only at delivery.

was given orally in the crossover phase of the study. Raemsch and Sommer,[7] using similar methods, reported a half-life after intravenous treatment of 1.26 ± 0.55 hours in seven volunteers and approximately 2½ hours after oral administration. The maternal half-life of 1.35 ± 0.43 hours that we observed when postpeak plasma concentration-versus-time data were evaluated after successive 10 mg sublingual doses of two to four nifedipine capsules does not differ greatly from others reported, even though the dose, route of administration, and physiologic milieu were different. The neonatal half-life was quite long; however, comparative data are not available. Immature neonatal hepatic detoxification mechanisms may be responsible for this finding. Raemsch and Sommer[7] noted a mean nifedipine level of 17 ng/ml at 6 hours in five fasting volunteers who received 20 mg of nifedipine orally. This value is somewhat greater than the mean trough value of 7.2 ng/ml we obtained in nine patients receiving 20 mg of nifedipine orally every 6 hours.

We further demonstrated that there are no time-dependent changes in plasma trough concentrations in patients continuing the same oral dose of nifedipine for up to 21 days after the initial study period. This finding is in agreement with previous reports. Raemsch and Sommer[7] studied nine male and female volunteers on an oral dosing regimen with 10 mg of nifedipine three times daily. They noted no change in the mean maximum or trough concentrations of nifedipine when concentrations on day 7 were compared with those of day 1. In this study involving pregnant women, there was also no apparent cumulative effect of nifedipine when administered orally in 20 mg doses every 6 hours. Because of the frequent need to administer oral tocolytic agents over a prolonged period of time, it is clinically useful to know this drug apparently may be given every 6 hours without fear of a cumulative effect.

This study represents the initial documentation that nifedipine undergoes transplacental passage. The transplacental passage of verapamil has recently been reviewed.[14] Given the similarities between these two calcium entry blockers, we were not surprised that nifedipine was detected in the fetal-neonatal circulation. Although substantial cord blood levels were noted in two patients in whom tocolysis failed and delivery occurred within 3 hours after a maternal nifedipine dose, neonatal nifedipine levels were ≤6 ng/ml in all instances in which ≥5 hours had elapsed since the most recent maternal nifedipine dose. As expected, the highest neonatal levels were found in newborns of mothers who had received the last dose of nifedipine in closest proximity to the time of delivery. Because neonatal nifedipine concentrations and fetal maternal ratios have not been previously determined in humans, only animal data are available for comparison. Harake et al.[15] infused 5 and 10 $\mu g \cdot kg^{-1} \cdot min^{-1}$ of nifedipine to eight pregnant ewes. Fetal and maternal nifedipine levels (mean ± SEM) during the 5 $\mu g \cdot kg^{-1} \cdot min^{-1}$ infusion rate were 29 ± 5 and 110 ± 12 ng/ml. After the 10 μg infusion rate the respective values were 91 ± 8 and 267 ± 35 ng/ml. Individual fetal/maternal ratios were not enumerated; however, the mean fetal/maternal ratios during the 5 and 10 μg infusion rates were 0.3 and 0.4, respectively. Although the limitations in comparing transplacental passage in an animal model to that in the human are well known, the overlapping range for

the plasma concentrations (at the 5 μg infusion rate) and the fetal/maternal ratios is interesting to note.

In summary, we have demonstrated that both sublingual and oral nifedipine treatment result in variable but usually measurable maternal plasma concentrations and that placental transfer of nifedipine occurs.

We thank Marion I. Brown, MS, and the labor and delivery nurses at Stanford for their able assistance with this study.

REFERENCES

1. Quilligan EJ, Little AB, Oh W, Catz C, Alexander D, Cornblath M. An evaluation and assessment of the state of the science. In: Pregnancy, birth and the infant. Bethesda, Maryland: National Institutes of Health, 1981; NIH publication no. 82-02304.
2. Benedetti TJ. Maternal complications of parenteral beta-sympathomimetic therapy for premature labor. AM J OBSTET GYNECOL 1983;145:1-6.
3. Ulmsten U. Treatment of normotensive and hypertensive patients with preterm labor using oral nifedipine, a calcium antagonist. Arch Gynecol 1984;236:69-72.
4. Read MD, Wellby DE. The use of a calcium antagonist (nifedipine) to suppress preterm labour. Br J Obstet Gynaecol 1986;93:933-7.
5. Ferguson JE II, Dyson DC, Holbrook RH Jr, Schutz T, Stevenson DK. Cardiovascular and metabolic effects associated with nifedipine and ritodrine tocolysis. AM J OBSTET GYNECOL 1989;161:788-95.
6. Kleinbloesem CH, van Brummelen P, van de Linde JA, Voogd PJ, Breimer DD. Nifedipine: kinetics and dynamics in healthy subjects. Clin Pharmacol Ther 1984;35:742-9.
7. Raemsch KD, Sommer J. Pharmacokinetics and metabolism of nifedipine. Hypertension 1983;5(suppl II):II-18–24.
8. Lederballe Pedersen O, Christensen CK, Mikkelsen E, Rämsch KD. Relationship between the antihypertensive effect and steady-state plasma concentration of nifedipine given alone or in combination with a beta-adrenoceptor blocking agent. Eur J Pharmacol 1980;18:287-93.
9. Ferguson JE II, Hensleigh PA, Gill P. Effects of betamethasone on white blood cells in patients with premature rupture of the membranes and preterm labor. AM J OBSTET GYNECOL 1984;150:439-41.
10. Holford NHG. MKMODEL—a mathematical modelling tool for the prophet system. In: Perry HM, ed. Prophet public procedures notebook. Cambridge, Mass.: Bolt, Beranek, and Newman, 1988:244-70.
11. McAllister RG Jr. Kinetics and dynamics of nifedipine after oral and sublingual doses. Am J Med 1986;81(suppl 6A):2-5.
12. Nakashima T, Inoki M, Nakanishi Y. Nifedipine serum concentration; effects upon blood pressure and heart rate in normotensive volunteers. Eur J Drug Metab Pharmacokinet 1984;9:73-8.
13. Kleinbloesem CH, Van Brummelen P, Faber H, Danhof M, Vermeulen NPE, Breimer DD. Variability in nifedipine pharmacokinetics and dynamics: a new oxidative polymorphism in man. Biochem Pharmacol 1984;33:3721-4.
14. Ferguson JE II, Siukola LVM, Albright GA. The use of verapamil for paroxysmal supraventricular tachycardia during epidural anesthesia for cesarean section. Am J Perintaol 1988;5:128-30.
15. Harake B, Gilbert RD, Ashwal S, Power GG. Nifedipine: effects on fetal and maternal hemodynamics in pregnant sheep. AM J OBSTET GYNECOL 1987;157:1003-8.

Bound volumes available to subscribers

Bound volumes of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY are available to subscribers (only) for the 1989 issues from the Publisher, at a cost of $56.00 ($79.00 international) for Vol. 160 (January-June) and Vol. 161 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue in the volume. The binding is durable buckram with the JOURNAL name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact The C.V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318, USA; phone (800) 325-4177, ext. 351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular JOURNAL subscription.

Prediction of hematocrit decline after intravascular fetal transfusion

Scott N. MacGregor, DO, Michael L. Socol, MD, Bruce W. Pielet, MD, John S. Sholl, MD, and Richard K. Silver, MD

Chicago and Evanston, Illinois

Fetal blood sampling and intravascular transfusion via cordocentesis allow more precise fetal evaluation and treatment in isoimmunized pregnancies. However, the timing of repeat transfusion has remained empiric. In this report we review our experience with fetal transfusions in isoimmunized pregnancies to evaluate the ability to predict fetal hematocrit decline and thereby determine the optimum timing for repeat transfusions. Between March 1986 and March 1988, 60 intravascular fetal transfusions were performed in 20 patients. Fetal transfusions were excluded from analysis if blood samples were unable to be obtained before and after transfusion, as well as at the beginning of the next transfusion or birth. Fifty-three procedures were analyzed. Two equations were used to predict the fetal hematocrit at the subsequent transfusion. The difference between observed and predicted hematocrits at the beginning of a subsequent transfusion or birth was −0.9% ± 5.8% with equation 2, which was the more accurate formula in the majority of fetuses. Prediction of fetal hematocrit decline may be used to determine the optimum timing of repeat transfusion. (AM J OBSTET GYNECOL 1989;161:1491-3.)

Key words: Fetal transfusion, isoimmunization, cordocentesis, fetal therapy

Fetal blood sampling and intravascular transfusion via cordocentesis allow more precise fetal evaluation and treatment in pregnancies complicated by blood group isoimmunization.[1-3] Fetal transfusion volume is determined by the severity of anemia, and the adequacy of therapy can be assessed by the posttransfusion fetal hematocrit. Despite these improvements in the management of the severely isoimmunized fetus, the timing of repeat transfusion has remained empiric. In this report we review our experience with fetal transfusions in isoimmunized pregnancies to evaluate the ability to predict fetal hematocrit decline and thereby determine the optimum timing for repeat transfusions.

Material and methods

Between March 1986 and March 1988, 60 intravascular fetal transfusions were performed in 20 patients with gestational ages between 21 and 35 weeks. Ultrasonography-guided intravascular transfusions were performed with methods previously described with a few modifications.[3] A detachable needle guide is no longer used and maternal administration of sedatives has been replaced by the intravenous infusion of pancuronium bromide (0.05 to 0.1 mg/kg) to the fetus after needle tip placement into the umbilical vein.

Transfusions were performed with cytomegalovirus-negative, frozen, washed, packed red blood cells cross-matched against maternal blood. Transfusion volumes ranged from 30 to 110 ml. Three minutes after completion of the transfusion, fetal blood was sampled for the posttransfusion hematocrit with the first 0.5 to 1.0 ml aliquot of this sample being discarded. The needle was left within the umbilical vessel during this period of donor-fetal blood mixing to avoid needle replacement. The desired fetal hematocrit after transfusion was approximately 45%, on the basis of the upper limits of normal fetal hematocrit between 26 and 30 weeks' gestation.[4] In those fetuses transfused either before 26 weeks' gestation or after 30 weeks' gestation, the desired posttransfusion hematocrit was slightly <45% or >45%, respectively. The pretransfusion and posttransfusion fetal hematocrits, as well as that of the donor blood, were determined with a Coulter S-IV or S-VI electronic cell counter (Coulter Electronics Inc., Hialeah, Fla.).

Fetal transfusions were excluded from analysis if blood samples were unable to be obtained after transfusion, as well as at the beginning of the next transfusion or birth. Fifty-three procedures were analyzed. Seventeen of the 20 patients were rhesus sensitized and 47 of the 53 transfusions were performed in these pregnancies. Kell, c, and E isoimmunization each complicated one pregnancy and the remaining six transfu-

From the Section of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Northwestern University Medical School, Prentice Women's Hospital and Evanston Hospital.

Sponsored by the Society For Gynecologic Investigation.

Reprint requests: Scott N. MacGregor, DO, 2650 Ridge Ave., Evanston, IL 60201.

6/6/16234

Table I. Gestational age distribution at the time of fetal intravascular transfusion

Gestational age (wk)	*Interval 1*	*Interval 2*
<24	3	1
24-27	9	9
28-30	7	11
>30	1	12
TOTAL	20	33

Table II. Differences between observed and predicted hematocrits at the subsequent transfusion or birth

Transfusion interval	*Observed-predicted hematocrit (mean ± SD)*	*Range*
Interval 1 (n = 20)		
Equation 1*	9.8 ± 9.3	−3.5-31.4
Equation 2	−3.6 ± 7.8	−14.1-13.9
Interval 2 (n = 33)		
Equation 2	0.5 ± 3.5	−8.0-8.0
Interval 1 + 2 (n = 53)		
Equation 2	−0.9 ± 5.8	−14.1-13.9

*$p < 0.05$.

Table III. Differences between observed and predicted hematocrits at subsequent transfusion or birth in interval 1 subgroups

Subgroup	*Observed-predicted hematocrit (mean ± SD)*	*Range*
Fetal hydrops		
Present (n = 5)		
Equation 1	3.3 ± 5.7	−6.4-9.4
Equation 2	−6.4 ± 6.6	−11.2-4.6
Absent (n = 15)		
Equation 1*	11.6 ± 9.4	−3.2-31.4
Equation 2	−2.9 ± 7.9	−14.1-13.9
Fetal red blood cells†		
≤10% (n = 10)		
Equation 1	5.4 ± 6.8	−3.5-21.0
Equation 2	−5.6 ± 7.1	−14.1-7.0
>10% (n = 10)		
Equation 1*	14.1 ± 9.5	−3.2-31.4
Equation 2	−1.7 ± 8.0	−12.2-13.9

*At subsequent transfusion or birth by Kleihauer-Betke testing.

†$p < 0.05$.

sions were performed in these patients. Gestational age assessment was based on menstrual dates and early ultrasonographic examinations. Fetal weight was estimated by ultrasonography-derived growth parameters obtained at each transfusion.[5, 6]

Two transfusion intervals were indentified: interval 1 between the first and second transfusions (n = 20) and interval 2 between subsequent transfusions or birth (n = 33). The hematocrit of either the fetal blood sample obtained at the beginning of the next transfusion or the umbilical cord blood sample obtained at birth was predicted with the following equations:

$$\text{Hct pred} = \text{Hct f} - \text{i} \times (\text{EFW1/EFW2}) \times (120 - \text{Days})/120 \quad \text{(Equation 1)}$$

$$\text{Hct pred} = \text{Hct f} \times (\text{EFW1/EFW2}) \times (120 - \text{Days})/120 \quad \text{(Equation 2)}$$

where Hct pred is the predicted hematocrit at the subsequent transfusion or birth, Hct f − i is the final hematocrit minus the initial hematocrit at the index transfusion, Hct f is the final hematocrit at the index transfusion, EFW1 is the ultrasonographically estimated fetal weight at the index transfusion, EFW2 is the ultrasonographically estimated fetal weight at the subsequent transfusion, and days is the number of days between the index and subsequent transfusion or birth. These equations assume that donor red blood cell survival in the fetal circulation is 120 days. In addition, equation 1 assumes that between the first and second transfusions few, if any, of the fetal red blood cells will survive.

The observed and predicted hematocrits at the beginning of a subsequent transfusion or birth were compared. Hematocrits were predicted with both equations 1 and 2 for the interval 1 transfusions. Since only two (6%) of the interval 2 transfusions had >10% fetal red blood cells (determined by Kleihauer-Betke testing) remaining at the subsequent transfusion or birth, hematocrits were predicted with only equation 2 for the interval 2 transfusions.

Interval 1 transfusions were further divided into those with presence versus absence of fetal hydrops and fetal red blood cells ≤10% versus >10% at the subsequent transfusion or birth. Observed and predicted hematocrits also were compared in these subgroups. Comparisons were performed with the paired t test with statistical significance assumed at $p < 0.05$.

Results

Gestational age distribution at the time of transfusion is summarized in Table I. The mean interval between subsequent transfusion or birth was 16.0 ± 6.4 days; the mean intervals in the interval 1 and interval 2 groups were 12.5 ± 3.9 and 17.7 ± 6.7 days, respectively.

The differences between observed and predicted hematocrits at the beginning of a subsequent transfusion or birth are shown in Table II. The mean differences for interval 1 transfusions were 9.8% ± 9.3% with equation 1 and −3.6% ± 7.8% with equation 2. For interval 2 and all (interval 1 plus interval 2) transfusions, the mean differences were only 0.5% ± 3.5% and −0.9% ± 5.8%, respectively, with equation 2. The

predicted hematocrits were only significantly different from those observed only for the interval 1 transfusions when equation 1 was used. The mean decline in hematocrit per day between subsequent transfusion or birth was 1.3% ± 1.1%; the mean declines in hematocrit per day for the interval 1 and interval 2 groups were 1.5% ± 1.6% and 1.2% ± 0.6%, respectively.

Comparisons of the differences between observed and predicted hematocrits among the interval 1 subgroups are summarized in Table III. If fetal hydrops was present at the initial transfusion or ≤10% fetal red blood cells remained at the subsequent transfusion or birth, equation 1 and equation 2 were comparable. Otherwise, equation 2 more accurately predicted the subsequent hematocrit.

Comment

Prediction of fetal hematocrit decline after intravascular transfusion is subject to systematic errors as well as variation among different fetuses. For example, a previous case report suggested that life span of transfused adult cells may be shorter in the isoimmunized fetus (approximately 100 days) than in adults (approximately 120 days).[7] Further studies are required to confirm this observation. Additionally, estimation of fetal weight by ultrasonographic parameters may introduce error. Finally, the severity of the fetal hemolytic anemia is variable even in those fetuses with similar blood group isoimmunization. The assumption that few fetal red blood cells survive after the first transfusion (equation 1) may be correct only in the most severely affected fetuses. Indeed, half of the fetuses in the interval 1 subgroup had >10% fetal red blood cells at subsequent transfusion or birth by Kleihauer-Betke testing. Despite difficulties involving interpretation and standardization observed with this test, it is considered sensitive to 0.01.

Our data suggest that the simple formulas evaluated help to predict the decline in fetal hematocrit and determine the optimum timing of repeat transfusion. This is most evident in interval 2 because by this time the fetal red blood cells have essentially all been replaced by donor red blood cells in the fetal circulation. Consequently, the decline in fetal hematocrit is dependent on the life span of the transfused blood cells and the rate of fetal growth rather than the hemolysis that is secondary to isoimmunization. The interval between first and second transfusions (interval 1) is more problematic because of the unpredictable hemolysis of the remaining fetal red blood cells. In those fetuses with more severe hemolytic anemia few fetal red blood cells will survive the transfusion interval. However, because the rate of hemolysis of the remaining fetal red blood cells is variable and cannot be controlled for, it will always be more difficult to predict the hematocrit decline in interval 1 than in interval 2. Nonetheless, these simple formulas offer an alternative to empiric decision making.

REFERENCES

1. Grannum PA, Copel JA, Plaxe SC, Scoscia AL, Hobbins JC. In utero exchange transfusion by direct intravascular injection in server erythroblastosis fetalis. N Engl J Med 1986;314:1431-4.
2. Berkowitz RL, Chitkara U, Goldberg JD, Wilkins I, Chervenak FA, Lynch L. Intrauterine intravascular transfusions for severe red blood cell isoimmunization: ultrasound-guided percutaneous approach. Am J Obstet Gynecol 1986;155:574-81.
3. Socol ML, MacGregor SN, Pielet BW, Tamura RK, Sabbagha RE. Percutaneous umbilical transfusion in severe rhesus isoimmunization: resolution of fetal hydrops. Am J Obstet Gynecol 1987;157:1369-75.
4. Forestier F, Daffos F, Galacteros F, Bardakjian J, Rainaut M, Beuzard Y. Hematological values of 163 normal fetuses between 18 and 30 weeks of gestation. Pediatr Res 1986; 20:342-6.
5. Shepard MJ, Richards VA, Berkowitz RL, Warsof SL, Hobbins JC. An evaluation of two equations for predicting fetal weight by ultrasound. Am J Obstet Gynecol 1982;142:47-54.
6. Hadlock FP, Harrist RB, Carpenter RJ, Deter RL, Park SK. Sonographic estimation of fetal weight. Radiology 1984; 150:535-40.
7. Jones HM, Linch DC, Nicolaides K, Rodeck CH. Survival of transfused adult cells in the fetus. Fetal Ther 1986; 1:193-5.

Effect of acute intravascular volume expansion on human fetal prostaglandin concentrations

Carl P. Weiner, MD,[a] and Jean E. Robillard, MD[b]
Iowa City, Iowa

Direct fetal intravascular transfusion is well tolerated by the human fetus. However, the rapid transfusion of high-hematocrit blood should increase vessel distention and the flow shear force. Each is known to stimulate the release of prostacyclin. We measured prostaglandins E_2 and $F_{2\alpha}$ and the stable metabolite of prostacyclin, 6-keto-prostaglandin $F_{1\alpha}$, by radioimmunoassay before, at the estimated midway point of, and at the completion of 40 umbilical venous transfusions performed because of immune hemolytic anemia. Umbilical venous pressures corrected for amniotic fluid pressure were measured at similar intervals during nine of the procedures. The mean (±SEM) length of gestation at transfusion was 29 ± 1 week, opening hematocrit 23% ± 1%, and total volume of 70% hematocrit red blood cells transfused 83 ± 5 ml. 6-Keto-prostaglandin $F_{1\alpha}$ was the principal circulating fetal prostanoid and its concentration was unrelated to gestational age. Intravenous transfusion was associated with an 84% increase in 6-keto-prostaglandin $F_{1\alpha}$ ($p = 0.03$) and a 68% increase in prostaglandin E_2 ($p < 0.05$). The rise for each strongly correlated with the rise in the fetal umbilical venous pressure (6-keto-prostaglandin $F_{1\alpha}$, $r = 0.94$, $p = 0.0005$; prostaglandin E_2, $r = 0.81$, $p < 0.03$). We conclude that 6-keto-prostaglandin $F_{1\alpha}$ is the principal circulating prostaglandin in the human fetus and that the release of venodilator prostaglandins may be one reason the human fetus can tolerate a large increase in intravascular volume without obvious sequelae. (AM J OBSTET GYNECOL 1989;161:1494-7.)

Key words: Cordocentesis, human, fetus, prostaglandins, intravascular transfusion, anemia

The most frequently performed therapeutic fetal procedure is an intravascular transfusion to treat immune hemolytic anemia. Though transfusion is an effective treatment for anemia, its effects on fetal homeostasis are not well documented. We have previously demonstrated that arginine vasopressin is reduced in the anemic fetus before transfusion[1] and that atrial natriuretic factor and umbilical venous pressure are greatly elevated in the fetus with immune hydrops.[2, 3] These observations suggest that the anemic fetus is in a state of relative hypervolemia. Thus it was surprising to observe that the fetal umbilical venous pressure increased only modestly during intravascular transfusion despite sometimes doubling the estimated fetal-placental intravascular volume.[4, 5] The placenta likely contributes to stability as a high-capacitance unit, but other factors may be involved. A variety of fetal tissues from humans and sheep are capable of producing large quantities of prostacyclin and prostaglandin E_2 (PGE_2).[6-9] Each can stimulate vasodilation at appropriate concentrations[10, 11] and is released in response to an increase in shear force and perfusion pressure occurring simultaneously with vessel distention.[12] Such changes might accompany a rapid infusion of packed red blood cells and trigger the release of prostacyclin and PGE_2. In support of this possibility, we have observed that the umbilical artery systolic/diastolic ratio, resistance index, and pulsatility index decline after transfusion.[13] Others have noted a similar acute decline in Doppler-determined resistance of the renal, cerebral, and femoral arteries after transfusion.[14] Thus we hypothesized that the fetus actively enhances vascular capacitance during intravascular transfusion by the release of vasodilator prostanoids. To test this hypothesis, we measured the stable metabolite of prostacyclin (6-keto-prostaglandin $F_{1\alpha}$, 6-keto-$PGF_{1\alpha}$), PGE_2, and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) before, during, and after completing the transfusion.

From the Fetal Diagnosis and Treatment Unit, Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology,[a] and the Division of Pediatric Nephrology, Department of Pediatrics,[b] University of Iowa College of Medicine.
This research was supported in part by grant HD24494 from the United States Public Health Service (C. P. W.).
Presented in part at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.
Reprint requests: Carl P. Weiner, MD, Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, The University of Iowa Hospitals and Clinics, Iowa City, IA 52242.
6/6/16515

Material and methods

This investigation was approved by the University Institutional Human Subjects Committee. Eighteen anemic fetuses underwent a total of 40 intravascular transfusions. Intravascular transfusions were performed as described previously[13] with a 5 MHz sector transducer (model 3600, General Electric, Ran-

cho Mirage, Calif.), an attached needle guide (CIVCO Inc., Kalona, Iowa), and a 22-gauge needle (Beckton-Dickinson, Rutherford, N.J.). Each fetus received pancuronium (0.3 mg/kg estimated fetal weight intravenously) for neuromuscular blockade and furosemide (4 mg/kg intravenously in two divided doses). The packed red blood cells were prepared from fresh type O-negative blood compatible with both mother and fetus, rendered buffy coat poor, irradiated, washed twice in normal saline solution, and resuspended in saline solution to yield a hematocrit of 70%. Adequate blood was infused over a 15- to 30-minute interval to give a closing hematocrit between 45% and 50%. Only the umbilical vein was used for transfusion and blood sampling. Blood specimens for hematocrit, venous blood gas parameters, 6-keto-$PGF_{1\alpha}$, PGE_2 and $PGF_{2\alpha}$ were aspirated before, at the estimated midway point of, and immediately after completing the transfusion. During nine of the procedures, the fetal umbilical venous pressure was recorded at the same sampling intervals. The interval between repeat transfusions was at least 3 weeks.

The fetal umbilical venous pressure was measured by connecting a saline solution–filled tube to a solid-state pressure transducer (Sorenson Transpac II, no. 468626-04, supplied by Abbott Critical Care Systems, North Chicago) and pressure monitor (no. 78833B, Hewlett-Packard, Houston). The transducer was placed at the level of the fetal heart as determined during the preceding ultrasonographic examination. The identity of the vessel was confirmed by observing both the lack of a pulsatile pressure waveform on the monitor screen and the direction the infusate flowed on ultrasonography. At the conclusion of the procedure, the needle was withdrawn from the vein into the amniotic cavity where the amniotic fluid pressure was recorded. There was neither maternal perception nor sonographic evidence of uterine activity during the time the pressure measurements were made. The reported umbilical venous pressures reflect subtraction of the amniotic fluid pressure from the umbilical venous pressure.

The heparinized specimen, which provided a sample for both the blood gas measurements and the prostaglandins, was placed directly onto wet ice. Blood gas values were measured immediately with automated equipment (model 1312, Instrumentation Laboratory, Pittsburgh). The pH, P_{CO_2} (millimeters of mercury), and P_{O_2} (millimeters of mercury) were measured directly. Base deficit (milliequivalents per liter), bicarbonate (milliequivalents per liter), and oxygen saturation (percent) were calculated by means of standard nomograms.

The heparinized plasma sample was then quickly acidified with citric acid and the prostaglandins were isolated with a C_{18} solid-phase column (Bond Elut, Analytichem International, Harbor City, Calif.) with ethyl acetate used as the eluting solvent. Once prostanoids are extracted, their concentration remains stable. Recovery of tritiated prostaglandins was as follows: 6-keto-$PGF_{1\alpha}$, 71.8% ± 1.7%; PGE_2, 75.1% ± 1.1%; and $PGF_{2\alpha}$, 8.30% ± 2.8%. The samples were taken to dryness and reconstituted in a mobile phase (water/acetonitrite/benzene/acetic acid, 772:225:2:1), clarified by microfiltration, and injected into the high-pressure liquid chromatography system (Waters free fatty acid HP column, 3.9 mm × 15 cm). Recovery of tritiated prostaglandin by high-pressure liquid chromatography was: 6-keto-$PGF_{1\alpha}$, 72.8% ± 0.3%; PGE_2, 71.6% ± 1.1%; $PGF_{2\alpha}$, 66.7% ± 0.6%. Fractions containing each of the respective prostaglandins were taken to dryness with argon and stored at −20° C until assayed at multiple volumes by highly specific and sensitive radioimmunoassays performed by the University of Iowa Cardiovascular Research Center Core Laboratory.[15-17]

All results are reported as the mean ± 1 SEM. Because of the small sample volumes, not all of the prostanoids could be measured for each transfusion. Adequate specimen to measure 6-keto-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ at each of the three sampling intervals was available in 19 procedures and PGE_2 in 22 procedures. The effect of transfusion on the study parameters at each sampling interval was compared with a repetitive measures analysis of variance. When significant differences were identified, Scheffé's test was applied. The relationship between the change in concentration of the three prostanoids during transfusion and gestational age, baseline hematocrit, change in hematocrit, and change in umbilical venous pressure was investigated by least-squares multiple linear regression analyses. Differences yielding $p \leq 0.05$ were considered significant.

Results

There were no procedure-related complications and all fetuses have since been delivered uneventfully. The effects of intravascular tranfusion on the various study parameters are summarized in Table I.

The mean length of gestation at transfusion was 29 ± 1 week (range 21 to 35 weeks). The opening hematocrit ranged from 5.5% to 34%, and the total volume of packed red blood cells infused ranged from 25 to 160 ml.

Levels of both 6-keto-$PGF_{1\alpha}$ and PGE_2 rose significantly during the course of the transfusion ($p < 0.04$ for each). $PGF_{2\alpha}$ remained stable. The three prostanoids also were measured in aliquots obtained from four of the units of blood used for a transfusion. In all samples the concentrations of 6-keto-$PGF_{1\alpha}$, PGE_2, and $PGF_{2\alpha}$ were less than the baseline concentrations and usually less than half. The umbilical venous pressure rose approximately 50% during the transfusion

Table I. Effects of intravascular transfusion on study parameters

	Baseline	*Midinterval*	*Closing*
Volume infused (ml)		42 ± 3	83 ± 5
Hematocrit (%)	23 ± 1	35 ± 1*	46 ± 1*
6-Keto-$PGF_{1\alpha}$ (pg/ml)	115.2 ± 20	154.0 ± 28	211.6 ± 41†
PGE_2 (pg/ml)	64.7 ± 11	55.4 ± 11	93.2 ± 14‡
$PGF_{2\alpha}$ (pg/ml)	60.9 ± 25	38.1 ± 6	43.9 ± 10
Umbilical venous pressure (mm Hg)	7 ± 1.1	9.2 ± 1.5	11.5 ± 1.7§
pH	7.39 ± 0.006	7.36 ± 0.009*	7.34 ± 0.008*
P_{CO_2} (mm Hg)	36.1 ± 0.4	36.4 ± 0.7	35.5 ± 0.7
P_{O_2} (mm Hg)	38.5 ± 1	38.2 ± 1	38.7 ± 2
Bicarbonate (mEq/L)	22.3 ± 0.3	20.9 ± 0.5‖	19.6 ± 0.5*
Base excess (mEq/L)	−1.5 ± 0.4	−3.3 ± 0.5*	−4.8 ± 0.5*
Oxygen saturation (%)	71.6 ± 1	69.5 ± 3	68 ± 3

*$p < 0.0001$ from baseline.
†$p = 0.03$ from baseline.
‡$p < 0.05$ from midinterval.
§$p = 0.005$ from baseline.
‖$p = 0.001$ from baseline.

($p < 0.0001$). Though there were considerable differences in the patterns of umbilical venous pressure responses by the eight different fetuses ($p < 0.01$), in most who began the transfusion with an umbilical pressure in the normal range, the pressure remained within the normal limits (<10 mm Hg).

The magnitude of the increase in 6-keto-$PGF_{1\alpha}$ and PGE_2 was unrelated to either gestational age, initial or change in hematocrit, or total volume of packed red blood cells infused. However, the increase in 6-keto-$PGF_{1\alpha}$ did correlate with the increase in PGE_2 ($r = 0.67$, $p = 0.003$) and each of these venodilator prostanoids correlated strongly with the change in the umbilical venous pressure that occurred during transfusion (6-keto-$PGF_{1\alpha}$; $r = 0.94$, $p = 0.0005$; PGE_2, $r = 0.81$, $p < 0.03$). The change in umbilical venous pressure was unrelated to either the total volume infused ($r = 0.20$, $p = 0.54$) or the change in hematocrit ($r = 0.38$, $p = 0.25$).

Comment

The rapid expansion of fetal intravascular volume by intravenous transfusion of high-hematocrit blood has numerous effects on fetal homeostasis other than correcting the anemia. We have previously demonstrated that, when preceded by the administration of pancuronium and furosemide, acute fetal volume expansion with packed red blood cells (1) stimulates the release of atrial natriuretic factor[2] and (2) increases umbilical venous pressure modestly[4] despite in some instances almost doubling the total fetal-placental intravascular volume.[5] There are several potential explanations why the increase in umbilical venous pressure is not more dramatic after fetal volume expansion. First, the placenta may act as a low-pressure, high-capacity reservoir. Second, atrial natriuretic factor produces vascular smooth muscle relaxation. Third, the fetal vasculature is capable of synthesizing and releasing a high concentration of PGE_2 and 6-keto-$PGF_{1\alpha}$.[6-9] Each can produce vasodilation in a variety of vessel beds. The rapid infusion of packed red blood cells increases both the flow rate and the shear force, which in some systems can stimulate the release of these prostanoids.[12] Further, PGE_2 itself can stimulate prostacyclin release.[18] This release would complement the action of atrial natriuretic factor and increase the systemic capacitance by venodilation.

This study documents that, similar to the human abortus,[6] 6-keto-$PGF_{1\alpha}$ is the principal circulating prostanoid in the anemic fetus. It also demonstrates that intravascular transfusion stimulates a significant rise in the concentration of 6-keto-$PGF_{1\alpha}$ and PGE_2 that is directly proportional to the rise in the fetal umbilical venous blood pressure. The release of these agents, which can produce systemic and placental vasodilation,[10, 11] may explain the acute decline in Doppler-determined placental, trunk, renal, and cerebral resistance that occurs after intravascular transfusion.[13, 14] Though a pharmacologic concentration of PGE_2 produces umbilical artery constriction in vitro,[15] the decline in the umbilical artery systolic/diastolic ratio with transfusion[13] would suggest that constriction is not a factor in the circumstances studied. Hypoxia[19] and acidosis[20] are unlikely explanations for their release since P_{O_2} and pH remained fairly constant. $PGF_{2\alpha}$ declined during transfusion by an amount not inconsistent with dilution.

This investigation used specific and sensitive radioimmunoassays although indomethacin was not used during sample collection. The measured levels are high but not dissimilar to those in prior studies of fetal prostaglandins. It has been suggested that high plasma con-

centrations especially of 6-keto-$PGF_{1\alpha}$ result from interfering plasma substances and that the 6-keto-$PGF_{1\alpha}$ would be much lower if measured by another method such as mass spectrography.[22] However, the present study was longitudinal in design and there was little prostaglandin-like material in the transfused packed red blood cells. Thus the increases in 6-keto-$PGF_{1\alpha}$ and PGE_2 are unlikely to be solely an artifact of the assay.

These findings have clinical application. One common problem either during or after transfusion is uterine irritability. If the release of prostacyclin and PGE_2 is an important adaptive mechanism for fetal adjustment to volume expansion, it would be unwise to use prostaglandin synthetase inhibitors either prophylactically before or therapeutically after transfusion.

In conclusion, the human fetus responds to a variety of stimuli as early as 0.5 of gestation. In this study intravascular transfusion stimulated the release of venodilator prostanoids, which may help stabilize the umbilical venous pressure.

REFERENCES

1. Robillard JE, Weiner CP, Smith F. Human fetal arginine vasopressin (AVP) and acute, intravascular volume expansion. Fetal Ther [In press].
2. Robillard JE, Weiner CP. Atrial natriuretic factor in the human fetus—affect of volume expansion. J Pediatr 1988;113:552-5.
3. Weiner CP, Heilskov J, Pelzer G, Grant S, Wenstrom K, Williamson RA. Normal values for human umbilical venous and amniotic fluid pressures and their alteration by fetal disease. AM J OBSTET GYNECOL 1989;161:714-7.
4. Weiner CP, Pelzer GD, Heilskov J, Wenstrom KD, Williamson RA. The effect of intravascular transfusion on umbilical venous pressure in anemic fetuses with and without hydrops. AM J OBSTET GYNECOL 1989;161: 1498-501.
5. Nicolaides KH, Clewell WH, Rodeck CH. Measurement of human fetoplacental blood volume in erythroblastosis fetalis. AM J OBSTET GYNECOL 1987;157:50-3.
6. MacKenzie IZ, MacLean DA, Mitchell MD. Prostaglandins in the human fetal circulation in mid-trimester and term pregnancy. Prostaglandins 1980;20:649-54.
7. Terragno NA, McGiff JC, Smigel M, Terragno A. Patterns of prostaglandin production in the bovine fetal and maternal vasculature. Prostaglandins 1978;16:847-56.
8. Skidgel RA, Friedman WF, Printz MP. Prostaglandin biosynthetic activities of isolated fetal lamb ductus arteriosus, other blood vessels, and lung tissue. Pediatr Res 1984; 18:12-8.
9. Friedman Z, Lunyong VE, Courtney J, Smith H, Berkowitz P, Sun F. Prostaglandin formation in the isolated human ductus arteriosus, aorta, pulmonary and umbilical arteries. Prostaglandins Leukot Med 1984;14:279-86.
10. Moncada S, Vane JR. The role of prostaglandins in vascular tissue. Fed Proc 1979;38:66.
11. Rankin JHG, Phernetton TM. Effect of prostaglandin E_2 on ovine maternal placental blood flow. Am J Physiol 1976;231:754.
12. Stanley JC, Graham LM, Burkel WE. Influence of pressure, flow rate, and pulsatility on release of 6-keto-PGF_1 alpha and thromboxane B2 in ex vivo-perfused canine veins. J Vasc Surg 1988;7:99-107.
13. Weiner CP, Anderson TA. The acute effect of cordocentesis with or without fetal curarization and of intravascular transfusion upon umbilical artery waveform indices. Obstet Gynecol 1989;73:219-24.
14. Mari G, Moise K, Kirshon B, Carpenter RJ, Deter RL. Doppler assessment of the cerebral and femoral blood flow before and after intravascular transfusion. AM J OBSTET GYNECOL [In press].
15. Van Orden DE, Farley DB. Prostaglandin F2a radioimmunoassay utilizing polyethylene glycol separation technique. Prostaglandins 1973;4:215-33.
16. Van Orden DE, Farley DB, Clancey CJ. Radioimmunoassay of prostaglandin E, and an approach to the specific assay of PGE_1. Prostaglandins 1977;13:437-53.
17. Farley DB, Van Orden BE. Effect of prostacyclin inhibition by tranylcypromine on uterine 6-keto-PGF_1 alpha levels during estrogen hyperemia in rats. Prostaglandins 1982;23:657-74.
18. Bjoro K. Prostacyclin and thromboxane formation in human umbilical arteries following stimulation with vasoactive autacoids. Prostaglandins 1986;31:699-714.
19. Bjoro K, Haugen G, Stray-Pedersen S. Altered prostanoid formation in human umbilical vasculature in response to variations in oxygen tension. Prostaglandins 1987;34:377-84.
20. Goldsmith JC, Kisker CT. Acidosis affects fetal vascular prostacyclin release. Thromb Res 1984;36:79-82.
21. Catella F, Nowak J, Fitzgerald GA. Measurement of renal and non-renal eicosanoid synthesis. Am J Med 1986; 81:23-9.

The effect of intravascular transfusion on umbilical venous pressure in anemic fetuses with and without hydrops

Carl P. Weiner, MD, Gay D. Pelzer, RN, Joni Heilskov, RN, Katharine D. Wenstrom, MD, and Roger A. Williamson, MD

Iowa City, Iowa

Human fetal umbilical venous pressure was measured during 20 intravascular transfusions performed for treatment of hemolytic anemia. The mean (±1 SEM) gestational age at the time of transfusion was 29.3 ± 1 weeks and the mean beginning hematocrit was 27% ± 2%. The mean volume of infused packed red blood cells (70% hematocrit) was 90.3 ± 7 ml. The mean hematocrit at completion of the procedure was 48% ± 1%. In nonhydropic fetuses umbilical venous pressure rose progressively from 6.7 ± 1 mm Hg at the start of transfusion to 10.9 ± 1 mm Hg at the completion of transfusion ($p < 0.002$). However, most fetuses who began the infusion with a normal umbilical venous pressure ended the transfusion with a normal umbilical venous pressure (<10 mm Hg). Fetuses with immune hydrops ($n = 2$) had elevated umbilical venous pressure values before the initiation of transfusion therapy when compared with the first transfusion of nonhydropic fetuses (12.5 ± 0.5 vs. 5.7 ± 1 mm Hg, $p = 0.01$). However, the umbilical venous pressure measurements declined into the normal range within 24 hours of the first transfusion; this normalization was too rapid to be explained by the reversal of liver hypertrophy or portal hypertension. There was no demonstrable relationship between the rise in umbilical venous pressure and either the gestational age, the volume transfused, or the rise in hematocrit. This study demonstrated: (1) In terms of the umbilical venous pressure, direct intravenous infusion of the human anemic fetus is well tolerated; (2) the elevated umbilical venous pressure associated with immune hydrops can correct rapidly with red blood cell replacement. (Am J Obstet Gynecol 1989;161:1498-501.)

Key words: Cordocentesis, rhesus disease, umbilical venous pressure

The fetal response to intravascular transfusion provides valuable information concerning both the ontogeny and the function of mechanisms involved with volume regulation and the cause(s) of hydrops fetalis. It also provides information necesary for the development of optimal methods of transfusion.

There are presently two forms of fetal intravascular transfusion—direct and exchange. The advantage of a direct transfusion is that it is faster. By reducing the time for operator error, the overall morbidity also should be reduced. However, concern has been expressed that direct transfusion might lead to volume overload and cardiac decompensation in an already stressed fetus.[1] The observations that arginine vasopressin is lower in the anemic fetus than in controls[2] and atrial natriuretic factor is very high in the fetus with immune hydrops[3] suggest that the anemic fetus has an expanded intravascular volume. On the other hand, we have previously reported that intravascular transfusion stimulates the release of atrial natriuretic factor[3] and vasodilator prostanoids.[4] Each could result in increased venous capacitance. This would be consistent with observations from our and other fetal therapy units that Doppler-measured resistance to blood flow in the umbilical, renal (Mari G, Moise K, Kirshon B, Carpenter RJ. Unpublished observations), femoral, and cerebral arteries declines transiently after intravascular transfusion.[5, 6] The effect of intravascular transfusion on the umbilical venous pressure of the anemic human fetus has not previously been reported. We hypothesized that a combination of high placental capacity and the activation of fetal venodilator mechanisms such as atrial natriuretic factor and prostaglandin release would stabilize the umbilical venous pressure during the infusion of large volumes of blood. To test this hypothesis, we measured umbilical venous pressure before, at the estimated midway point of, and after completion of the transfusion.

Material and methods

Eight fetuses undergoing 20 intravascular transfusions for treatment of anemia as a result of isoimmunization were studied. Two of the 8 (25%) were hy-

From the Fetal Diagnosis and Treatment Unit, Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of Iowa College of Medicine.

This research was supported in part by grant HD24494 (C. P. W.) from the United States Public Health Service.

Presented in part at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.

Reprint requests: Carl P. Weiner, MD, Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, The University of Iowa Hospitals and Clinics, Iowa City, IA 52242.

6/6/16514

Table I. Effect of simple intravascular transfusion on nonhydropic fetus (mean ± SEM)

	Baseline	*Midinterval*	*Posttransfusion*
Hematocrit (%)	28 ± 1	37 ± 1*	48 ± 1*
Volume infused (ml)	—	42.5 ± 2	93.5 ± 2
Umbilical venous pressure (mm Hg)	6.7 ± 1	8.1 ± 1.6*	10.9 ± 1.4*
Umbilical vein pH	7.40 ± 0.005	7.38 ± 0.004*	7.35 ± 0.004*
Umbilical vein Po_2 (mm Hg)	40.9 ± 1.5	40.8 ± 1.7	41.0 ± 2.1
Umbilical vein Pco_2 (mm Hg)	36.8 ± 0.6	36.6 ± 0.5	35.9 ± 0.9

*$p < 0.05$, compared with baseline value.

dropic at the time of the first transfusion. Our method for intravascular transfusion has been described previously in detail.[5] For each procedure we used a 22-gauge spinal needle (Beckton-Dickinson, Rutherford, N.J.) and a 5 MHz sector transducer (model 3600, General Electric, Rancho Mirage, Calif.) with an attached needle guide (CIVCO, Kalona, Iowa). All fetuses received pancuronium (0.3 mg.kg estimated fetal weight intravenously) for neuromuscular blockade and furosemide (4 mg/kg intravenously in two divided doses) to dilate capacitance vessels.[7] Transfusion therapy was initiated when the fetal hematocrit declined below 30%. After the first procedure, a repeat transfusion was performed at 3- to 4-week intervals until the final transfusion was performed at 34 to 35 weeks, if appropriate. Our goal was a term delivery. The packed red blood cells were prepared from fresh type O, negative blood compatible with both mother and fetus, rendered buffy coat-poor, irradiated, washed twice with 0.9% sodium chloride, and resuspended in 0.9% sodium chloride to yield a hematocrit of 70%. Blood was infused into the nonhydropic fetus over 15 to 30 minutes to produce a closing hematocrit of 45% to 50%. The hydropic fetuses described here were managed differently at the beginning of therapy. During the first transfusion, the hematocrit increased only to 25%; 24 hours later the hematocrit had increased to 45% to 50%.

The hematocrit and fetal umbilical vein blood gas parameters were measured before, at the estimated midway point of, and at the end of the transfusion. After the required blood specimens had been obtained, the needle was connected to a saline solution–filled tube attached to a solid-state pressure transducer (Sorenson Transpac II, No. 46862-04, supplied by Abbott Critical Care Systems, North Chicago, Ill.) and pressure monitor (No. 78833B, Hewlett-Packard, Houston). The transducer was placed at the level of the fetal heart as determined during the preceding ultrasonographic examination. The needle was purged briefly to clear the shaft of blood, and the pressure was recorded at its nadir. This process took approximately 30 seconds. The identity of the vessel punctured was confirmed to be the umbilical vein by observations of both the flow direction of the injected saline solution and the lack of a pulsatile pressure waveform on the monitor screen. At the conclusion of the transfusion, the needle was withdrawn from the vein into the amniotic cavity. The nadir pressure was recorded. There was neither maternal perception nor sonographic evidence of a uterine contraction during the time any of the pressure measurements were recorded. The umbilical venous pressure reported in this article reflects subtraction of the amniotic fluid pressure from the umbilical venous pressure.

A complete blood count was performed on blood anticoagulated with 1% ethylenediaminetetraacetic acid with a Technicon H-1 analyzer (Technicon, Tarreytown, N.Y.). Venous blood gas measurements were performed with blood anticoagulated with heparin on a Blood Gas Manager No. 1312 (Instrumentation Laboratory, Lexington, Mass.). The pH, Pco_2 (millimeters of mercury), and Po_2 (millimeters of mercury) were measured directly. The base excess (milliequivalents per liter), bicarbonate (milliequivalents per liter), and oxygen saturation (percent) wre calculated by use of standard nomograms.

The results are presented as the mean ± 1 SEM. The three values for baseline umbilical venous pressure, hematocrit, and posttransfusion umbilical venous pressure were compared with a repetitive measures analysis of variance. When significant differences were identified, Scheffé's test was applied. Differences in discrete variables between nonhydropic and hydropic fetuses were tested for significance with an independent t test. The relationship between the baseline umbilical venous pressure and gestational age and between the change in umbilical venous pressure and gestational age, total volume infused, and change in hematocrit were tested by least-squares regression. A $p \leq 0.05$ was considered to represent a significant difference.

Results

There were no complications associated with any of the 20 procedures. The mean gestation at transfusion was 29.3 ± 1 weeks (range 21.5 to 34.5) and the mean beginning hematocrit was 27% ± 2% (range 5% to 37%). There was no demonstrable relationship between the beginning umbilical venous pressure and gestational age ($r = 0.10$; $p = 0.68$). The mean volume of packed red blood cells infused was 90.3 ± 7 ml (range

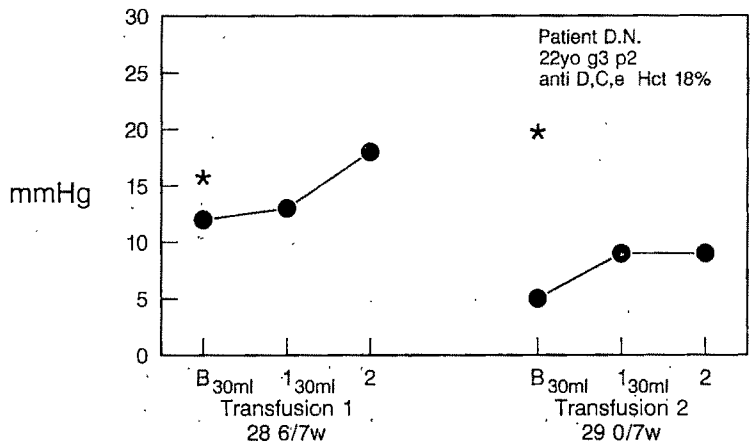


Fig. 1. Effect of intravascular transfusion on umbilical venous pressure in fetus with hydrops. *Asterisks* indicate amniotic fluid pressure. Umbilical venous pressure (in millimeters of mercury) corrected for amniotic fluid pressure is indicated by *solid circles*. Normal umbilical venous pressure is <10 mm Hg.

35 to 120). Umbilical venous pressure values with paired amniotic fluid pressure values at all three sampling times were available for 15 procedures.

The baseline umbilical venous pressure exclusive of hydropic fetuses (6.7 ± 1 mm Hg) was within normal limits (2 SD ≥ 10 mm Hg[8]). The umbilical venous pressure rose significantly ($p < 0.002$) during transfusion, reaching a level just above the normal range (10.9 ± 1 mm Hg) (Table I). The increase observed for the umbilical venous pressure during transfusion was similar in fetuses undergoing the first transfusion as opposed to a subsequent transfusion (4 ± 2 vs 4.3 ± 1 mm Hg). Most fetuses who began the transfusion with a normal umbilical venous pressure still had a normal umbilical venous pressure at the end of the procedure. The baseline umbilical venous pressure of the two fetuses that were hydropic at the time of first transfusion was significantly elevated (12.5 ± 0.5 mm Hg) compared with all nonhydropic fetuses undergoing their first transfusion (5.7 ± 1 mm Hg, $p = 0.01$, two-tail t test). The umbilical venous pressures of these two hydropic fetuses responded differently during the transfusion when compared with those of the nonhydropic fetuses. In one fetus previously described,[8] the umbilical venous pressure normalized *during* the first transfusion and remained normal thereafter. The umbilical venous pressure of the second fetus rose during the first transfusion but was normal by the time of the second transfusion 1 day later (Fig. 1). The hydrops itself resolved far more slowly.

There was no relationship observed between the rise in umbilical venous pressure during transfusion and either gestational age ($r = 0.14$, $p = 0.72$), the volume of packed red blood cells infused ($r = 0.21$, $p = 0.57$), or the change in hematocrit achieved by transfusion ($r = 0.36$, $p = 0.25$).

Comment

Blood returning through the umbilical vein passes via the ductus venosus into the inferior vena cava where it streams preferentially through the foramen ovale into the left atrium.[9] We assume that the umbilical venous pressure is an approximation of the fetal central venous pressure, which also may be sensitive to left-sided cardiac alterations. Our present and prior experience[8] measuring the umbilical venous pressures of fetuses with a variety of problems supports this assumption. Fetuses with elevated umbilical venous pressures had pathologic conditions consistent with cardiac failure, hepatosplenomegaly, or acute bradycardia.

Direct intravascular transfusion significantly increased the umbilical venous pressure, though most pressures were still within the normal range at the completion of the transfusion. Thus, despite a preexisting, relative increase in fetal intravascular volume before transfusion,[2, 3] acute volume expansion was well tolerated by the fetus. The finding that umbilical venous pressure rose during transfusion is similar to our animal investigation,[10] where there was a 50% rise in the central venous pressure of the sheep fetus that had been rendered anemic by phlebotomy and then transfused 24 hours later. The magnitude of the volume expansion should not be underestimated. With a mean transfused volume of almost 100 ml in nonhydropic fetuses, we often doubled the estimated fetal intravascular volume during the course of this study.[10] The tolerance of the fetus to volume expansion likely reflects both a high placental capacitance and other adaptive fetal mechanisms.[3, 4] We presently have little information concerning the length of time the umbilical venous pressure remains elevated after the transfusion. In two fetuses studied after the preparation of this manuscript, the needle was left in place pending a final

blood count. The umbilical venous pressure had already begun to decline by the time the result was received 5 to 10 minutes later. The umbilical venous pressures of all fetuses repetitively studied here had returned to the baseline value by the next transfusion 3 weeks later. These findings, however, may be applicable only to the fetus who is transfused by a procedure consisting of pancuronium, furosemide, and 70% hematocrit blood and whose closing hematocrit is between 45% and 52%.

The two fetuses with immune hydrops studied at the time transfusion therapy was initiated each had an elevated umbilical venous pressure that corrected far too rapidly with transfusion for us to conclude that the hydrops resulted solely from hepatic parenchymal hypertrophy and resulting portal hypertension. This observation was unexpected and would seem at odds with the prevailing opinion that portal hypertension and hypoalbuminemia that are secondary to hepatoerythropoiesis are important in the genesis of hydrops.[12, 13] Thus it seems likely that hypoxic myocardial dysfunction led to increased umbilical venous pressure in these two fetuses. In this circumstance intravascular transfusion would have a positive inotropic effect by increasing the fetal oxygen-carrying capacity. This might explain prior clinical studies reporting an increase in the survival rate of hydropic fetuses treated by intravascular transfusion rather than intraabdominal transfusion, where red blood cell absorption from the peritoneal cavity can be delayed.[14]

On the basis of results in these two fetuses, no conclusion can be made concerning the appropriate method for intravascular transfusion. However, these observations suggest that fetal vascular overload can be prevented by monitoring the umbilical venous pressure. In conclusion, though direct fetal intravenous transfusion, as described here, significantly raises the umbilical venous pressure, the pressure remains within clinically acceptable norms.

Addendum

We have now examined 16 fetuses undergoing 40 intravascular transfusions as described. The results are virtually identical.

REFERENCES

1. Grannum PA, Copel JA, Plaxe SC, Scioscia AL, Hobbins JC. In utero exchange transfusion by direct intravascular injection in severe erythroblastosis fetalis. N Engl J Med 1986;314:1431-4.
2. Robillard JE, Weiner CP, Smith F. Human fetal arginine vasopressin (AVP) and acute, intravascular volume explansion. Fetal Ther [In press].
3. Robillard JE, Weiner CP. Atrial natriuretic factor in the human fetus—effect of volume expansion. J Pediatr 1988;113:552-5.
4. Weiner CP, Robillard JE. Effect of acute intravascular volume expansion on human fetal prostaglandin concentrations. AM J OBSTET GYNECOL 1989;161:1494-7.
5. Weiner CP, Anderson TL. The acute effect of cordocentesis with or without fetal curarization and of intravascular transfusion upon umbilical artery waveform indices. Obstet Gynecol 1989;73:219-24.
6. Mari G, Moise K, Kirshon B, Carpenter RJ, Deter RL. Doppler assessment of the cerebral and femoral blood flow before and after intravascular transfusion. AM J OBSTET GYNECOL [In press].
7. McEvoy GK, ed. Furosemide. In: American hospital formulary service drug information 87. Bethesda: American Society of Hospital Pharmacists, 1987:1355.
8. Weiner CP, Heilskov J, Pelzer G, Grant S, Wenstrom K, Williamson R. Normal values for human umbilical venous and amniotic fluid pressures and their alteration by fetal disease. AM J OBSTET GYNECOL 1989;161:714-7.
9. Lind J, Stern L, Wgelius C. Human fetal and neonatal circulation. Springfield, Illinois: Charles C Thomas, 1964.
10. Chestnut DH, Pollack KL, Weiner CP, Robillard J, Thompson CS, DeBruyn CS. Does furosemide alter the hemodynamic response to rapid intravascular transfusion of the anemic fetal lamb? AM J OBSTET GYNECOL 1989; 161:1571-5.
11. Nicolaides KH, Clewell WH, Rodeck CH. Measurement of human fetoplacental blood volume in erythroblastosis fetalis. AM J OBSTET GYNECOL 1987;157:50-3.
12. James LS. Shock in the newborn in relation to hydrops. In: Robertson JG, Dambrosio F, eds. International symposium on the management of the Rh problem. Ann Ostet Ginecol Med Perinat 1971;92:599-601.
13. Phibbs RH, Johnson P, Tooley WH. Cardio-respiratory status of erythroblastic infants. II. Blood volume, hematocrit and serum albumin concentration in relation to hydrops fetalis. Pediatrics 1974;53:13-8.
14. Rodeck CH, Nicolaides KH, Warsof SL, Fysh WJ, Gamsu RH, Kemp JR. The management of severe rhesus isoimmunization by fetoscopic intravascular transfusions. AM J OBSTET GYNECOL 1984;150:769-74.

Duplex Doppler ultrasonographic evaluation of the fetal renal artery in normal and abnormal fetuses

J. C. Veille, MD, and C. Kanaan, MD
Cleveland, Ohio

Using duplex Doppler ultrasonography in asymmetrically grown fetuses with decreased amniotic fluid, we found the renal artery waveform pattern was different from that in the normally grown fetus. Such an abnormal waveform may reflect an increase in vascular resistance within the renal bed in oligohydramnios and intrauterine growth retardation. This would suggest that local mechanisms are operational and may influence renal blood flow in the human fetus with intrauterine growth retardation. (AM J OBSTET GYNECOL 1989;161:1502-7.)

Key words: Duplex Doppler ultrasonography, fetal renal artery, intrauterine growth retardation

Pulsed Doppler ultrasonography with real-time imaging and on-line spectral analysis (duplex imaging) has wide clinical applications in the evaluation of blood flow velocity. Duplex ultrasonographic examination of the kidney has been used to investigate renovascular resistance in patients with renal transplant to noninvasively evaluate the increase in resistance that occurs during acute rejection.[1-5] In patients with episodes of acute renal rejection, the renal arterial diastolic flow velocity is significantly reduced. We hypothesized that in fetuses affected by asymmetric intrauterine growth retardation (IUGR), fetal renal blood flow may similarly be affected as a result of an elevated intravascular resistance, leading to a decrease in renal perfusion and to oligohydramnios.

Material and methods

Patient population. Patients from our clinical practice or from our antenatal testing unit at MacDonald Hospital for Women in Cleveland, Ohio, were asked to participate in this study. Informed consent was obtained as approved by the Institutional Review Board of University Hospitals of Cleveland. None of the normal patients studied was taking any medication at the time of the study except for prenatal vitamins. A total of 22 normal pregnant patients (mean gestational age at the time of the study ± SD = 31.2 ± 4.5 weeks) and 11 pregnant patients who were suspected and were later proved to have asymmetric IUGR of the fetus (mean gestational age at the time of the study, 30.0 ± 3.1 weeks) were studied.

Of the eleven patients with IUGR, three had pregnancy-induced hypertension, two were heavy smokers, one had postdate syndrome, one had intractable nausea and vomiting with no weight gain during her pregnancy, one had gestational diabetes with pregnancy-induced hypertension, and there was no apparent cause for IUGR in three patients. Of the 11 patients with IUGR seven had oligohydramnios, whereas the other four had normal amniotic fluid. It is of interest that the highest values for the systolic/diastolic ratio (S/D) of the fetal renal artery were found in the group with oligohydramnios. Statistical analysis of the waveform was done between these two subgroups of patients with IUGR and is shown in Table I. Among the group of seven patients who had IUGR and oligohydramnios, four had reverse umbilical artery diastolic flow and nonreactive nonstress test results. All four had an emergency cesarean section because of fetal distress, and the neonates' birth weights were <1500 gm (780, 900, 1100, 1400 gm, respectively). All four neonates had respiratory distress syndrome, apnea, bradycardia, jaundice, and difficulty in heat control. The other seven patients had reactive nonstress test results and negative contraction stress test results and were vaginally delivered of infants at 36.8 ± 1.2 weeks. Except for being small for gestational age (SGA) all seven infants did well in the neonatal period.

Instrumentation. All patients had a routine ultrasonographic examination. Measurements of biparietal diameter, femur length, and abdominal circumference were obtained. Placental grading and amniotic fluid determination were noted. The amniotic fluid volume determination was performed with a semiquantitative technique as previously described.[6] The estimated fetal weight was then determined with previously published tables.[7] Birth weights 2 SDs below the mean[8] were con-

From the Department of Reproductive Biology, Case Western Reserve University.
Supported in part by National Institutes of Health Research grant HL-38296, from the National Institute of Child Health and Human Development (J. C. V.).
Presented at the Thirty-fifth Annual Meeting of the Society for Gynecologic Investigation, Baltimore, Maryland, March 17-20, 1988.
Reprints not available.
6/6/15967

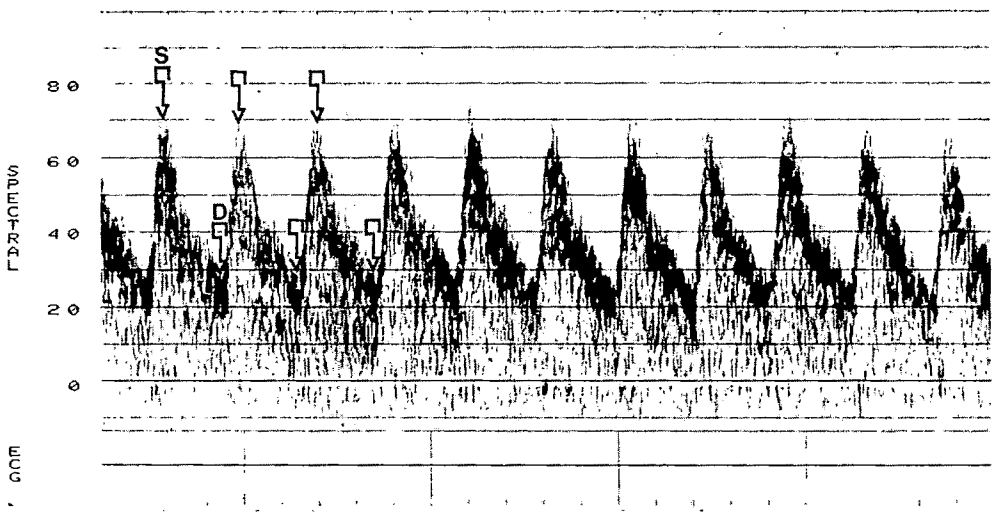


Fig. 1. Duplex waveform of normally grown fetus. *S*, Systolic mark; *D*, diastolic mark.

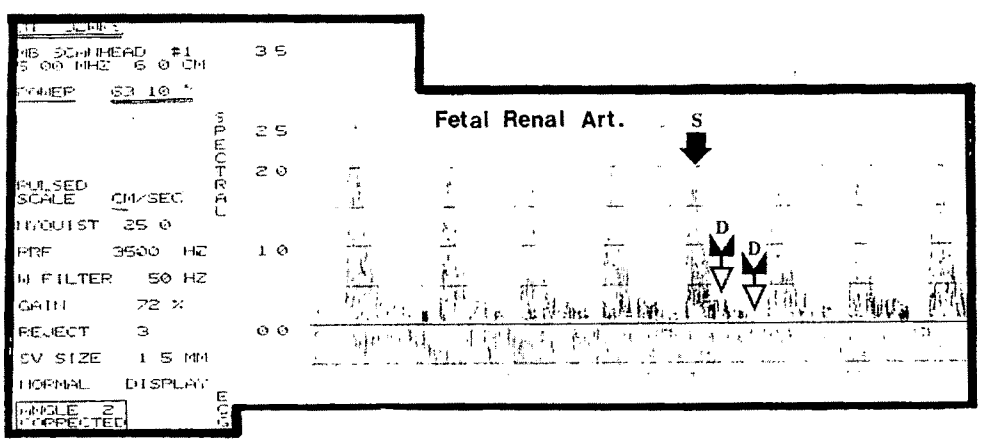


Fig. 2. Duplex waveform of fetal renal artery. *S*, Systolic mark; *D*, diastolic mark.

Table I. Fetal renal artery of IUGR fetuses (mean ± SEM)

	Without signs of hypoxia (n = 7)	*With signs of hypoxia (n = 4)*	*p Value*
S/D ratio	5.3 ± 0.59	8.07 ± 0.36	<0.02
Pulsatility index	1.98 ± 0.15	2.46 ± 0.30	NS
Time-velocity integral	2.93 ± 0.23	2.67 ± 0.57	NS
Area under curve (cm^2)	4.47 ± 0.63	10.40 ± 5.54	NS
Half-time (msec)	60.8 ± 5.04	42 ± 11.6	NS

sidered to be SGA. Duplex ultrasonographic examinations were performed with a 3 or 5 mHz real-time pulsed-wave Doppler system (UM 8, Advanced Technology, Bothell, Wash.). The Doppler cursor was placed in the lumen of the vessel and the sample volume was kept to a minimal size (1.5 mm).

The wall filter was also kept to minimal settings (50 Hz) to obtain the full Doppler spectrum during diastole. With the use of a foot pedal, the two-dimensional image was intermittently frozen on one screen while the Doppler signal was displayed on the opposite screen. When an adequate waveform was displayed, a hard copy for subsequent analysis was made on a strip-chart recorder set at a speed of 50 mm/sec. Maximum power intensity was previously set by the company to meet the Food and Drug Administration's preenactment level requirements (i.e., I_{SPTA} = 46 mW/cm^2, I_{SPPA} = 65 W/cm^2, and Im = 160 W/cm^2).* Power was further decreased to 10% of its maximum intensity.

*I_{SPTA} = Maximum value of spatial peak temporal average intensity, I_{SPPA} = maximum value of spatial peak pulsed average intensity, Im = spatial peak maximum intensity.

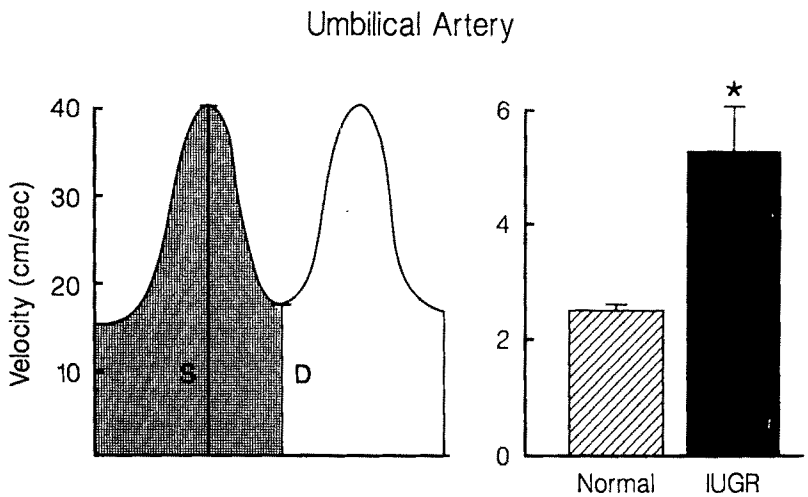


Fig. 3. Summary of umbilical artery S/D ratio. Fetuses affected by IUGR are shown to have significantly higher value. *Denotes p value <0.01.

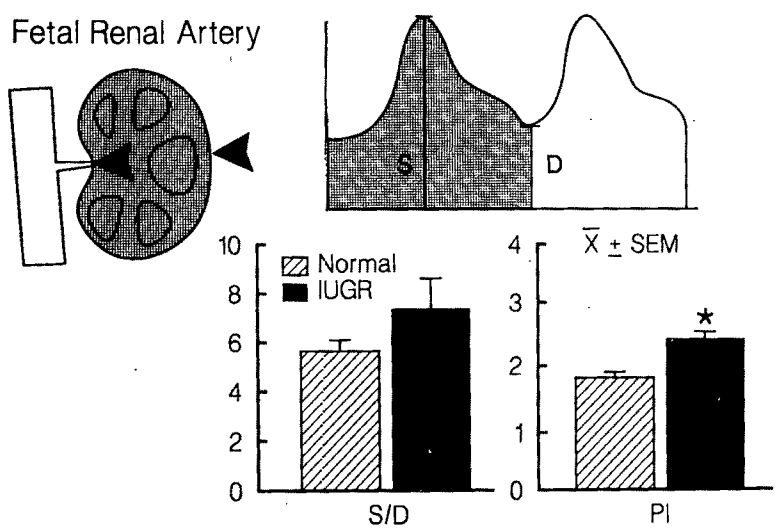


Fig. 4. Summary of fetal renal artery S/D ratio and pulsatility index *(PI)* in normal and IUGR fetuses. No difference in S/D ratio was found. Pulsatility index was significantly elevated in IUGR fetuses. *Denotes p value <0.01.

Duplex examination

Umbilical artery. The placental cord insertion was localized and the umbilical artery identified. The sample volume was placed in the lumen of the vessel and recorded on a strip-chart recorder. Signals were recorded with the fetus in a quiet state and during apnea. Six cycles were calculated for the S/D ratio value and averaged (Fig. 1).

Renal artery. An axial view of the fetus was obtained at the level of the kidneys. The abdominal aorta was identified. Doppler sample volume was placed at the hilus renalis, and then carefully positioned as parallel as possible to the renal vessel to obtain a maximum signal. At times it was necessary to use a larger Doppler sample volume to obtain an adequate waveform. This was then decreased to obtain a signal with the least amount of artifact. Although we could not determine the precise angle of the Doppler beam to the renal artery, repositioning of the transducer and the Doppler sample was accomplished to maximize the waveform signal. The abdominal aorta gave a significantly different signal that helped to differentiate between the two waveforms. A minimum of three consecutive cardiac cycles were analyzed (Fig. 2).

Waveform analysis. The waveforms were analyzed with a digital image processing system (Digisonics, Inc., Houston, Tex.). Each waveform was traced from the hard copy on a tablet digitizer and entered in the com-

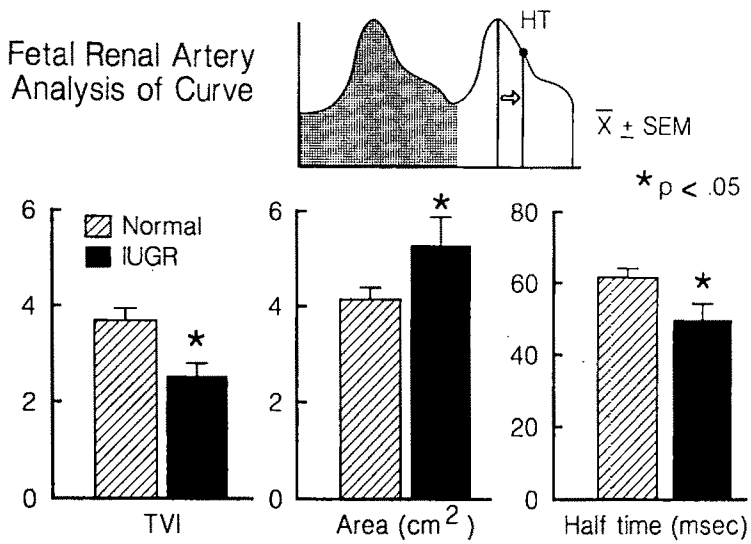


Fig. 5. Time-velocity integral *(TVI)*, area of curve, and half-time of curve between normal and IUGR fetuses. Time-velocity integral and half-time were significantly lower in IUGR fetuses. However, area was significantly higher in IUGR fetuses.

puter program. The systolic and diastolic peak flow velocities were analyzed for both the umbilical artery and the fetal renal artery.

The area under the waveform and the time-velocity integral of the curve were derived by planimetry. The velocity curves were digitalized with the use of the darkest portion of the curve as well as its external contour. The time-velocity integral is derived by the summation of all the different velocity components of the curve (i.e., the variation of the velocity distribution, or the narrow band of frequencies on the spectral display). The area half-time of the traced waveform is the time taken for the Doppler curve to decrease from its peak value to half of its peak value.

Statistical analysis was performed with the nonpaired Student t test with a p value of <0.05 used to reject the null hypothesis. Mean and SEM are reported.

Results

The S/D ratio of the umbilical artery was significantly different in the normal fetuses, as compared with the asymmetrically grown fetuses (2.5 ± 0.11 versus 5.3 ± 0.79, respectively; $p < 0.01$) (Fig. 3). However, the S/D ratio of the waveform for the fetal renal artery was not significantly different between the two groups (5.64 ± 0.45 versus 7.38 ± 1.24, respectively) (Fig. 4). The pulsatility index (i.e., the systolic peak velocity minus the diastolic peak velocity divided by the mean velocity of the waveform) was significantly higher in the abnormal group (1.89 ± 0.08 versus 2.34 ± 0.13; $p < 0.05$) (Fig. 4). Both the time-velocity integral and the area half-time were significantly higher in the normal group, as compared with the abnormally grown fetuses time-velocity integral, 3.69 ± 0.25 versus 2.54 ± 0.26 cm/sec and half-time, 61.5 ± 2.48 versus 49.7 ± 4.59 msec, respectively) (Fig. 5). The total area under the fetal renal artery waveform was significantly larger in the IUGR fetuses (4.15 ± 0.25 versus 5.29 ± 0.59 cm^2, respectively) (Fig. 5).

Comment

Significant differences in the analysis of the umbilical artery and the fetal renal artery waveform were discovered between normally grown fetuses and the fetuses affected by asymmetric IUGR. In the normally grown fetuses the S/D ratio and the pulsatility index of the umbilical artery were found to be significantly higher in the IUGR group. This was largely because of a decrease in the amplitude of the diastolic component of the waveform in the IUGR group. Similar findings have been reported in human pregnancies and agree with our study.[9-11] In fact, a similar decrease in the diastolic component of the umbilical artery waveform has been reported in pregnant ewes after repeated placental embolization through the umbilical artery.[12] With progressive embolization, the end-diastolic velocity waveform progressively decreased and reversed flow was observed before fetal death. This suggests that the abnormal waveform pattern of the umbilical artery is caused by a progressive increase in vascular resistance within the placental bed (Morrow RJ. Unpublished observations).

The S/D ratio of the fetal renal artery was not significantly higher in the IUGR group. With the use of the mean value of the flow velocity curve, the pulsatility index was calculated and found to be significantly el-

evated in the fetuses affected by IUGR. Similarly, significant differences were found when the time-velocity integral and the area half-time of the fetal renal artery waveform were analyzed. This not only indicates that the S/D ratio cannot discriminantly identify impaired fetal renal blood flow, but also suggests that the way the fetal kidney is perfused in the IUGR fetuses is different from that in the normally grown fetus. The majority of the fetuses (4/7) with IUGR and oligohydramnios had signs suggestive of acute fetal hypoxia. It is always possible that the difference between the IUGR and the normally grown fetuses could be explained by the four fetuses with acute hypoxia rather than the growth retardation. Analysis of the data into those with and those without signs of acute fetal hypoxia did not show major differences in the fetal renal waveform between these two groups except for the S/D ratio. Such results suggest that the changes between the IUGR and the normally grown fetuses are mostly a result of the growth retardation process. Similar findings were reported by Rigsby[3, 4] in the renal artery of transplanted kidneys during acute rejection. They speculated that the abnormal renal waveform may be a result of structural, neurogenic, or humoral process mechanism. Experimental work in dog, lamb, and primate fetuses with the use of radioactive microspheres during acute and chronic preparations suggests that both neurogenic and humoral mechanisms are indeed operational and are responsible for local changes in renal blood flow.[12-15] With embolization of the dog's kidneys with microspheres, a good correlation between progressive increase in the renal vascular resistance and decreased organ perfusion was found.[1]

In the instrumented lamb, the renal blood flow has been estimated to be between 2% and 3% of the combined cardiac output during physiologic conditions.[12, 13] During either hypoxemia or hypercapnia, renal blood flow was shown to be reduced by 25% to 50% of the baseline values.[14] Vasoconstriction occurred abruptly when the fetal arterial Po_2 value fell <20 mm Hg. Although the exact mechanism for this decrease in renal blood flow is not clear, it is thought to be mediated by the local action of prostaglandins.[16] In our study the decrease in the time-velocity integral indicates that the quantity of blood perfusing the kidney of the IUGR fetus is decreased but that the decrease found in the area half-time of the renal waveform suggests that the kidney is being perfused at a faster rate. Another possible explanation for the above findings is that there is a significant increase in the vascular resistance distal to the point of measurement. This is in keeping with experimental evidence that vasoconstriction of the renal vascular bed occurs in fetuses with an inappropriate uterine environment. The fact that in our study the total area under the fetal renal artery waveform is higher in the IUGR group may reflect local mechanisms trying to maintain adequate renal perfusion under such circumstances of poor growth.

Our results of an elevated pulsatility index seem paradoxical. If the area of the curve is larger in the IUGR fetuses and the mean value for the curve increases, the pulsatility index should be lower in this group. However, the systolic component of the curve was markedly elevated in this group. The net result was that the difference in the S/D value increased, giving a higher value to the pulsatility index. This would imply that instead of a local vasodilatation on the renal vasculature, the fetal renal blood flow may be maintained by a combination of increase in arterial pressure and intrarenal action of catecholamines, vasopressin, and prostaglandins as previously reported.[17, 18]

It is always possible that technical differences occurred during the sampling of the fetal renal artery waveform. Variability in the Doppler angle could, in turn, affect the shape of the waveform and create significant problems in the interpretation of the data. Although such bias could have occurred because we did not visualize the fetal renal artery per se, we were careful to maintain the Doppler sample volume parallel to the fetal hilus renalis to keep the Doppler angle as close as possible to zero in all views. This study is qualitative in nature as quantification of Doppler echocardiographic data implies that the accurate estimation of the vessel studied can be obtained. Qualitative analysis of the waveform is not angle-dependent and gives an estimate of resistance to flow rather than an estimate of total flow.

Our results suggest that the overall perfusion of the kidney in the fetus affected with IUGR is maintained inasmuch as the diastolic component of the waveform was found to be normal, but the way the kidneys are perfused is different from the normally grown fetus. The oligohydramnios observed in cases of severe IUGR may then be a result of an inability of the kidney to compensate for the decrease in blood flow velocities and increased vascular resistance, which results in an overall decrease in renal blood flow and glomerular filtration rate.[17]

We thank Beth Wilson, RN, for her coordination efforts and Joyce Olson for her assistance in this study.

REFERENCES

1. Norris CS, Barnes RW. Renal artery flow velocity analysis, a sensitive measure of experimental and clinical renovascular resistance. J Surg Res 1984;36:230-6.
2. Wood RFM, Nasmyth DG. Doppler ultrasound in the diagnosis of vascular occlusion in renal transplantation. Transplantation 1982;33:547-51.
3. Rigsby CM, Burns PN, Weltin GG, et al. Doppler signal quantitation in renal allographs: comparison in normal and rejecting transplants with pathologic correlation. Radiology 1987;162:39-42.

4. Rigsby CM, Taylor KJW, Weltin GG, et al. Renal allographs in acute rejection: evaluation using Duplex sonography. Radiology 1986;158:375-8.
5. Arima M, Takahara S, Ihara H, et al. Predictability of renal allograph prognosis during rejection crisis by ultrasonic Doppler flow technique. Urology 1982;19:389-94.
6. Hill LM, Breckle R, Wolfgram KR, O'Brien PC. Oligohydramnios: ultrasonically detected incidence and subsequent fetal outcome. Am J Obstet Gynecol 1983; 147:407-10.
7. Warsof SL, Gohari P, Berkowitz RL, Hobbins JC. The estimation of fetal weight by computer-assisted analysis. Am J Obstet Gynecol 1977;128:881-92.
8. Usher RH, McLean F. Intrauterine growth of live born caucasian infants at sea level: standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. J Pediatr 1969;74:901-10.
9. Fleischer A, Schulman H, Farmakides G, Bracero L, Blattner P, Randolph G. Umbilical artery velocity waveforms and intrauterine growth retardation. Am J Obstet Gynecol 1986;151:502-5.
10. Trudinger BJ, Giles WB, Cook CM. Flow velocity waveforms in the maternal uteroplacenta and fetal umbilical circulation. Am J Obstet Gynecol 1985;152:155-63.
11. Reuwer PJHM, Bruinse HW, Stoutenbeek P, et al. Doppler assessment of the fetoplacental circulation in normal and growth retarded fetuses. Eur J Obstet Gynecol Reprod Biol 1984;18:199-205.
12. Rudolph AM, Heymann MA. Circulatory changes during growth in the fetal lamb. Circ Res 1970;26:289-99.
13. Paton JB, Fisher DE, Peterson, et al. Cardiac output and organ blood flow in baboon fetus. Biol Neonate 1973; 22:50-7.
14. Cohn EH, Sacks EJ, Heymann MA, Rudolph AM. Cardiovascular responses to hypoxemia and acidemia in fetal lamb. Am J Obstet Gynecol 1973;120:817-24.
15. Peters LLH, Sheldon RE, Jones MD Jr, Makowski EL, Meschia G. Blood flow to fetal organs as a function of arterial oxygen content. Am J Obstet Gynecol 1979; 135:637-46.
16. Millard RW, Borg H, Vatner SF. Prostaglandin control of the renal circulation in response to hypoxemia in the fetal lamb in utero. Circ Res 1979;45:172-9.
17. Robillard JE, Weitzman RE, Burmeister L, Smith FG Jr. Developmental aspects of the renal response to hypoxemia in the lamb fetus. Circ Res 1981;48:128-37.
18. Rurak DW. Plasma vasopressin levels during hypoxemia and the cardiovascular effects of exogenous vasopressin in fetal and adult sheep. J Physiol (Lond) 1978;277:341-57.

Intrapartum Doppler velocimetry, amniotic fluid volume, and fetal heart rate as predictors of subsequent fetal distress

I. An initial report

Albert P. Sarno, Jr., MD, MAJ, USA, Myoung Ock Ahn, MD, MPH, Harbinder S. Brar, MD, Jeffrey P. Phelan, MD, and Lawrence D. Platt, MD
Los Angeles, California

This study examines the usefulness of umbilical artery Doppler velocimetry, amniotic fluid volume assessment, and fetal heart rate data in the early intrapartum period as predictors of subsequent fetal distress. A total of 109 patients seen in the latent phase of labor in the labor and delivery area were studied. Both an abnormal initial fetal heart rate and an amniotic fluid index ≤5.0 cm were associated with a significant increase in the incidence of intrapartum fetal distress. Conversely, a systolic/diastolic ratio >3.0 by Doppler ultrasonography was not associated with increased fetal morbidity. Overall, the sensitivities, specificities, and positive predictive values of the fetal heart rate tracing and the amniotic fluid volume assessment were comparable. Doppler systolic/diastolic ratios showed very poor sensitivity and positive predictive value. We conclude that the fetal heart rate tracing or the assessment of amniotic fluid volume in the early intrapartum period are reasonable predictors of subsequent fetal condition. The lack of patients with the absence of or reverse umbilical velocity preclude conclusions with regard to Doppler systolic/diastolic ratios for this purpose. (Am J Obstet Gynecol 1989;161:1508-14.)

Key words: Doppler velocimetry, amniotic fluid volume, fetal heart rate, intrapartum fetal distress

The routine use of intrapartum fetal heart rate (FHR) monitoring has been addressed by the Executive Board of the American College of Obstetricians and Gynecologists.[1] The recently published statement states that intermittent fetal auscultation "is as effective as electronic fetal monitoring in monitoring high risk patients during labor."

However, physicians trained in the era of fetal monitoring may be uncomfortable in following up patients in this manner. In addition, staffing requirements and medical-legal risks are practical issues to be confronted. Therefore studies aimed at further identification of the correct patients for auscultation alone seem indicated.

This information could then be used to triage patients to either continuous intrapartum electronic fetal monitoring or intermittent auscultation. The evaluation, if rapid and easily performed, could be done by the labor and delivery nurse with the results relayed to the physician.

Should the physician prefer to monitor all fetuses with electronic monitoring, this same test may provide information to alert the physician to the increased likelihood of fetal distress. More intensive monitoring and preparations for cesarean delivery may then be indicated. Because most community hospitals do not have 24-hour inhouse obstetric and anesthesia coverage, earlier notification of appropriate personnel may be helpful.

The concept of a "fetal admission test" has been addressed with either the initial FHR monitor strip[2] or the fetal response to acoustic stimulation.[3] Other parameters that may potentially be useful as an admission test include amniotic fluid volume and umbilical artery blood flow as measured by Doppler velocimetry.

This study examines the usefulness and predictive values of Doppler velocimetry, amniotic fluid volume assessment, and FHR patterns early in the course of labor for the subsequent development of intrapartum fetal morbidity.

Material and methods

The study group consisted of 109 patients seen at the labor and delivery unit of Women's Hospital,

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of Southern California School of Medicine and Women's Hospital, Los Angeles County/University of Southern California Medical Center.
Computational assistance was provided by the CLINFO Project, funded by the Division of Research Resources of the National Institutes of Health under Grant No. RR-0043.
Presented in part at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.
The opinions expressed herein are those of the authors and are not necessarily those of the United States Army or Department of Defense.
Reprint requests: Albert P. Sarno, Jr., MD, MAJ, USA, Department of Obstetrics and Gynecology, Tripler Army Medical Center, Honolulu, HI 96859-5000.
6/6/15653

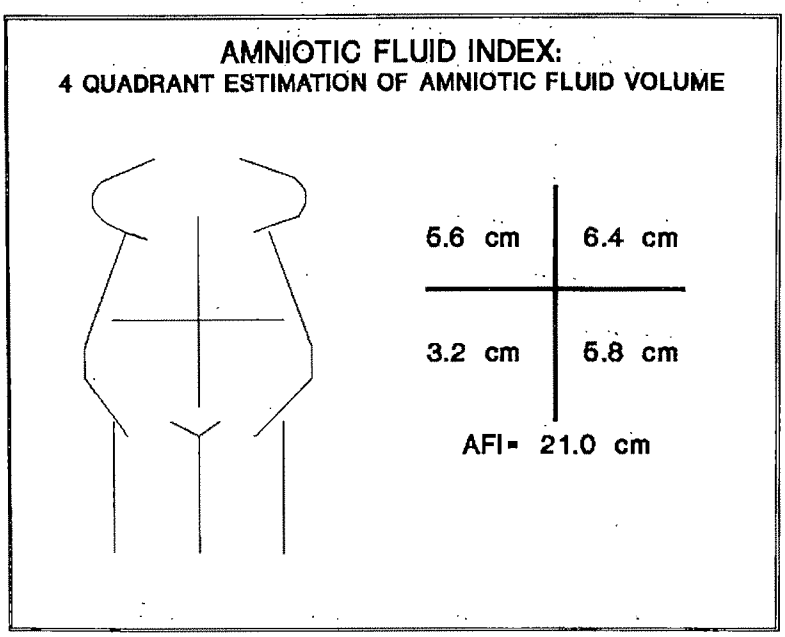


Fig. 1. Schematic representation of four-quadrant estimation of amniotic fluid volume. Greatest vertical diameters of largest pocket of amniotic fluid in each quadrant are summed to form amniotic fluid index. (Hypothetical numbers are shown).

Los Angeles County/University of Southern California Medical Center from August 1, 1987, to December 1, 1987. Inclusion criteria included term or post-term pregnancy (≥36 completed weeks' gestation) as assessed by usual clinical criteria, singleton fetus, vertex presentation, and latent phase of labor. The presence of ruptured membranes was not exclusionary. All patients were evaluated by a single examiner (A. S.).

FHR tracings were examined in both the first and last 40 minutes of monitoring and were evaluated for baseline rate, reactivity, beat-to-beat variability, and the presence of severe variable decelerations, late decelerations, and bradycardia. Severe variable decelerations were graded by the criteria of Kubli et al.[4] Severe variable decelerations and late decelerations were diagnosed only if present with >50% of contractions. Fetal bradycardia was defined as a FHR of 90 beats/min or a decline in heart rate of 40 beats/min from baseline, persisting for 1 minute. FHR reactivity was present if at least a single acceleration of the FHR of 15 beats/min from baseline lasting 15 seconds was noted.[5] Baseline variability was normal if >5 beats/min. For purposes of the study, an abnormal FHR tracing was deemed to be present when any of the following were present: (1) abnormal FHR baseline; (2) decreased baseline variability; (3) absence of reactivity; (4) variable decelerations; (5) late decelerations; or (6) bradycardia.

Real-time ultrasonographic examination was performed with either a General Electric 2600 (Waukesha, Wisc.) or an ADR/ATL Ultramark 4 (Bothell, Wash.) with a 3.5 mHz linear transducer. Amniotic fluid volume was assessed by the amniotic fluid index, which divides the maternal abdomen into four quadrants and measures the greatest verticle diameter of the largest pocket of amniotic fluid in each quadrant and adds the measurements (Fig. 1). An amniotic fluid index >5.0 cm was considered to reflect adequate fluid.[6] The results of the amniotic fluid index were not revealed to the physicians who managed the patient's labor.

Umbilical artery velocimetry was then performed with continuous-wave Doppler ultrasonography (Angioscan III, Unigon Industries, Yonkers, N.Y., or Multigon 500A, Multigon Industries, New York, N.Y.) with a 4 mHz transducer with a power output of 6.5 mW/cm^2 and an effective depth of 8 cm. The umbilical velocity waveform, obtained transabdominally, was recognized by the characteristic signature with the arterial and venous flow in opposite directions (Fig. 2). The screen was frozen and peak systolic to lowest end-diastolic ratios (S/D) were computed. Three different recordings were performed, waveforms were analyzed, and the mean value was calculated for a given patient and used for analysis. The results of the Doppler analyses were not revealed to the physicians who managed the patient's labor. An S/D ratio >3.0 was considered abnormal for term gestation.[7]

Pregnancy outcome was assessed with respect to the incidence of meconium staining, fetal distress that required cesarean delivery, Apgar scores at 1 or 5 minutes <7, birth weight less than the 10th percentile for gestational age, subsequent abnormal FHR patterns, and perinatal mortality. Sensitivity, specificity, and predictive values for fetal distress requiring

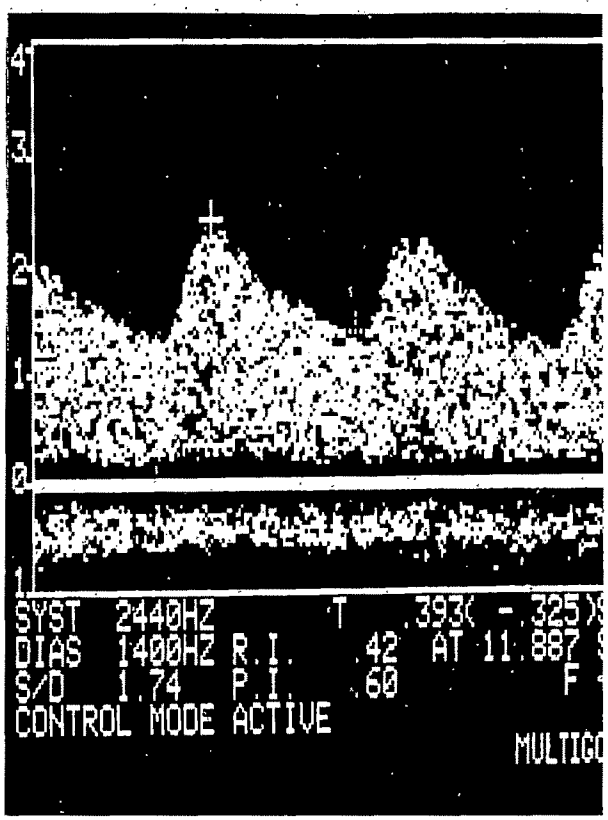


Fig. 2. Umbilical waveform with characteristic arterial and venous flow in opposite directions. Cursors show peak systolic and lowest end-diastolic flow velocity.

Table I. Maternal variables (n = 109)

	Mean ± SD	*Range*
Age (yr)	26.0 ± 5.3	15-41
Gravidity	2.8 ± 1.9	1-6
Parity	1.3 ± 1.5	0-5
Estimated gestational age (wk)	40.2 ± 2.0	36-44
Duration of rupture of membranes (hr)	13.2 ± 18.0	1.5-35
Labor (hr)	16.4 ± 8.1	2.1-32.0

Membrane status on admission: intact = 57 (52.3%); ruptured = 52 (47.7%).

cesarean delivery were calculated for each testing modality. Statistical analysis was carried out with the Fisher exact test or χ^2 analysis, and significance was considered at $p < 0.05$.

Results

Table I shows the maternal variables for the entire study population. Equal numbers of patients were first seen with either intact or ruptured membranes. Suspected postdate pregnancy occurred in 31.5% of patients, but most of these had poor clinical dating criteria.

Table II describes pregnancy outcome related to the interpretation of the initial FHR tracing, the amniotic fluid index and Doppler S/D ratio. Of 109 patients, 21 were noted to have abnormalities of the initial FHR tracing, an incidence of 19.3%. Although all indexes of morbidity are higher in the abnormal tracing group, there is a significant increase in fetal distress that requires cesarean delivery (p = 0.0009, RR = 21.6). A total of 17 of 109 patients were found to have oligohydramnios, for an incidence of 15.6%. All indexes of morbidity (except small for gestational age) are increased in the group with oligohydramnios and this reaches statistical significance for fetal distress that requires cesarean delivery (p = 0.047, RR = 5.3) and Apgar score <7 at 1 minute (p = 0.002, RR = 3.3). There was no relationship in the incidence of oligohydramnios to postdate and nonpostdate pregnancies (20.0% and 21.4%, respectively).

Abnormal Doppler flow was found in eight of 109 patients (7.3%). None of these eight patients had the absence of or reverse end-diastolic flow velocity (range of S/D ratios, 3.03 to 6.5). Seven of these eight values were between 3.0 and 3.6, with a single value more significantly elevated at 6.5. An abnormal S/D ratio was not associated with an increase in any index of fetal morbidity. Both fetuses that were small for gestational age had normal S/D ratios. The mean S/D ratio in the group of patients in whom fetal distress developed was 2.26 (range, 1.36 to 2.66), whereas the mean S/D ratio for the group without fetal distress was 2.30 (range, 1.45 to 6.50).

Though equal numbers of patients were seen with intact and ruptured membranes, 5 of 57 patients with intact membranes had oligohydramnios (8.8%), whereas 12 of 52 patients with ruptured membranes had oligohydramnios (23.1%), a significant difference (p = 0.04, χ^2).

Table III shows pregnancy outcome in patients with oligohydramnios in relation to the status of membranes on admission. Morbidity is equal between the two groups, which suggests that oligohydramnios as a result of ruptured membranes has equal significance with preexisting oligohydramnios.

Table IV describes FHR patterns in the last 40 minutes of labor in relation to the results of the initial FHR tracing, amniotic fluid index, and Doppler velocimetry. Patients with abnormal initial FHR patterns had significant increases in the incidence of severe variable decelerations and late decelerations with decreased variability. Patients with oligohydramnios had significant increases in the incidence of baseline tachycardia, loss of reactivity, and bradycardia. An abnormal S/D ratio was associated with a significant increase in the incidence of bradycardia in subsequent labor.

Table V shows the sensitivity, specificity, positive pre-

Table II. Pregnancy outcome in relation to the initial FHR tracing, amniotic fluid index, and Doppler S/D

	Normal FHR	*Abnormal FHR*	*Amniotic fluid index >5.0 cm*	*Amniotic fluid index <5.0 cm*	*S/D < 3*	*S/D > 3*
No.	88	21	92	17	101	8
Meconium (%)	28.4	52.4	29.3	52.9	32.7	0
Cesarean delivery because of fetal distress (%)	1.1	23.8*	3.3	17.6†	5.9	0
Apgar <7 (%)						
1 minute	15.9	28.6	14.1	47.1‡	20.8	0
5 minutes	1.1	0	0	5.9	1.0	0
Small for gestational age (%)	2.3	0	2.2	0	2.0	0
Perinatal mortality (%)	0	4.8	0	5.8	1.0	0

*$p < 0.001$ When compared with normal FHR.
†$p < 0.05$ When compared with amniotic fluid index >5.0 cm.
‡$p < 0.005$ When compared with amniotic fluid index >5.0 cm.

Table III. Pregnancy outcome in patients with oligohydramnios in relation to membrane status

	Intact (n = 5)		*Ruptured (n = 12)*	
	n	%	*n*	%
Meconium	3	60.0	6	50.0
Cesarean delivery because of fetal distress	0	0	3	25.0
Apgar scores <7				
1 minute	2	40.0	6	50.0
5 minutes	0	0	1	8.3
Small for gestational age	0	0	0	0
Perinatal mortality	0	0	1	8.3

dictive value, and negative predictive value of the initial FHR, amniotic fluid index, and Doppler velocimetry for subsequent fetal distress that required cesarean delivery.

Comment

The concept of an admission test performed early in the course of labor is attractive because it provides an assessment of current fetal condition and risk. This evaluation would be important even in routine obstetric populations because intrapartum morbidity develops in 10% to 20% of patients at "low risk."[8,9] A fetal admission test may provide a mechanism to triage patients to a "low-risk" or "high-risk" status. Should the patient and her health care provider desire, this may allow intermittent auscultation or intermittent monitoring. Reassuring admission test results may obviate the need for telemetry in the patient who desires to ambulate through the latent phase of labor. Nonreassuring test results would alert the physician to an increased risk of fetal intolerance of labor. Continuous monitoring and physician vigilance may then be indicated.

Diagnostic modalities evaluated thus far include the initial FHR strip and the fetal response to acoustic stimulation. Ingemarrson et al.[2] have studied the usefulness of a short FHR strip in early labor and have found a low rate of intrauterine asphyxia (0.9%) when the tracing was reassuring and a high rate (50%) when the tracing was ominous. In a subsequent study, the same authors evaluated the fetal response to acoustic stimulation and found this technique to be a useful adjunct to the initial FHR strip in the exclusion of fetal compromise in early labor.[3] An ominous FHR strip in association with a nonreactive response to acoustic stimulation was associated with a 75% risk for fetal distress.

Amniotic fluid volume has been found to aid in the assessment of fetal condition in antepartum surveillance.[11] Antepartum oligohydramnios with intact membranes has been associated with postdate pregnancy,[11] intrauterine growth retardation,[12] fetal anomalies,[13] abnormal antepartum FHR patterns,[10] and increased perinatal morbidity and mortality.[11] Therefore, it would seem a logical extension to evaluate amniotic fluid volume in the early intrapartum period as a predictor of subsequent fetal morbidity.

Doppler umbilical artery velocimetry has been used to evaluate high-risk pregnancies. Previous studies by Fleischer et al.,[14] Trudinger et al.,[15] and Brar et al.[16]

Table IV. Late FHR patterns in relation to initial FHR, amniotic fluid index, and Doppler S/D

	Normal FHR (%) n = 88	*Abnormal FHR (%) n = 21*	*Amniotic fluid index >5.0 cm (%) n = 92*	*Amniotic fluid index <5.0 cm (%) n = 17*	*S/D <3.0 (%) n = 101*	*S/D >3.0 (%) n = 8*
Baseline tachycardia	8.0	4.8	3.3	29.4*	6.9	12.5
Decreased baseline variability	3.4	4.8	3.3	11.8	5.0	0
Loss of reactivity	12.5	9.5	8.7	35.3†	13.9	0
Severe variable decelerations	10.2	57.1‡	18.5	29.4	20.8	12.5
Late decelerations	0	9.5§	0	5.9	1.0	0
Bradycardia	9.1	14.3	7.6	23.5‖	7.9	37.5¶

*$p < 0.001$ When compared with amniotic fluid index >5.0 cm.
†$p < 0.005$ When compared with amniotic fluid index >5.0 cm.
‡$p < 0.0005$ When compared with normal FHR.
§$p < 0.05$ When compared with normal FHR.
‖$p < 0.05$ When compared with amniotic fluid index >5.0 cm.
¶$p < 0.05$ When compared with S/D <3.0.

have established a relationship between impaired end-diastolic flow velocity, intrauterine growth retardation, and adverse fetal outcome. Abnormal Doppler flow may also be an earlier indicator of fetal compromise than is the nonstress test in intrauterine growth retardation.[17]

Brar et al.[18] have evaluated placental vascular resistance by Doppler S/D ratios in patients with late decelerations and control patients with reactive tracings. Patients with late decelerations had mean S/D ratios significantly higher than those of the control group (4.32 versus 2.32) as well as higher incidences of meconium, cesarean section because of fetal distress, low Apgar scores and admissions to the neonatal intensive care unit. However, the morbidity in this group was largely a result of those patients with late decelerations and elevated S/D ratios as opposed to those with late decelerations and normal S/D ratios. They concluded that Doppler assessment may be an important adjunct during intrapartum monitoring of patients with late decelerations and may help discriminate "false-positive" from "true-positive" late decelerations.

This study suggests that both the initial FHR tracing and amniotic fluid volume assessment may be reasonable choices for an admission test. The sensitivities, specificities, and positive and negative predictive values are comparable, although the FHR strip has somewhat better sensitivity and positive predictive value. The FHR tracing certainly is the most readily available and most physicians are familiar with its interpretation, which makes this technique readily applicable.

However, amniotic fluid volume assessment may provide information not obtainable by evaluation of the FHR strip: the fetus at risk because of cord compression as a result of oligohydramnios. The association noted between ruptured membranes, oligohydramnios, and increased morbidity in this study suggests that fetal morbidity may occur as a result of oligohydramnios after membrane rupture. To identify this population, ultrasound assessment of amniotic fluid volume should be performed on all patients who are admitted to the hospital in labor with ruptured membranes. It would seem logical that prophylactic amnioinfusion may decrease morbidity in this group of patients because the technique has been shown to relieve variable decelerations,[19] and decrease morbidity in preterm premature rupture of the membranes.[20] These approaches are being investigated.

The lack of correlation between abnormal Doppler S/D ratios and adverse fetal outcome is not surprising given the relatively small number of patients in the study. Although eight patients were found to have elevated S/D ratios, there were no cases of absent or reverse end-diastolic flow velocity, the abnormalities most predictive of poor fetal outcome. The mean S/D ratios in patients with and without fetal distress (2.26 versus 2.30) were not different, which suggests that elevated placental vascular resistance was not a common cause of fetal distress in the study population. In keeping with the findings of Brar et al.[18] it would appear that the role of Doppler velocimetry in the intrapartum period would be one as an adjunctive test in the presence of abnormal FHR patterns that are suggestive of uteroplacental insufficiency, rather than one as a screening test. Correlation of Doppler S/D ratios with a large number of patients who exhibit decreased variability and lack reactivity seems indicated given the association between late decelerations and abnormal S/D ratios. The lack of correlation between Doppler S/D ratios and variable decelerations is predictable because abnormal flow velocity in the umbilical artery is reflective of a primary lesion within the placental vascular bed,[21] whereas variable decelerations reflect acute cord compression,[22] two very distinct pathophysiologic mechanisms.

The value of a screening test is measured by its sen-

sitivity, specificity, and predictive value for abnormal outcome. With reference to a screening test for subsequent fetal distress, the clinician is most interested in the positive predictive value of the test (i.e., given abnormal test results, what is the risk for adverse outcome?). However, the positive predictive value of a test varies with the incidence of the disease. For example, when the incidence of the disease is 5% and sensitivity and specificity are kept constant at 95%, the maximum positive predictive value obtainable is 50% (i.e., 50% of patients with abnormal test results will have the abnormal outcome). In the current study 6 of 109 patients (5.9%) required cesarean delivery because of clinical fetal distress. This low incidence may account for the relatively low positive predictive values noted for all three testing modalities. Therefore, it would appear that the utility of abnormal screening test results would be to provide the clinician with the information that the risk of intrapartum fetal distress is increased. However, immediate intervention by cesarean delivery would not be indicated.

Conversely, the predictive value of negative (normal) test results can be clinically useful in the evaluation of a test that predicts fetal condition in subsequent labor. The high negative predictive values noted for all three testing modalities should reassure the clinician when the test results are normal. In this instance the finding of normal or "reassuring" test results may then allow triage of the patient to intermittent auscultation.

Sensitivity (the probability that the test results will be abnormal in all cases of adverse outcome) and specificity (the probability that the test results will be normal in all cases of normal outcome) are also important indicators of the accuracy of the screening test. Whereas the initial FHR tracing and the amniotic fluid index identified 83.3% and 50% of patients with abnormal outcome, respectively, Doppler S/D was not abnormal in any case of adverse outcome (mean S/D, 2.26; range, 1.36 to 2.66). However, all three testing modalities showed comparable specificity when compared to each other.

Overall, it would appear that both the initial FHR tracing and the amniotic fluid index may be of value as screening tests within the scheme outlined above; however, the lack of sensitivity and positive predictive value with Doppler S/D ratios mitigate against its use as a primary screening tool in this situation.

It would seem that the combination of initial FHR (or fetal acoustic stimulation) with amniotic fluid index assessment into a comprehensive admission test with Doppler velocimetry as an adjunctive test in the presence of an abnormal initial FHR or oligohydramnios may provide even better sensitivity, specificity, and predictive values than any single test. The finding of oligohydramnios in the presence of ruptured membranes increases the risk for fetal distress most likely as a result of acute cord compression; therefore, prophylactic amnioinfusion may be effective in decreasing fetal morbidity.

Table V. Sensitivity, specificity, and positive and negative predictive value of initial FHR, amniotic fluid index, and Doppler S/D for subsequent fetal distress requiring cesarean delivery (%)

	Sensitivity	*Specificity*	*Positive predictive value*	*Negative predictive value*
Initial FHR	83.3	84.5	23.8	98.9
Amniotic fluid index	50.0	86.4	17.6	96.7
Doppler S/D	0	92.2	0	94.1

In conclusion, our study suggests that the initial FHR strip or amniotic fluid index in early labor may be reasonable to use as a fetal admission test to guide subsequent labor management. Doppler velocimetry does not appear to be of use in this setting, but may be of value as an adjunctive test in cases in which abnormal heart rate patterns, decreased amniotic fluid volume, or reverse flow are seen or if flow is absent. Unfortunately, none of the latter were seen in this study; therefore, a larger study is being carried out.

REFERENCES

1. ACOG Newsletter. Washington, DC: American College of Obstetricians and Gynecologists, 1988;32:(11)1.
2. Ingemarsson I, Arulkumaran S, Ingemarsson E, Tambyraja RL, Ratnam SS. Admission test: a screening test for fetal distress in labor. Obstet Gynecol 1986;68:800-4.
3. Ingemarsson I, Arulkumaran S, Paul RH, Ingemarsson E, Tambyraja RL, Ratnam SS. Fetal acoustic stimulation in early labor in patients screened with the admission test. Am J Obstet Gynecol 1988;158:70-4.
4. Kubli FW, Hon EH, Khazin AF, Takemura H. Observations on heart rate and pH in the human fetus during labor. Am J Obstet Gynecol 1969;104:1190-206.
5. Krebs H-B, Petres RE, Dunn LJ, Smith PJ. Intrapartum fetal heart rate monitoring. VI. Prognostic significance of accelerations. Am J Obstet Gynecol 1982;142:297-305.
6. Phelan JP, Ahn MO, Smith CV, Rutherford SE, Anderson E. Amniotic fluid index measurements during pregnancy. J Reprod Med 1987;32:601-4.
7. Thompson RS, Trudinger BJ, Cook CM, Giles WB. Umbilical artery velocity waveforms: normal reference values for A/B ratio. Br J Obstet Gynaecol 1988;95:589-91.
8. Westgren M, Ingemarsson E, Ingemarsson I, Solum T. Intrapartum electronic fetal monitoring in low risk pregnancies. Obstet Gynecol 1980;56:301-4.
9. Hobel C, Hyvarinen M, Okana D, Oh W. Prenatal and intrapartum high risk screening. Am J Obstet Gynecol 1973;117:1-9.
10. Rutherford SE, Phelan JP, Smith CV, Jacobs N. The four-quadrant assessment of amniotic fluid volume: an adjunct to antepartum fetal heart rate testing. Obstet Gynecol 1987;70:353-6.
11. Phelan JP, Platt LD, Yeh S-Y, Broussard P, Paul RH. The role of ultrasound assessment of amniotic fluid volume in the management of the postdate pregnancy. Am J Obstet Gynecol 1985;151:304-8.
12. Manning FA, Hill LM, Platt LD. Qualitative amniotic fluid volume determination by ultrasound: antepartum detec-

tion of intrauterine growth retardation. AM J OBSTET GYNECOL 1981;139:254-8.
13. Hill LM, Breckle R, Wolfgram K, Obrien P. Oligohydramnios: ultrasonically detected incidence and subsequent fetal outcome. AM J OBSTET GYNECOL 1983;147:407-10.
14. Fleischer A, Schulman H, Farmakides G, Bracero L, Blattner M, Randolph G. Umbilical artery velocity waveforms and intrauterine growth retardation. AM J OBSTET GYNECOL 1985;151:502-5.
15. Trudinger BJ, Giles WB, Cook C, Bombardieri J, Collins L. Fetal umbilical artery flow velocity waveforms and placental resistance: clinical significance. Br J Obstet Gynaecol 1985;92:23-30.
16. Brar HS, Platt LD. Reverse-end diastolic flow velocity on umbilical artery velocimetry in high-risk pregnancies: an ominous finding with adverse pregnancy outcome. AM J OBSTET GYNECOL 1988;159:559-61.
17. Trudinger BJ, Cook CM, Jones L, Giles WB. A comparison of fetal heart rate monitoring and umbilical artery waveforms in the recognition of fetal compromise. Br J Obstet Gynaecol 1986;93:171-5.
18. Brar HS, Platt LD, Paul RH. Fetal umbilical velocity waveforms using Doppler ultrasonography in patients with late decelerations. Obstet Gynecol 1989;73:363-6.
19. Miyazaki FS, Nevarez F. Saline amnioinfusion for relief of repetitive variable decelerations: a prospective randomized study. AM J OBSTET GYNECOL 1985;153:301-6.
20. Nageotte MP, Freeman RK, Garite TJ, Dorchester W. Prophylactic intrapartum amnioinfusion in patients with preterm premature rupture of membranes. AM J OBSTET GYNECOL 1985;153:557-62.
21. Giles WB, Trudinger BJ, Baird PJ. Fetal umbilical artery flow velocity waveforms and placental resistance: pathological correlation. Br J Obstet Gynaecol 1985;92:31-8.
22. Gabbe SG, Ettinger BB, Freeman RK, Martin CV. Umbilical cord compression associated with amniotomy: laboratory observations. AM J OBSTET GYNECOL 1976;126:353-5.

Doppler assessment of the fetal and uteroplacental circulation during nifedipine therapy for preterm labor

Giancarlo Mari, MD, Brian Kirshon, MD, Kenneth J. Moise, Jr., MD, Wesley Lee, MD, and David B. Cotton, MD

Houston, Texas

To investigate the effects of nifedipine on the human fetal circulation, 11 fetuses whose mothers were treated with nifedipine for treatment of preterm labor were studied. Maximum velocity waveforms were obtained in the middle cerebral artery (n = 8), renal artery (n = 6), ductus arteriosus (n = 8), and umbilical artery (n = 10). Transvalvular maximal velocity waveforms were obtained across the aortic (n = 11) and pulmonary (n = 7) valves. Maternal uterine arteries also were studied (n = 7). Doppler data were collected before and 5 hours after nifedipine therapy. Patients received an oral loading dose of 30 mg of nifedipine followed by a second oral dose of 20 mg 4 hours later. No significant difference in the flow velocity waveforms was found in the vessels studied 5 hours after the initial dose. These results suggest that short-term nifedipine therapy does not influence either fetal or uteroplacental circulation as evaluated with the Doppler technique. (AM J OBSTET GYNECOL 1989;161:1514-8.)

Key words: Doppler, nifedipine, fetal blood flow

Nifedipine is a calcium channel blocker with hemodynamic effects, including negative inotropic action, inhibition of atrioventricular conduction, and coronary and peripheral vasodilation.[1] Nifedipine has been used in the treatment of pregnancy-induced hypertension[2,3] and preterm labor.[4,5] In view of potential adverse effects of calcium channel blockers in the rhesus monkey[6] and fetal lamb,[7] this drug has not been used extensively in human pregnancy. The purpose of this study was to evaluate Doppler blood flow velocity waveforms of various regional blood flows in fetuses whose mothers were given nifedipine for treatment of preterm labor. Additionally, the uteroplacental circulation was studied.

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Baylor College of Medicine.
Presented at the Thirty-sixth Annual Meeting of the Society For Gynecologic Investigation, San Diego, California, March 15-18, 1989.
Reprint requests: Giancarlo Mari, MD, Department of Obstetrics and Gynecology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030.
6/6/16509

Material and methods

Image-directed pulsed Doppler techniques were used to obtain maximum blood velocity waveforms in 11 normal fetuses whose mothers received nifedipine for treatment of preterm labor. All of the patients ini-

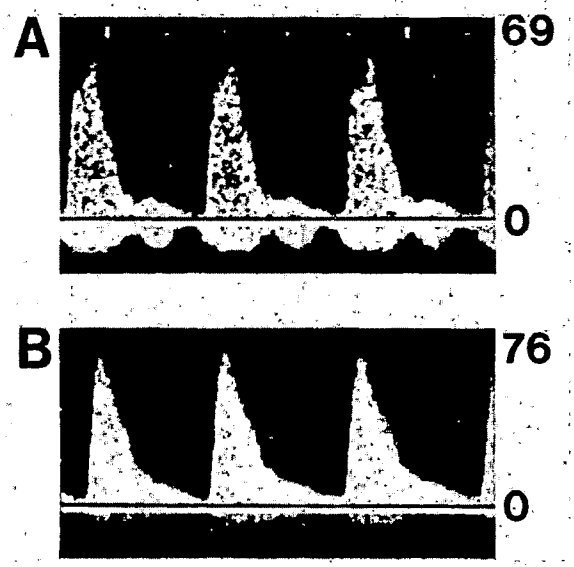


Fig. 1. Doppler waveforms of fetal renal artery and vein **(A)** and middle cerebral artery **(B).** Values are in centimeters per second.

tially received a 30 mg dose of oral nifedipine, followed by a 20 mg oral dose 4 hours later. The studies were performed before and 5 hours after the initial nifedipine dose (1 hour after the second dose) in the following vessels: middle cerebral artery (n = 8), renal artery (n = 6), umbilical artery (n = 10), ductus arteriosus (n = 8), pulmonary (n = 7) and aortic (n = 11) valves, and uterine arteries (n = 7). The decision to assess the fetal status by Doppler technique at 5 hours after the loading dose (30 mg) and 1 hour after the second dose was based on the fact that the half-life of nifedipine is 2 hours with peak blood levels occurring approximately 30 minutes after oral administration.[8] Because gastric absorption is delayed in pregnancy, we elected to study the fetus at 60 minutes instead of 30 minutes after the second dose of nifedipine. In so doing it was thought that an adequate maternal and fetal nifedipine level would be obtained.

The patients were studied between 10 AM and 10 PM and remained in a fasting state on entry into the study. All the patients had preterm painful uterine contractions when they were first seen, and the diagnosis was preterm labor in all. All patients gave written consent for the study, which was approved by the Baylor Human Investigational Review Board. After the initial dose of nifedipine, continuous fetal heart rate monitoring was performed. The maternal heart rate and pressure were recorded before nifedipine and thereafter at 15, 30, and 60 minutes and hourly thereafter. Nifedipine levels were determined (n = 8) by reverse-phase high-performance liquid chromatography analysis after Doppler evaluation (approximately 6 hours after the initial nifedipine dose).

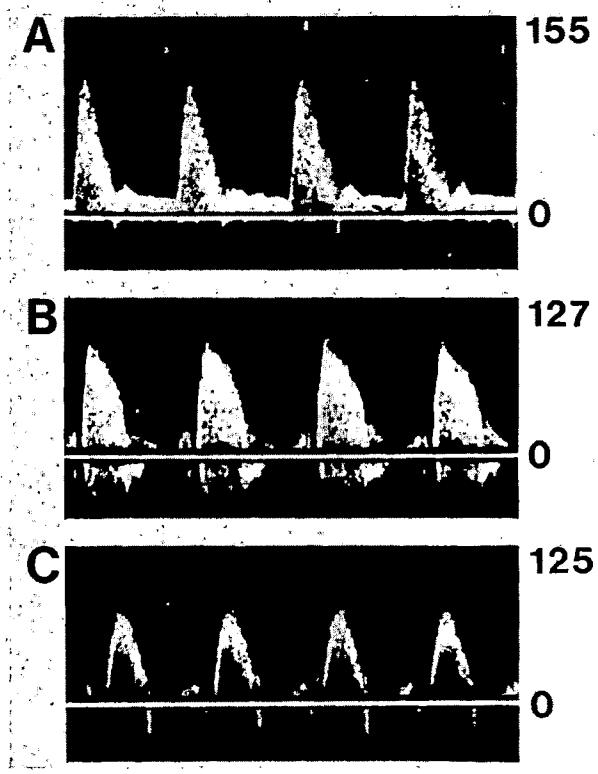


Fig. 2. Doppler waveforms of fetal ductus arteriosus **(A),** pulmonary valve **(B),** and aortic valve **(C).** Values are in centimeters per second.

Doppler ultrasonographic studies were carried out with a General Electric PASS II cardiac ultrasonography system. A 2.5 MHz transducer was used with a 2 mm sample volume. A 50 Hz pass filter was used. Doppler ultrasonographic energy was 114 mW/cm^2 spatial peak temporal average intensity. The fetuses were examined with the mothers in a semirecumbent position. Doppler examination of the renal artery was performed in the following manner: a sagittal section of the fetal body was visualized, and the ultrasonographic beam was directed laterally to the spine, to obtain a coronal section of the fetal body. By moving the transducer anteriorly, a coronal section of the descending aorta and kidneys was imaged. As the renal artery could not be visualized clearly, the sample volume was positioned in the renal hilum and then moved toward the aorta, enabling pulsed Doppler waveforms to be recorded at different points along the renal vessels. Doppler waveforms of the artery and vein were detected simultaneously and displayed on either side of the baseline (Fig. 1, *A*). Doppler examination of the middle cerebral artery (Fig. 1, *B*) was performed as reported previously.[9] The ductus arteriosus (Fig. 2, *A*) was studied as described by Huhta et al.[10] and Moise et al.[11] Umbilical artery waveforms were recorded by placing the sample volume in the cord; both artery and

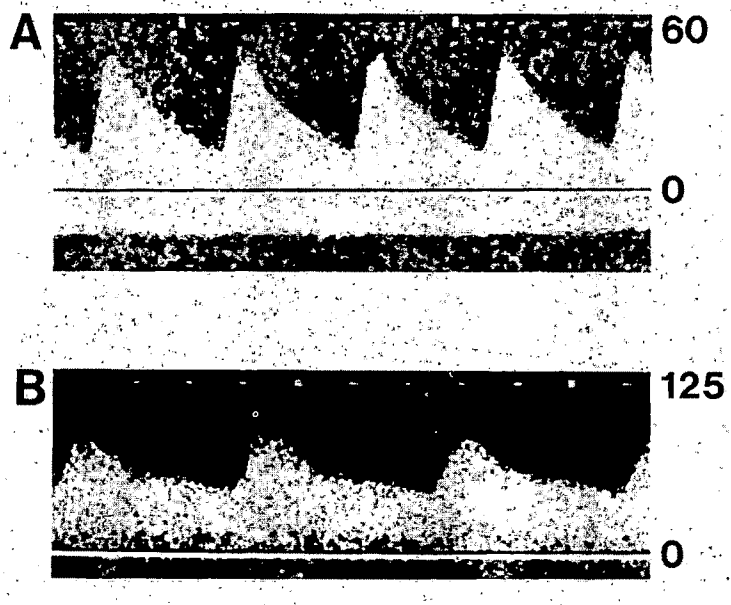


Fig. 3. Doppler waveforms of umbilical artery and vein **(A)** and uterine artery **(B)**.

Table I. Comparison of various index values for different fetal arteries

Vessel	*n*	*Baseline (mean ± SD)*	*After nifedipine (mean ± SD)*	*p Value**
MCA (PI)	8	2.36 ± 0.61	2.20 ± 0.28	NS
RA (PI)	6	2.49 ± 0.56	2.52 ± 0.40	NS
UA (PI)	10	1.06 ± 0.20	1.09 ± 0.29	NS
Ut A (PI)	7	0.77 ± 0.28	0.71 ± 0.21	NS
DA (PSV)	8	111 ± 14	110 ± 14	NS
AoV (TVI) × HR	10	1171 ± 267	1129 ± 210	NS
PuV (TVI) × HR	7	1032 ± 280	1109 ± 174	NS

MCA, Middle cerebral artery; *RA*, renal artery; *UA*, umbilical artery; *Ut A*, uterine artery; *DA*, ductus arteriosus; *PSV*, peak systolic velocity (centimeters per second); *AoV*, aortic valve; *PuV*, pulmonary valve; *PI*, pulsatility index; *TVI*, time velocity integral; *HR*, heart rate.

*Differences evaluated by *t* test. *NS*, Not significant.

vein could be recorded at the same time (Fig. 3, *A*). The uterine arteries (Fig. 3, *B*) were studied as described by Schulman et al.[12] Doppler velocity waveforms were recorded on videotape and a computer system was used to calculate the pulsatility index (peak velocity − end-diastolic velocity/mean velocity)[13] of the middle cerebral artery, renal artery obtained at level of the aorta, umbilical artery, and uterine artery. The peak velocity of the ductus arteriosus was also obtained. The time velocity integral, defined as the area under the Doppler velocity curve, was measured in the pulmonary artery at or slightly above the pulmonary valve (Fig. 2, *B*) and at the aortic valve (Fig. 2, *C*). The relationship between aortic and pulmonary time velocity integrals multiplied by heart rate (a parameter proportional to ventricular cardiac output)[14] was studied. Three consecutive waveforms were analyzed and the results averaged. Measurements were obtained only during fetal apnea. We defined apnea as the absence of visible changes in the waveform patterns for at least 15 waveforms.

Paired Student's *t* test was used to compare the baseline pulsatility index values with those obtained after the start of nifedipine therapy. Analysis of variance was used to compare the maternal blood pressure and heart rate at different time intervals. A probability value of <0.05 was considered to indicate statistical significance.

Results

The maternal age ranged between 17 and 39 (mean ± SD 22.3 ± 6). Parity ranged between 0 and 5. The gestational age of the fetuses ranged from 26 to 34 weeks (mean ± SD 31 ± 2.5 weeks).

The short-term effects of nifedipine on the fetal circulation as evaluated by the Doppler method were examined in the fetuses. The results are summarized in Table I.

The maternal nifedipine levels 6 hours after the initial dose (2 hours after the second dose) were between 23 and 145 ng/ml (51.2 ± 38 ng/ml) ($n = 8$). Preterm labor abated in all patients. Analysis of variance showed no significant difference in the maternal blood pressure

and heart rate ($p > 0.05$). No evidence of fetal distress was observed on the fetal heart rate monitor during the study period. The gestational age at delivery ranged between 33 and 41 weeks (mean ± SD 38.5 ± 2.54 weeks). The birth weight ranged between 1860 and 3950 gm (3026 ± 692 gm). Apgar scores at 1 and 5 minutes ranged between 6 and 9 (8.2 ± 0.9) and 8 and 10 (9.2 ± 0.6), respectively.

Comment

Oral nifedipine has been used to treat pregnancy-induced hypertension[2, 3] and preterm labor.[4, 5] Read and Wellby[4] found oral nifedipine to be more effective than ritodrine in suppressing premature uterine activity. Walters and Redman[2] observed a rapid and significant fall in maternal blood pressure by an average of 26/20 mm Hg 20 minutes after oral administration in 21 women with acute episodes of severe hypertension during pregnancy or in the puerperium. These authors observed no adverse fetal effects. Veille et al.[15] found no significant changes in maternal uterine blood flow, arterial blood pressure, and heart rate after intravenous administration of nifedipine in pregnant, unanesthetized pygmy goats. Ahokas et al.[16] investigated the short-term effects of nifedipine in 12 hypertensive term-pregnant rats. Nifedipine (200 μg/kg) lowered mean arterial pressure 25% by decreasing total peripheral resistance 38%. Cardiac output was increased 15%. Placental blood flow was not significantly altered, but resistance was decreased.

Ducsay et al.,[6] during periods of spontaneous uterine contractility, administered nicardipine (another calcium channel blocker) to chronically catheterized rhesus monkeys, at 128 and 132 days' gestation. Fetal heart rate and blood pressure were unaffected, however, fetuses became acidotic and hypoxemic after maternal nicardipine treatment. Harake et al.[7] administered nifedipine intravenously for 30 minutes to chronically instrumented pregnant ewes. Infusion of 5 μg/kg/min resulted in a 30% to 50% increase in total and regional fetal cerebral blood flow. Infusion of 10 μg/kg/min decreased uterine blood flow by 21% and fetal arterial oxygen content by 15%, with no further increase in fetal cerebral blood flow. Maternal arterial blood pressure decreased and heart rate increased with no change in maternal arterial blood gas values. The decrease of uterine blood flow reported by Harake et al.[7] could be a consequence of the redistribution of the blood flow to other maternal circulatory regions. The acidosis and hypoxemia observed by Ducsay et al.[6] after administration of calcium channel blockers could be a consequence of the reduced uterine blood flow as reported by Skillman et al.[17] in the fetal lamb.

Doppler studies have been shown to be a valuable tool to assess fetal well-being. Fetal Doppler studies have shown a correlation between abnormal flow velocity waveforms of the umbilical artery and an increased incidence of complications in the fetus and/or infant.[18] A significant relationship has been found between the pulsatility index of the umbilical artery (>1.5) in high-risk pregnant patients and pH, P_{CO_2}, and lactate concentrations obtained from umbilical blood sampled in utero.[19] The pulsatility index of cerebral waveforms has been found to be a powerful indicator for the development of neonatal neurologic abnormalities.[20] Pathologic uterine artery waveforms are associated with high-risk pregnancy.[21] Additionally, studies have demonstrated the ability of the Doppler technique to diagnose tricuspid regurgitation and fetal ductus arteriosus constriction, as a consequence of maternal indomethacin therapy used in the treatment of preterm labor.[10, 11]

Our results show no significant difference in the pulsatility index of the vessels studied. Additionally no significant changes were observed in the peak velocity of the ductus arteriosus and the product of the time velocity integral and heart rate in the pulmonary and aortic valves. The lack of change in the latter suggests that no significant change in cardiac output occurred during nifedipine. It is possible that higher doses of nifedipine and/or continuous intravenous administration could modify the pulsatility index values.

Plasma levels of nifedipine during the period of study were calculated with the effective half-life of the drug taken into account. Therapeutic effects of nifedipine (decrease of diastolic blood pressure) have been reported with plasma values in the range of 15 ng/ml.[22] Others, have reported a therapeutic action of nifedipine (decrease of mean arterial pressure) with plasma values as low as 3 ng/ml.[23] Finally, a large interindividual variation has been reported in the plasma level of nifedipine for different doses tested.[24] Our maternal plasma nifedipine levels ranged from 23 to 145 ng/ml. Thus absence of waveform changes occurred at a time when nifedipine was present in the circulation and presumably exerting other actions.

This study demonstrates that it is possible to study the effects of a calcium channel blocker on various human fetal areas with a noninvasive method. Our results suggest that nifedipine does not adversely affect uteroplacental and human fetal blood flow as evaluated by the Doppler method in the doses used for treatment of preterm labor in this study. Whether the same results will be obtained during long-term therapy or with a more aggressive dosage regimen remains to be established.

REFERENCES

1. Nayler WG, Horowitz JD. Calcium antagonists: a new class of drugs. Pharmacol Ther 1983;20:203-62.
2. Walters BNJ, Redman CWG. Treatment of severe

pregnancy-associated hypertension with the calcium antagonist nifedipine. Br J Obstet Gynaecol 1984;91:330-6.
3. Constantine G, Beevers DG, Reynolds AL, Luesley DM. Nifedipine as a second line antihypertensive drug in pregnancy. Br J Obstet Gynaecol 1987;94:1136-42.
4. Read MD, Wellby DE. The use of a calcium antagonist (nifedipine) to suppress preterm labour. Br J Obstet Gynaecol 1986;93:933-7.
5. Ulmsten U, Andersson KE, Wingerup L. Treatment of premature labour with the calcium antagonist nifedipine. Arch Gynecol 1980;229:1-5.
6. Ducsay CA, Thompson JS, Wu AT, Novy MJ. Effects of calcium entry blocker (nicardipine) tocolysis in rhesus macaques: fetal plasma concentrations and cardiorespiratory changes. Am J Obstet Gynecol 1987;157:1482-7.
7. Harake B, Gilbert RD, Ashwal S, Power GG. Nifedipine: effects on fetal and maternal hemodynamics in pregnant sheep. Am J Obstet Gynecol 1987;157:1003-8.
8. Physicians' Desk Reference. Oradell, New Jersey: Medical Economics, 1988:1440-1.
9. Mari G, Moise KJ, Deter RL, Kirshon B, Carpenter RJ, Huhta JC. Doppler assessment of the pulsatility index in the cerebral circulation of the human fetus. Am J Obstet Gynecol 1989;160:698-703.
10. Huhta JC, Moise KJ, Fisher DJ, et al. Detection and quantitation of constriction of the fetal ductus arteriosus by Doppler echocardiography. Circulation 1987;75:406-12.
11. Moise KJ, Huhta JC, Sharif DS, et al. Indomethacin in the treatment of premature labor. Effects on the fetal ductus arteriosus. N Engl J Med 1988;319:327-31.
12. Schulman H, Fleischer A, Farmakides G, et al. Development of uterine artery compliance in pregnancy as detected by Doppler ultrasound. Am J Obstet Gynecol 1985;155:1031-6.
13. Gosling RG, King DH. Ultrasound angiology In: Marons AW, Adamson L, eds. Arteries and veins. Edinburgh: Churchill Livingstone, 1975:61-98.
14. Alverson DC, Eldridge M, Dillon T, Yabek SM, Berman W. Noninvasive pulsed Doppler determination of cardiac output in neonates and children. J Pediatr 1982;101:46-50.
15. Veille JC, Bissonnette JM, Hohimer AR, Knopp S, Koch P. The effect of a calcium channel blocker (nifedipine) on uterine blood flow in the pregnant goat. Am J Obstet Gynecol 1986;154:1160-3.
16. Ahokas RA, Sibai BM, Mabie WC, Anderson GD. Nifedipine does not adversely affect uteroplacental blood flow in the hypertensive term-pregnant rat. Am J Obstet Gynecol 1989;159:1440-5.
17. Skillman CA, Plessinger MA, Woods JR, et al. Effect of graded reductions in uteroplacental blood flow on the fetal lamb. Am J Physiol 1985;249:H1098-105.
18. Rochelson BL, Schulman H, Fleischer A, et al. The clinical significance of Doppler umbilical artery velocimetry in the small for gestational age fetus. Am J Obstet Gynecol 1987;156:1223-6.
19. Ferrazzi E, Pardi G, Bauscaglia M, et al. The correlation of biochemical monitoring versus umbilical flow velocity measurements of the human fetus. Am J Obstet Gynecol 1988;159:1081-7.
20. Rizzo G, Arduini D, Luciano R, et al. Prenatal cerebral Doppler ultrasonography and neonatal neurologic outcome. J Ultrasound Med 1989;8:238-40.
21. Fleischer A, Schulman H, Farmakides G, et al. Uterine artery Doppler velocimetry in pregnant women with hypertension. Am J Obstet Gynecol 1986;154:806-12.
22. Kleinbloesem CH, van Brummelen P, van de Linde JA, Voogd PJ, Breimer DD. Clin Pharmacol Ther 1984; 35:742-9.
23. Banzet O, Colin JN, Thibonnier M, Singlas E, Alexandre JM, Corvol P. Acute antihypertensive effect and pharmacokinetics of a tablet preparation of nifedipine. Eur J Clin Pharmacol 1983;24:145-50.
24. Stern Z, Zylber-Katz E, Levy M. Nifedipine plasma concentration in patients treated for angina pectoris. Int J Clin Pharmacol Ther Toxicol 1984;22:198-203.

Maternal and fetal blood flow velocity waveforms in patients with preterm labor: Relationship to outcome

Harbinder S. Brar, MD, Arnold L. Medearis, MD, Greggory R. DeVore, MD, and Lawrence D. Platt, MD

Los Angeles, California

Elevated systolic/diastolic ratios obtained by umbilical and uterine velocimetry have been used to predict adverse pregnancy outcome. We performed pretherapy umbilical and uterine velocimetry by means of continuous-wave Doppler ultrasonography on 92 patients who came for treatment in preterm labor. Fourteen (15.2%) and 12 (13%) patients had elevated uterine (>2.6) and umbilical (>3.5) systolic/diastolic ratios, respectively, and 9 (9.8%) patients had both ratios elevated. Overall 17 (18.5%) patients had at least one abnormal systolic/diastolic ratio. Patients with abnormal Doppler waveforms had a significantly shorter gestation, infants with lower birth weights, and a higher incidence of adverse pregnancy outcome as determined by meconium, cesarean section for fetal distress, low 1- and 5-minute Apgar scores, and days in the neonatal intensive care unit, compared with patients with normal systolic/diastolic ratios. There was no significant difference in the ability of uterine, umbilical, or combined velocimetry to predict preterm birth or adverse pregnancy outcome. Doppler studies in preterm labor patients may help to identify patients at increased risk for preterm birth and poor pregnancy outcome. Further studies are warranted to assess its usefulness in the evaluation of these patients. (Am J Obstet Gynecol 1989;161:1519-22.)

Key words: Doppler ultrasonography, preterm labor

Perinatal morbidity and mortality have decreased over the last decade. However, further reduction in the incidence and complication of preterm birth continues to be a major goal in perinatal medicine.[1] Some recent reports have suggested retarded fetal growth in preterm deliveries.[2] The recent introduction of Doppler ultrasonography[3] has made it possible to study the fetoplacental[4] and uteroplacental[5] circulation in a noninvasive manner. Umbilical artery waveforms show increasing end-diastolic velocity with advancing gestation, probably as a result of increased compliance of the placenta.[4] Uterine artery compliance is achieved late in the second trimester, reflected by a peak systolic/end-diastolic (S/D) ratio of <2.6 and the absence of diastolic notching.[5] Umbilical and uterine artery waveforms obtained by Doppler ultrasonography have been useful for the surveillance of high-risk pregnancies including hypertension,[5] twins,[6] diabetes,[7] and placenta previa.[8] Abnormalities in waveforms also have been correlated with adverse pregnancy outcome in the preterm[9] and term[10] small-for-gestational-age fetus. Our previous studies have shown the effect of tocolytic agents on these waveforms[11] and an association between abnormal waveforms and failure of tocolysis.[12] The principal objective of this study was to determine the incidence of uterine and umbilical artery waveform abnormalities in patients with preterm labor and their ability to predict preterm birth and adverse pregnancy outcome in such pregnancies.

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of Southern California School of Medicine and Women's Hospital, Los Angeles County–University of Southern California Medical Center.

Presented at the Thirty-fourth Annual Meeting of the Society for Gynecologic Investigation, Atlanta, Georgia, March 18-21, 1987.

Reprint requests: Harbinder S. Brar, MD, Pomona Valley Hospital, 142 Nemaha Ave., Pomona, CA 91767.

6/6/15652

Material and methods

Ninety-two consecutive patients in preterm labor who were seen at the labor and delivery suite of Women's Hospital, Los Angeles County–University of Southern California Medical Center were studied. All patients signed informed consent forms before entry into the study. Inclusion criteria included premature uterine contractions with a frequency of ≥2 in 10 minutes, <36 completed weeks of gestation, documented cervical change, and cervical dilation <4 cm. Patients with ruptured membranes, known intrauterine growth retardation, medical complications of pregnancy, or a mature lung profile were excluded from the study. Pretherapy umbilical and uterine artery waveforms were obtained transabdominally with continuous-wave Doppler ultrasonography (Angioscan III, Ultrasonix Inc.) according to previously described techniques.[13] S/D ratios of three different umbilical waveforms were computed and the mean calculated. The mean of the right and left uterine artery S/D ratios was also calculated and used in the statistical analysis. Patients were then treated with ritodrine or magnesium sulfate according to the managing physician's choice. Magnesium

sulfate tocolysis was given as a 4 gm intravenous bolus over 15 minutes and maintenance therapy ranging from 1 to 4 gm/hr to suppress uterine contractions. Ritodrine was given as an intravenous infusion with a starting dose of 50 μg/min that was increased by 50 μg/min every 15 minutes until uterine contractions were suppressed, a maximum dose of 350 μg/min was reached, or significant side effects occurred. Data collected included age, parity, gestational age at entry and delivery, meconium at delivery, cesarean section for fetal distress, birth weight, and its classification for gestational age by California curves (small for gestational age was defined as <10th percentile), Apgar scores, Dubowitz score, neonatal death, and number of neonatal intensive care unit days. Patients were grouped for analysis according to whether or not they had normal or abnormal uterine and umbilical artery waveforms. A mean S/D ratio of >2.6 or the presence of diastolic notching of the uterine artery waveform was considered abnormal,[5] whereas the cutoff value for abnormal umbilical S/D ratio was 3.5.[12] This value was arbitrarily set higher than the traditional 3.0 value[14] because the patient population was preterm and the umbilical S/D ratio decreases with advancing gestation. The study group was also evaluated according to whether both uterine and umbilical waveforms were abnormal or normal. Comparison of outcome measures consisting of birth weight, preterm delivery at <37 completed weeks of gestation, small for gestational age, meconium, cesarean section for fetal distress, number of neonatal intensive care unit days, and neonatal deaths were performed in the different groups with χ^2 or Fisher's exact test as appropriate. A p value <0.05 was considered statistically significant. Descriptive statistics were calculated in the usual manner. The sensitivity, specificity, and predictive values of umbilical, uterine, and combined velocimetry for the prediction of adverse pregnancy outcome and preterm birth were also calculated.

Results

Fifty-eight patients received magnesium sulfate and 34 patients received ritodrine tocolysis. The mean maternal age was 24.5 ± 2.6 years (range, 18 to 32), and the mean parity was 1.8 ± 0.8 (range, 0 to 5). The mean gestational age at entry was 32.7 ± 1.8 (range, 27 to 36) weeks; women had a mean cervical dilation of 1.2 ± 0.5 cm (range, 0.5 to 4). The incidences of abnormality in umbilical and uterine S/D ratios were 12 patients (13%) and 14 patients (15.2%), respectively. Nine (9.8%) patients had an abnormal umbilical S/D ratio and an abnormal uterine S/D ratio. Seventeen (18.5%) patients had at least one abnormal S/D ratio. There was no significant difference in the mean gestational age and cervical dilation at entry in the normal and abnormal groups. The relationship of the umbilical S/D ratios to long-term pregnancy outcome is shown in Table I. A significantly higher number of patients with elevated umbilical S/D ratios were delivered preterm, and consequent their infant had lower birth weight when compared with patients with normal ratios. A significantly higher number of patients with abnormal umbilical S/D ratios had outcome parameters suggestive of adverse pregnancy outcome, including meconium in amniotic fluid, cesarean section for fetal distress, and low Apgar scores, compared with patients with normal ratios. Neonates of patients with abnormal umbilical S/D ratios spent a significantly longer time in the neonatal intensive care unit compared with patients with normal ratios. The single neonatal death in the group studied occurred in a patient with both abnormal ratios.

An association similar to umbilical S/D ratios was found between abnormal uterine S/D ratios and adverse pregnancy outcome (Table I). The mean gestational age and birth weight at delivery of patients with abnormal uterine S/D ratios was significantly lower ($p < 0.05$) than patients with normal ratios. There was a significant association between adverse pregnancy outcome as assessed by meconium, low Apgar scores, and cesarean section for fetal distress and abnormal uterine S/D ratios. In addition, neonates of patients with abnormal uterine ratios spent significantly ($p < 0.05$) more days in the neonatal intensive care unit compared with patients with normal ratios.

When both uterine and umbilical velocimetry are combined, nine patients had both ratios abnormal. There was a significant association between adverse pregnancy outcome and both ratios being abnormal (Table I).

The sensitivity, specificity, and positive and negative predictive values of umbilical, uterine, and combined velocimetry for the prediction of adverse pregnancy outcome and preterm birth are shown in Table II. There were no statistically significant differences in the predictive values, sensitivity, or specificity when different methods were compared.

Comment

Our previous study[12] showed the association between elevated umbilical and uterine S/D ratios and the failure of tocolysis with delivery within 48 hours. This study evaluates the role of screening Doppler waveform analysis to subsequent pregnancy outcome in preterm labor patients. Although we do not have a control population without preterm labor, the overall incidence (18.5%) of abnormal umbilical and uterine ratios in this group of patients is higher than that expected in a low-risk population, but similar to that reported in other high-risk pregnancy conditions.[14] The presence of el-

Table I. Relationship of pregnancy outcome to pretherapy S/D ratios obtained from umbilical, uterine, and combined velocimetry in patients with perterm labor

	Umbilical S/D ratio		*Uterine S/D ratio*		*Both ratios*	
	Normal (n = 80)	*Abnormal (n = 12)*	*Normal (n = 78)*	*Abnormal (n = 14)*	*Normal (n = 75)*	*Abnormal (n = 9)*
Meconium	9 (11.3%)	7 (58.3%)*	10 (12.8%)	6 (42.9%)*	10 (13.3%)	5 (55.6%)*
Cesarean section for fetal distress	9 (11.3%)	4 (33.3%)*	8 (10.3%)	5 (35.7%)*	8 (10.7%)	4 (46.4%)*
1 min Apgar score <7	10 (12.5%)	6 (50%)*	11 (14.1%)	5 (35.7%)*	10 (13.3%)	5 (55.6%)*
5 min Apgar score <7	5 (6.3%)	4 (33.3%)*	5 (6.4%)	4 (28.6%)*	5 (6.7%)	3 (33.3%)*
Preterm birth	25 (31.3%)	10 (83.3%)†	24 (30.8%)	11 (78.6%)†	24 (32%)	8 (88.9%)†
Birth weight (gm)‡	3100 ± 322	2240 ± 220*	3105 ± 326	2210 ± 235*	3090 ± 312	1920 ± 209*
SGA	0	3 (25%)	0	3 (21.4%)	0	3 (33.3%)
NICU (days)‡	12.5 ± 4.8	29.1 ± 8.6*	13.0 ± 4.6	29.8 ± 8.8*	12 ± 5.2	31.3 ± 9.7*
Neonatal deaths	0	1 (8.3%)	0	1 (7.1%)	0	1 (11.1%)

Comparisons were done between normal and abnormal groups. *SGA*, Small for gestational age; *NICU*, neonatal intensive care unit.

*$p < 0.05$.

†$p < 0.01$.

‡Values expressed as mean ± SD.

Table II. Comparison of umbilical, uterine, and combined velocimetry for prediction of preterm birth and adverse pregnancy outcome in patients with preterm labor*

	Preterm birth				*Adverse pregnancy outcome*			
	Sensitivity	*Specificity*	*NPV*	*PPV*	*Sensitivity*	*Specificity*	*NPV*	*PPV*
Uterine	31.4	94.7	69.2	78.6	39.1	92.8	82.1	64.3
Umbilical	28.6	96.5	68.8	83.3	34.8	94.2	81.3	66.7
Combined	25	98.1	68	88.9	40	98.4	84	88.9

Differences not significant in comparing methods. *NPV*, Negative predictive value; *PPV*, positive predictive value.

*Preterm birth was defined as <37 weeks. Adverse pregnancy outcomes included small-for-gestational-age fetus, cesarean section for fetal distress, meconium, and low 1- and 5-minute Apgar scores.

evated S/D ratios presumably represents elevated downstream vascular resistance.[15] There appears to be a progressive increase in the incidence of poor perinatal outcome and increase in S/D ratios with progressive changes within the placental vasculature.[16] Therefore the association between elevated S/D ratios and the presence of adverse pregnancy outcome in patients with preterm labor is consistent with other reports that suggest a similar association in other high-risk pregnancies, including pregnancy-induced hypertension,[5] twins,[6] diabetes,[7] and placenta previa.[8]

Abnormal uterine and umbilical S/D ratios have both been associated with retarded fetal growth.[16, 17] Abnormal (retarded) growth has also been observed in neonates delivered after preterm birth and premature rupture of membranes.[2] Although patients with prenatally diagnosed intrauterine growth retardation were excluded from the study, three neonates were classified as having intrauterine growth retardation after delivery; all three had abnormal umbilical and uterine S/D ratios. It is possible that other neonates who were not overtly growth retarded had abnormal slowing of the growth but had not fallen below the 10th percentile. Therefore the presence of abnormal S/D ratios in patients with preterm labor may be another indicator of decreased fetal growth, requiring careful assessment of fetal growth for the duration of pregnancy and the initiation of antepartum fetal assessment protocols.

There does not appear to be any significant difference in the predictive values or sensitivity and specificity of use of either the fetoplacental (umbilical) or the uteroplacental (uterine) S/D ratios or a combination of both in predicting adverse pregnancy outcome and preterm birth. Many of the fetuses we studied had both waveforms abnormal, and therefore would suggest that increased resistance on either side of the placenta has an equally adverse effect on the fetus.

Whereas abnormal ratios appear to predict preterm birth (<37 weeks) reasonably well, their sensitivity appears to be lower when compared with the prediction of failed tocolysis (delivery <48 hours after onset of preterm labor), as suggested in our previous study.[12] The patients with abnormal and normal S/D ratios had similar gestational ages and cervical dilation at time of

entry into the study. Therefore the higher incidence of preterm birth in patients with abnormal umbilical and uterine S/D ratios cannot be attributed to these variables. The role of uterine and umbilical velocimetry as a screening test in predicting preterm delivery in the general population is unknown and requires further investigation.

The association of abnormal waveforms with preterm birth is intriguing. It is possible that the stress of increased uterine or umbilical resistance and decreased flow may be linked to altered prostaglandin synthesis and production.[18] This may also explain the high incidence of preterm labor and birth in other high-risk pregnancies that also have a high incidence of abnormal uterine and umbilical ratios.[14] Another possible explanation for this relationship may be that preterm labor patients have a reduced expansion of intravascular volume that is also seen in patients with preeclampsia.[19] It is interesting to speculate that this more limited intravascular volume expansion could contribute to abnormal waveforms seen in preterm labor and preeclampsia of pregnancy.[5] Clearly further study is needed in this area to answer these questions.

The relationship of the success of tocolytic therapy to Doppler waveform has previously been shown.[12] Whether or not an abnormal Doppler waveform will prove to be a contraindication to tocolytic therapy has not been studied. However, from our previous work[11] it appears that there is no direct adverse effect on S/D ratios when tocolytic agents are used. In fact, the lowering of the S/D ratio caused by increasing heart rate[20] might somehow benefit the fetus. These hypotheses require well-designed prospective studies that include standard methods of fetal assessment, such as the nonstress testing, contraction stress test, or biophysical profile, and assessment of biochemical parameters of pregnancy outcome, such as umbilical cord gas measurements. In conclusion, it appears that incorporating umbilical and uterine artery velocimetry in the initial assessment of patients suspected to be in preterm labor may be a helpful adjunct in managing these pregnancies. Further study is warranted before its use can be considered standard for evaluation of preterm labor patients.

REFERENCES

1. Main DM, Gabbe SG, Richardson S, Strong S. Can preterm deliveries be prevented? Am J Obstet Gynecol 1985;151:892-8.
2. Westgren MD, Beall M, Divon M, et al. Fetal femur length/abdominal circumference ratio in preterm labor patients with and without successful tocolytic therapy. J Ultrasound Med 1986;5:243-5.
3. Campbell S, Diaz RJ, Griffin DR, et al. New Doppler technique for assessing uteroplacental blood flow. Lancet 1983;1:675-7.
4. Trudinger BJ, Giles WB, Cook CM, et al. Fetal umbilical artery flow velocity waveforms and placental resistance: clinical significance. Br J Obstet Gynaecol 1985;92:23-30.
5. Fleischer A, Schulman H, Farmakides G, et al. Uterine artery Doppler velocimetry in pregnant women with hypertension. Am J Obstet Gynecol 1986;154:806-13.
6. Giles WB, Trudinger BJ, Cook CM. Fetal umbilical artery flow velocity time waveforms in twin pregnancies. Br J Obstet Gynaecol 1985;92:490-7.
7. Bracero L, Schulman H, Fleischer A, et al. Umbilical artery velocimetry in diabetes and pregnancy. Obstet Gynecol 1986;68:654-8.
8. Brar HS, Platt LD, DeVore GR, et al. Surveillance of pregnancies complicated by placenta previa using fetal umbilical velocimetry. J Reprod Med 1988;33:741-4.
9. Fleischer A, Schulman H, Farmakides G, Bracero L, Blattner P, Randolph G. Umbilical artery velocity waveforms and intrauterine growth retardation. Am J Obstet Gynecol 1985;151:502-5.
10. McCowan LM, Erskine LA, Ritchie JWK. Umbilical artery Doppler blood flow studies in the preterm, small for gestational age fetus. Am J Obstet Gynecol 1987;156:655-9.
11. Brar HS, Medearis AL, DeVore GR, et al. Maternal and fetal blood flow velocity waveforms in patients with preterm labor. Effect of tocolytics. Obstet Gynecol 1988;72:209-14.
12. Brar HS, Medearis AL, DeVore GR, Platt LD. Maternal and fetal blood flow velocity waveforms in patients with preterm labor: prediction of successful tocolysis. Am J Obstet Gynecol 1988;159:947-50.
13. Brar HS, Platt LD, DeVore GR, Horenstein J, Medearis AL. Qualitative assessment of maternal uterine and fetal umbilical artery blood flow and resistance in laboring patients by Doppler velocimetry. Am J Obstet Gynecol 1988;158:952-6.
14. Schulman H. The clinical implication of Doppler ultrasound analysis of the uterine and umbilical arteries. Am J Obstet Gynecol 1987;156:889-93.
15. Giles WB, Trudinger BJ, Baird PJ. Fetal umbilical artery flow velocity waveforms and placental resistance. Pathological correlation. Br J Obstet Gynaecol 1985;92:31-8.
16. Rochelson BL, Schulman H, Fleischer A, et al. The clinical significance of Doppler umbilical artery velocimetry in the small for gestational age fetus. Am J Obstet Gynecol 1987;156:1223-5.
17. Brar HS. The use of Doppler ultrasound to assess intrauterine growth retardation in the fetus. Semin Perinatol 1988;12:40-51.
18. Venuto RC, O'Dorisio T, Stein JH. Uterine prostaglandin E secretion and uterine blood flow in the pregnant rabbit. J Clin Invest 1975;55:193.
19. Goodlin RL, Quaife MA, Dirksen JW. The significance, diagnosis and treatment of maternal hypovolemia associated with fetal/maternal illness. Semin Perinatol 1981;5:163.
20. Brar HS, Medearis AL, Platt LD. Relationship of systolic/diastolic ratios from umbilical velocimetry to fetal heart rate. Am J Obstet Gynecol 1989;160:188-91.

Clinical management of the fetus with markedly diminished umbilical artery end-diastolic flow

Michael Y. Divon, MD, Barbara A. Girz, DO, Richard Lieblich, MD, and Oded Langer, MD

Bronx, New York

This study was conducted to evaluate prospectively a management protocol for fetuses with a markedly abnormal umbilical artery velocity waveform. The study population consisted of fetuses whose systolic/diastolic ratio was >2 SD above the mean for gestational age. The matched control population consisted of fetuses with similar gestational ages, indications for testing, and estimated fetal weights with normal systolic/diastolic ratios. Abnormal Doppler results were used only to determine the frequency of fetal testing. Biophysical profile testing was performed semiweekly on all patients. Patients with absent or reversed end-diastolic flow were admitted for daily testing. The following criteria were used as indications for delivery: (1) worsening maternal condition, (2) oligohydramnios, (3) intrauterine growth retardation with lung maturity, and (4) biophysical profile score ≤4. Fifty-one patients (7%) had abnormal Doppler blood flow velocity studies. When the study population was compared with the control population at the time of delivery, there were no differences in umbilical artery pH, Apgar score, or incidence of intrauterine growth retardation. However, study patients were delivered at a significantly lower gestational age and lower birth weight and experienced a higher likelihood of neonatal intensive care unit admission. When study patients with documented end-diastolic flow were compared with study patients with no end-diastolic flow, there were no differences in umbilical artery pH, Apgar score, or incidence of intrauterine growth retardation. However, fetuses with no end-diastolic flow had a significantly shorter test-to-delivery interval, lower gestational age, lower birth weight, and more neonatal intensive care unit admissions. There were no perinatal deaths among the study patients. The range of systolic/diastolic ratios for the five patients who failed to follow our protocol for intensive maternal-fetal surveillance was 4.3 to infinity; all experienced fetal death within 18 days. These results suggest that immediate delivery of the fetus with diminished end-diastolic flow may not be mandatory. The combined use of fetal biophysical testing and commonly used criteria for delivery results in acceptable fetal outcome and prolongation of gestational age. (Am J Obstet Gynecol 1989;161:1523-7.)

Key words: Fetal Doppler, umbilical artery velocimetry management

Abnormal umbilical artery velocity waveforms have been associated with an increased risk for perinatal morbidity and mortality. Such waveforms probably reflect increased placental impedance to blood flow and are consistent with histologic evidence of a placental vascular lesion.[1] Recent reports have described the ominous fetal outcome associated with a markedly abnormal umbilical artery velocity waveform. Some authors have suggested that perinatal management of these pregnancies should be "aggressive" because perinatal mortalities range from 50% to 90%.[2, 3] Woo et al.[4] described eight perinatal deaths in nine fetuses with absent or reversed umbilical artery end-diastolic blood flow and concluded that this situation mandates immediate delivery. Schulman[5] suggested that absent end-diastolic flow is an emergency situation in which "frequent fetal surveillance" is mandatory. Despite the increased interest in the use of Doppler techniques in obstetrics, guidelines for clinical management of such pregnancies have not been established. The purpose of this study was to evaluate a management protocol for pregnancies complicated by a markedly abnormal umbilical artery velocity waveform.

From the Department of Obstetrics and Gynecology, The Albert Einstein College of Medicine of Yeshiva University.

Presented in part at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.

Reprint requests: Michael Y. Divon, MD, Albert Einstein College of Medicine, Department of Obstetrics and Gynecology, Rm. 703, 1825 Eastchester Road, Bronx, NY 10461.

6/6/15660

Material and methods

A prospective, matched control study of two groups of fetuses was designed. The study population consisted of patients with markedly abnormal umbilical artery end-diastolic flow; the control population consisted of patients with similar indications for fetal testing, gestational ages, and estimated fetal weights with a normal umbilical artery blood flow pattern. No attempt was made to match for maternal age and parity.

The study population was drawn from a private,

Table I. Indications for fetal testing*

Hypertensive disorders of pregnancy	49%
Intrauterine growth retardation	35%
Diabetes mellitus	11%
Other	5%

*Study and control patients.

Table II. Indication for delivery

	Study group (n = 51)	*Control group (n = 51)*	*p*
Fetal indications (%)	65	20	<0.01
Maternal indications (%)	19	12	NS
Spontaneous onset of labor (%)	16	68	<0.01

middle-class patient population evaluated at the Maternal-Fetal Assessment Center of the Albert Einstein College of Medicine. Patients with congenital anomalies, postdate pregnancies, or gestational age <26 weeks were excluded from the study. The study period extended from July 1, 1985, through Jan. 31, 1989. Umbilical artery velocity waveforms were obtained with a continuous-wave Doppler device (Fetal-Flo, Medasonics, Calif., or Angioscan III, Multigon, N.Y.). The location of the umbilical cord was determined by a real-time ultrasonographic scanner, and the Doppler transducer was aimed in the direction of the umbilical cord. We accepted recordings for analysis only after obtaining a steady state of at least five consecutive pulsatile arterial waveforms. The umbilical artery flow velocity waveform was characterized by the ratio of peak systolic/end-diastolic frequencies (S/D ratio). Three S/D ratios were determined per patient, and the results were averaged. No waveforms were obtained during periods of fetal breathing movements, body movements, or cardiac arrhythmias.[6] An S/D ratio >2 SD above the mean for gestational age for this population was used to define a markedly abnormal test result.[7] The absence of end-diastolic flow was confirmed by turning off the thump filter during the examination. Alterations of end-diastolic flow were used only to determine the frequency of fetal testing. Biophysical profile testing was performed semiweekly on all patients. Patients with absent or reversed end-diastolic flow were admitted for bed rest and daily fetal testing. The following criteria were used as indications for delivery: (1) worsening maternal condition, (2) oligohydramnios (largest pocket of amniotic fluid <2 cm), (3) severe intrauterine growth retardation (IUGR) (when the sonographic estimated fetal weight was <3rd percentile for gestational age) with established lung maturity,[8] (4) biophysical profile score ≤4 (abnormal fetal heart rate was not used as a sole criterion for delivery). Patients with abnormal S/D ratios were not considered candidates for tocolysis; steroids were administered if delivery was planned or expected between 28 and 32 weeks' gestation. Outcome data were collected and included diagnosis-to-delivery interval, indication for delivery, mode of delivery, Apgar score, umbilical artery pH, gestational age at birth, birth weight, neonatal intensive care unit admission, and perinatal mortality. Gestational age was determined by the last menstrual period and an ultrasonographic examination before 22 weeks' gestation. IUGR was identified when the birth weight was below the 10th percentile for gestational age according to the Brenner curve.[9] Results were compared by Student's *t* test, Wilcoxon's test, or χ^2 as appropriate and considered significant at $p < 0.05$.

Results

During the study period 1186 singleton pregnancies were evaluated. Four hundred sixty-eight patients were excluded (17 with significant fetal anomalies and 451 with postdate pregnancies). Fifty-one patients (7%) had an S/D ratio >2 SD above the mean for gestational age. Fifteen of these patients had no end-diastolic flow; 3 of these 15 had reversed end-diastolic flow. The indications for fetal testing and delivery for the study patients and their matched controls are presented in Tables I and II, respectively. Most study patients (and therefore their matched controls) had either hypertension, diabetes, or IUGR. Study patients were significantly more likely to be delivered because of fetal indications and were therefore much less likely to experience spontaneous onset of labor.

Table III compares patient characteristics and fetal outcome for study and control patients. There were no significant differences in umbilical artery pH, Apgar score, or incidence of IUGR. However, study patients were delivered at a significantly lower gestational age, lower birth weight, and with a higher incidence of neonatal intensive care unit admissions. Table IV compares study patients with and without end-diastolic flow. There were no significant differences in umbilical artery pH, incidence of IUGR, or Apgar score <7 at 5 minutes. However, those fetuses with no end-diastolic flow had a significantly shorter test-to-delivery interval, lower mean gestational age, lower birth weight, and higher incidence of neonatal intensive care unit admissions.

There were no perinatal deaths among the study or control patients. However, there were five patients with abnormal S/D ratios who failed to follow our protocol for intensive maternal-fetal monitoring. Fetal death was confirmed within 18 days of the abnormal test. The range of S/D ratios for these patients was 4.3 to infinity.

Table III. Patient characteristics and fetal outcome

	Study group (n = 51)	*Control group (n = 51)*	*p*
S/D ratio* (mean ± SD)	5.50 ± 1.5	2.57 ± 0.52	<0.01
Test-to-delivery interval (days; median, range)	21 (0-113)	43 (0-96)	<0.01
Cesarean sections (%)	67	29	<0.01
Umbilical artery pH (mean ± SD)	7.24 ± 0.045	7.28 ± 0.05	NS
Apgar score <7 at 5 min	2	1	NS
Gestational age at birth (wk; mean ± SD)	35.4 ± 3.6	38.5 ± 2.5	<0.05
Birth weight (gm; mean ± SD)	1860 ± 713	2980 ± 560	<0.01
IUGR (<10th percentile for gestational age) (%)	70	59	NS
NICU admissions (%)	57	31	<0.05
Perinatal deaths	0	0	NS

NICU, Neonatal intensive care unit.
*To calculate this mean, an S/D ratio of 8 was assigned to all cases of S/D ratio = infinity.

Table IV. Characteristics and fetal outcome in study patients with and without end-diastolic flow

	End-diastolic flow		
	Present (n = 36)	*Absent (n = 15)*	*p*
S/D ratio (mean ± SD)	4.72 ± 0.43	Infinity	
Test-to-delivery interval (days; median, range)	24 (3-113)	4 (0-11)	<0.01
Cesarean sections (%)	63	87.5	NS
Umbilical artery pH (mean ± SD)	7.25 ± 0.07	7.21 ± 0.047	NS
Apgar score <7 at 5 min	1	1	NS
Gestational age at birth (wk; mean ± SD)	36 ± 3	33 ± 3.9	<0.05
Birth weight (gm; mean ± SD)	2002 ± 650	1376 ± 701	<0.05
IUGR (<10th percentile for gestational age) (%)	69	75	NS
NICU admissions (%)	47	80	<0.05

NICU, Neonatal intensive care unit.

All patients were hypertensive, and fetal testing at the time of the Doppler study was indicative of a nonasphyxiated fetus.

Comment

The fetal biophysical profile is commonly used for antepartum fetal assessment. Several studies have shown that a high biophysical profile score predicts fetal health with excellent accuracy.[10] Doppler velocimetry has been recently discussed as a potential means of fetal surveillance.[11-13] Several investigators have shown that abnormal umbilical artery velocity waveforms can be detected before the development of abnormalities noted on traditional fetal testing. Furthermore, recent reports have emphasized the ominous pregnancy outcome associated with markedly abnormal umbilical artery velocity waveforms.[2-5] The usefulness of fetal biophysical profile testing in patients with markedly abnormal umbilical artery velocity waveforms has not been previously assessed. The results of this study indicate that intensive monitoring of patients with very abnormal umbilical artery velocity waveforms, combined with the use of commonly accepted criteria for delivery, results in acceptable fetal outcome.

Our results demonstrate that fetuses with an abnormal umbilical artery velocity waveform are at a significantly increased risk for early delivery, decreased birth weight, and neonatal intensive care unit admission. Berkowitz et al.[14] evaluated fetal outcome among growth-retarded fetuses and have shown that those with an abnormal S/D ratio were at increased risk for early delivery, reduced birth weight, decreased amniotic fluid at birth, and neonatal intensive care unit admission. Similar results have been reported by other investigators.[15, 16]

The mean gestational age at delivery for the study patients was significantly lower than that for the control patients (35.4 ± 3.6 vs. 38.5 ± 2.5 weeks, respectively). In addition, patients with no end-diastolic flow were delivered at a significantly lower gestational age than study patients in whom some end-diastolic flow could be documented (33 ± 3.9 vs. 36 ± 3 weeks, respectively). These findings are probably related to the fact that as end-diastolic flow diminishes, the need to deliver the patient increases. The median test-to-delivery interval for study patients with documented end-diastolic flow was 24 days, compared with a median of 4 days for study patients with absent end-diastolic flow. Three

patients with reversed end-diastolic flow were all delivered on the day of the Doppler test. The same phenomenon is also reflected by the neonatal birth weights. The mean birth weights for control subjects, study patients with documented end-diastolic flow, patients with no end-diastolic flow, and patients with reversed end-diastolic flow were 2980, 2002, 1376, and 1150 gm, respectively. Thus, as the end-diastolic flow diminishes, the birth weight and the birth weight percentile are also smaller. On the basis of these data, it seems that umbilical artery diastolic flow should be considered as a continuum. This phenomenon is also time dependent. Although most study patients were delivered because of fetal or maternal indications, a few (16%) reached term and began labor spontaneously before the ill effects of the increased placental impedance could be documented by traditional end points, such as abnormal fetal testing, abnormal cord gas values, or presence of IUGR.

Should patients with no end-diastolic flow be delivered immediately? The range of diagnosis to delivery in the study group was 0 to 11 days, with a mean gestational age of 33 weeks. Although they were delivered within a median of 4 days of the abnormal test, these preterm fetuses would probably benefit from a no-intervention policy as long as other fetal testing modalities indicate fetal health. Brar and Platt[17] evaluated 31 fetuses with no end-diastolic flow whose diagnosis-to-delivery interval was 2 to 29 days and concluded that "it appears premature to intervene in a pregnancy solely on the basis of abnormal waveforms because antepartum improvement can occur." However, their perinatal mortality rate was 32.3%. The diagnosis-to-delivery interval in our study is much shorter than that reported by Brar and Platt and is the probable explanation for the fact that we report a lower perinatal death rate.

Brar and Platt[2] reported their experience with 12 fetuses who demonstrated umbilical artery reversed end-diastolic flow. Their test-to-delivery interval was 5 to 7 days and their perinatal mortality was 50%. Our experience with this group of patients is limited. Three of the study patients had reversed end-diastolic flow. These fetuses were delivered on the day of the Doppler test because of a biophysical profile score ≤4, were mildly acidotic at birth (with cord arterial pH values of 7.11, 7.14, and 7.21), were admitted to the neonatal intensive care unit, and had birth weights below the 5th percentile for gestational age. All mothers were hypertensive and one had superimposed preeclampsia. There were no perinatal deaths. Thus it seems unlikely that the duration of pregnancy can be significantly prolonged once the placental impedance is increased to the extent that it causes a rebound of blood flow during diastole.

Five study patients failed to follow our protocol for intensive fetal-maternal assessment. All five experienced intrauterine fetal death. The perinatal morbidity of the monitored study patients was zero, and their umbilical arterial pH was not statistically significant from that of the control patients (7.24 ± 0.045, range 7.11 to 7.32, vs. 7.29 ± 0.04, range 7.18 to 7.36, respectively). Furthermore, the fact that the lowest pH value obtained on the study patients was 7.11 seems to indicate that, despite the presence of very abnormal velocity waveforms, intensive monitoring combined with commonly accepted criteria for delivery was useful in preventing severe forms of fetal acidosis. The other management option would have been to deliver these fetuses immediately after documentation of a markedly abnormal umbilical arterial waveform. If this had been done, study patients would have been delivered at an undesirably low mean gestational age of 32.2 weeks instead of the actual gestational age at delivery, 35.4 weeks (Table II).

Because the purpose of our study was to evaluate commonly used fetal monitoring techniques in fetuses with abnormal umbilical artery velocity waveforms, patients with either significant congenital anomalies or gestational age <26 weeks were excluded from this study. Several investigators reported that abnormal waveforms can be detected in these patient populations.[3, 18, 19] Our reasons for exclusion of these patients seem obvious: (1) The management of significant congenital anomalies is complex and not always related to the ability to document fetal well-being; (2) we did not intend to study the efficacy of fetal surveillance before 26 weeks of gestation. Patients with postdate pregnancies were excluded from the study on the basis of our previous experience showing that "umbilical artery velocimetry is unlikely to be useful for the antenatal assessment of the postdate fetus."[20] Clearly, fetal outcome reported by investigators who include lethal congenital anomalies or gestational ages <26 weeks, or both, is expected to be less satisfactory than that reported in our study.

In conclusion, umbilical artery diastolic blood flow should be considered on a continuum. As the end-diastolic flow decreases, perinatal morbidity increases. The difficulty with delivery of every fetus with an abnormal umbilical artery velocity waveform is that these waveforms are often detected at early gestational ages when delivery may result in iatrogenic prematurity. The use of Doppler velocimetry, fetal biophysical testing, and commonly used criteria for delivery may result in a prolonged gestational age with acceptable fetal outcome.

REFERENCES

1. Giles WB, Trudinger BJ, Baird PJ. Fetal umbilical artery flow velocity waveforms and placental resistance: pathological correlations. Br J Obstet Gynaecol 1985;92:31.

2. Brar HS, Platt LD. Reverse end diastolic flow velocity on umbilical artery velocimetry in high risk pregnancies: an ominous finding with adverse pregnancy outcome. AM J OBSTET GYNECOL 1988;159:559.
3. Hsieh FJ, Chang FM, Ko TM, Chen HY, Chen YP. Umbilical artery flow velocity waveforms in fetuses dying with congenital anomalies. Br J Obstet Gynaecol 1988;95:478.
4. Woo JSK, Liang ST, Lo RLS. Significance of an absent or reversed end diastolic flow in Doppler umbilical artery waveforms. J Ultrasound Med 1987;6:291.
5. Schulman H. The clinical implications of Doppler ultrasound analysis of the uterine and umbilical arteries. AM J OBSTET GYNECOL 1987;156:889.
6. Divon MY, Guidetti DA, Braverman JJ, Oberlander E, Langer O, Merkatz IR. Intrauterine growth retardation—a prospective study of the diagnostic value of real-time sonography combined with umbilical artery flow velocimetry. Obstet Gynecol 1988;72:611.
7. Schulman H, Fleischer A, Stern W, Farmakides G, Jagani N, Blattner P. Umbilical velocity wave ratios in human pregnancy. AM J OBSTET GYNECOL 1984;148:985.
8. Guidetti DA, Divon MY, Braverman JJ, Langer O, Merkatz IR. Sonographic estimates of fetal weight in the IUGR population. Am J Perinatol 1989;6:457.
9. Brenner WE, Edelman DA, Hendricks CH. A standard of fetal growth for the United States of America. AM J OBSTET GYNECOL 1976;126:555.
10. Manning FA, Morrison I, Harman CR, Lange IR, Menticoglou S. Fetal assessment based on fetal biophysical profile scoring: experience in 19,221 referred high-risk pregnancies. II. An analysis of false-negative fetal deaths. AM J OBSTET GYNECOL 1987;157:880.
11. Trudinger BJ, Cook CM, Jones L, Giles WB. A comparison of fetal heart rate monitoring and umbilical artery waveforms in the recognition of fetal compromise. Br J Obstet Gynaecol 1986;93:171.
12. Farmakides G, Schulman H, Winter D, Ducey J, Guzman E, Penny B. Prenatal surveillance using nonstress testing and Doppler velocimetry. Obstet Gynecol 1988;71:184.
13. Schulman H, Winter D, Farmakides G, et al. Pregnancy surveillance with Doppler velocimetry of uterine and umbilical arteries. AM J OBSTET GYNECOL 1989;160:192.
14. Berkowitz GS, Mehalek KE, Chitkara U, Rosenberg J, Cogswell C, Berkowitz RL. Doppler umbilical velocimetry in the prediction of adverse outcome in pregnancies at risk for intrauterine growth retardation. Obstet Gynecol 1988;71:742.
15. Trudinger BJ, Giles WB, Cook CM. Flow velocity waveforms in the maternal uteroplacental and fetal umbilical placental circulations. AM J OBSTET GYNECOL 1985; 152:155.
16. Rochelson BL, Schulman H, Fleischer A, et al. The clinical significance of Doppler umbilical artery velocimetry in the small for gestational age fetus. AM J OBSTET GYNECOL 1987;156:1223.
17. Brar HS, Platt LD. Antepartum improvement of abnormal umbilical artery velocity: does it occur? AM J OBSTET GYNECOL 1989;160:36.
18. Reed KL, Anderson CF, Shenker L. Changes in intracardiac Doppler blood flow velocities in fetuses with absent umbilical artery diastolic flow. AM J OBSTET GYNECOL 1987;157:774.
19. Rochelson B, Schulman H, Farmakides G, et al. The significance of absent end diastolic velocity in umbilical artery velocity waveforms. AM J OBSTET GYNECOL 1987; 156:1213.
20. Guidetti DA, Divon MY, Cavalieri RL, Langer O, Merkatz IR. Fetal umbilical artery flow velocimetry in postdate pregnancies. AM J OBSTET GYNECOL 1987;157:1521.

Doppler assessment of the pulsatility index of the middle cerebral artery during constriction of the fetal ductus arteriosus after indomethacin therapy

Giancarlo Mari, MD, Kenneth J. Moise, Jr., MD, Russell L. Deter, MD, Brian Kirshon, MD, James C. Huhta, MD, Robert J. Carpenter, Jr., MD, and David B. Cotton, MD

Houston, Texas

To investigate the effects of constriction of the ductus arteriosus on the pulsatility index of the middle cerebral artery, maximum velocity waveforms were obtained in 13 fetuses (one set of twins) whose mothers were treated with indomethacin for preterm labor (n = 9) or polyhydramnios (n = 3). Eleven of the fetuses manifested ductal constriction within 48 hours of therapy, whereas two fetuses had constriction after 1 week of therapy. Six of the 13 fetuses also manifested tricuspid insufficiency in association with constriction of the ductus arteriosus. All abnormal cardiac changes resolved in utero after discontinuation of indomethacin. No difference in the pulsatility index values of the middle cerebral artery was observed in the fetuses with ductal constriction but without tricuspid regurgitation (n = 7) when the values were compared with those obtained in absence of ductal constriction (1.87 ± 0.37 vs. 1.88 ± 0.33). In the fetuses that manifested both ductal constriction and tricuspid insufficiency (n = 6), the pulsatility index values in the middle cerebral artery were significantly lower in the presence of ductal constriction when compared with the values obtained in the absence of ductal constriction (2.22 ± 0.26 vs. 1.57 ± 0.34). These results indicate that a response to indomethacin sufficient to cause both ductal constriction and tricuspid insufficiency decreases the pulsatility index of the middle cerebral artery. (AM J OBSTET GYNECOL 1989;161:1528-31.)

Key words: Fetal Doppler, fetal cerebral circulation, pulsatility index, middle cerebral artery, ductus arteriosus

Indomethacin has been used during pregnancy in the management of preterm labor[1,2] and polyhydramnios.[3,4] At our institution Doppler assessment of the fetal ductus arteriosus is performed in all women treated with indomethacin because of its ability to cause fetal ductal constriction.[5,6] The constriction of the fetal ductus arteriosus may redistribute the blood flow from the right to the left ventricle as has been reported in animal work.[7] In these circumstances an increase in blood flow to the brain could occur because of the greater amount of blood ejected by the left ventricle toward the brain.[8]

The purpose of this study was to investigate blood flow velocity waveforms in the fetal middle cerebral artery before and during indomethacin treatment to determine whether ductal constriction caused by this drug alters hemodynamic parameters in the cerebral circulation.

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, and the Lillie Abercrombie Section of Pediatric Cardiology, Department of Pediatrics, Baylor College of Medicine.

Presented at the Thirty-sixth Annual Meeting of the Society For Gynecologic Investigation, San Diego, California, March 15-18, 1989.

Reprint requests: Giancarlo Mari, MD, Department of Obstetrics and Gynecology, Division of Maternal-Fetal Medicine, One Baylor Plaza, Houston, TX 77030.

6/6/15659

Material and methods

Image-directed pulsed and continuous-wave Doppler techniques were used to obtain maximum fetal blood velocity waveforms from the ductus arteriosus, tricuspid valve, and middle cerebral artery in 13 fetuses (one set of twins) whose mothers were treated with indomethacin for preterm labor (n = 9) or polyhydramnios (n = 3). The menstrual age varied between 25 and 33 weeks (mean ± SD: 30 ± 2.5 weeks). Seven fetuses manifested ductal constriction (group A); six fetuses also manifested tricuspid insufficiency in association with constriction of the ductus arteriosus (group B). Nine fetuses were noted to have normal baseline echocardiograms with subsequent constriction of the ductus arteriosus. Baseline echocardiograms were not obtained in four fetuses. In these four cases values obtained within 4 days from the resolution of ductal constriction were used as baseline values. The indomethacin dose in all the patients was 25 mg orally every 6 hours.

Doppler ultrasonographic studies were done with a

General Electric PASS II dynamic image scanner. A 2.5 MHz transducer was used with a 2 mm sample volume. A 50 Hz high-pass filter was used. The maximal Doppler ultrasonographic energy was 114 mW/cm², spatial peak temporal average. The fetuses were examined with the mothers in a semirecumbent position.

Doppler examination of the middle cerebral artery was performed as reported previously.[9] After imaging the ductus arteriosus in a sagittal plane as described by Huhta et al.[5] and Moise et al.,[6] pulsed and continuous-wave Doppler examinations of the ductus were performed and the maximal velocities were obtained. The tricuspid valve was examined in the four-chamber view of the heart.

The Doppler velocity waveforms were recorded on videotape and a computer system was used to calculate the following variables: peak systolic velocity, peak diastolic velocity, end-diastolic velocity, and mean velocity. Three consecutive waveforms were analyzed and the results averaged. The mean pulsatility index (peak systolic velocity − end diastolic velocity/mean velocity)[10] was then calculated for the middle cerebral artery. Ductal constriction was defined as the presence of a fetal ductal systolic velocity >1.4 m/sec in conjunction with a diastolic velocity of >0.35 m/sec.[5] Tricuspid regurgitation was considered to be present if a holosystolic regurgitation wave with a velocity >2 m/sec was detected with continuous-wave Doppler. Measurements were obtained only during fetal apnea. We considered apnea to be the absence of visible changes in the waveform patterns for at least 15 waveforms.

Paired Student's *t* tests were used to compare the pulsatility index values in the middle cerebral artery in the presence and absence of ductal constriction. A probability value <0.05 was considered to indicate statistical significance.

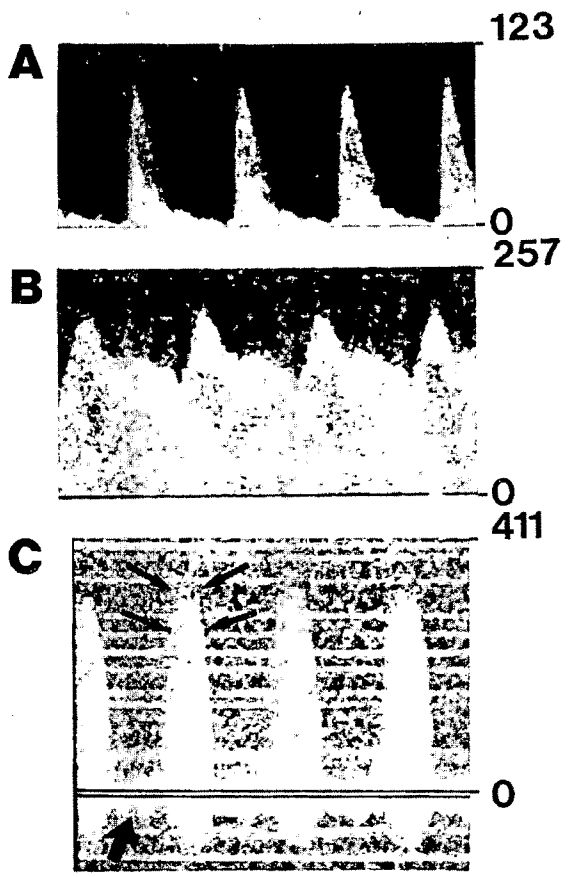


Fig. 1. Doppler waveforms of the ductus arteriosus before **(A)** and during **(B)** indomethacin treatment. (Diastolic velocity in **B** is 40 times the value recorded in **A**). Panel **C** shows the normal tricuspid valve waveform away from the transducer *(large arrow)*, with a regurgitant wave *(four arrows)* (4 m/sec) into the right atrium toward the transducer. All values are reported in centimeters per second.

Results

Eleven fetuses manifested ductal constriction within 48 hours after the initiation of therapy (Fig. 1). In two fetuses the increased blood velocity in the ductus was observed after 1 week of therapy. Ductal constriction and tricuspid insufficiency resolved in all fetuses within 48 hours of discontinuation of the therapy. No adverse fetal or neonatal effects were observed as a result of changes in ductal or tricuspid valve flow velocity waveforms.

No significant difference in the values of pulsatility index of the middle cerebral artery was seen in the seven fetuses with ductal constriction but without tricuspid insufficiency (1.87 ± 0.37 vs. 1.88 ± 0.33) (Tables I and III). A significant difference was observed in group B with both ductal constriction and tricuspid regurgitation (2.22 ± 0.26 vs. 1.57 ± 0.34; $p < 0.05$; Tables II and III). An evaluation of the power of this test indicated that differences ≥19% could have been detected with a probability of 90%.[11]

Comment

Indomethacin may cause constriction of the fetal ductus arteriosus during pregnancy in animals,[12] and the resulting pulmonary arterial hypertension may cause rapid death after delivery.[13] Recently a Doppler method has been validated for recording the blood velocity waveform in the human fetal ductus arteriosus that allows detection of ductal constriction in utero.[5, 6] From our experience in more than 500 studies of the human fetal ductus arteriosus, tricuspid insufficiency (Fig. 1, *C*) is often associated with ductal constriction when peak systolic and diastolic velocities in the Doppler waveforms of the ductus arteriosus are almost identical to those in Fig. 1, *B*. Tricuspid regurgitation probably results from elevated pressure in the right ventricular outflow tract produced by severe ductal

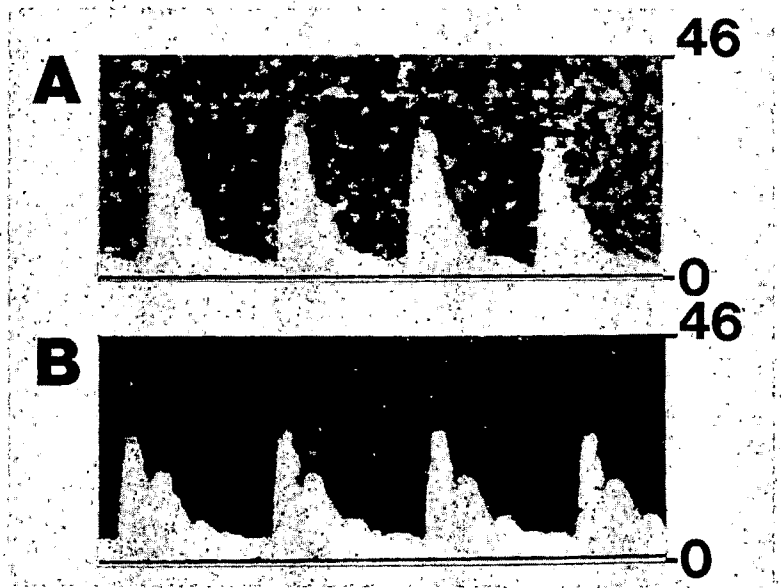


Fig. 2. Doppler waveforms of the middle cerebral artery before (**A**) and during (**B**) ductal constriction with tricuspid insufficiency.

Table I. Pulsatility index values of middle cerebral artery in seven fetuses with ductal constriction without tricuspid insufficiency

Patient No.	*Baseline index*	*Index after indomethacin treatment*
1	2.49	2.14
2	2.08	1.79
3	2.02	2.10
4	1.67	1.51
5	1.57	2.20
6	1.36	1.37
7	1.90	2.09

Table II. Pulsatility index values of middle cerebral artery in six fetuses with ductal constriction and tricuspid insufficiency

Patient No.	*Baseline index*	*Index after indomethacin treatment*
1	2.10	1.20
2	2.40	1.70
3	2.30	2.00
4	2.60	1.70
5	1.90	1.10
6	2.00	1.70

constriction that leads to mild endocardial ischemia with papillary muscle dysfunction.[14]

Our results indicate that when both ductal constriction and tricuspid insufficiency occur simultaneously, the pulsatility index of the middle cerebral artery is significantly reduced (Tables II and III; Fig. 2), but this change is not seen if only ductal constriction is present

Table III. Pulsatility index values (mean ± SD) of middle cerebral artery in the two groups of fetuses

Patient No.	*Baseline index (mean ± SD)*	*Index after indomethacin treatment (mean ± SD)*	*p Value*
7	1.87 ± 0.37	1.88 ± 0.33	NS
6	2.22 ± 0.26	1.57 ± 0.34	<0.05

NS, Not significant.

(Tables I and III). The lower pulsatility index observed in these circumstances suggests hemodynamic changes consistent with an increase of cerebral blood flow. This change in flow would be a result of a redistribution of venous return from the right to the left ventricle, as has been demonstrated experimentally in fetal dogs after ligation of the ductus arteriosus.[7] Blood ejected by the fetal left ventricle is directed principally to the head and upper trunk.[8] If an increase of venous return to the left ventricle occurs as a result of severe constriction of the ductus arteriosus, an increase in cerebral blood flow would be expected.

Such changes in the pulsatility index of the middle cerebral artery are considered by several investigators to indicate fetal hypoxia. In a longitudinal study of 14 fetuses, Woo et al.[15] assessed the velocity waveforms of the middle cerebral artery by determining the peak systolic velocity/end diastolic velocity (A/B) ratio (often used as an alternative to the pulsatility index). Hypoxic fetuses were found to have a lower A/B value. Wladimiroff et al.[16] observed that in fetuses with intrauterine growth retardation there was an increase of the end-diastolic velocity in the internal carotid artery and

a decrease of the pulsatility index when compared with normal fetuses. These studies in the human fetus are consistent with experimental studies[17] showing that hypoxia induces increased cerebral blood flow by decreasing peripheral resistance.

Patients treated with indomethacin may also have disease processes that increase the risk of fetal hypoxia. If ductal constriction and tricuspid insufficiency are present, Doppler studies of cerebral arteries may be misleading because changes consistent with hypoxia could occur in the absence of hypoxia as a result of the blood redistribution caused by ductal constriction. In such situations other indicators of hypoxia must be evaluated.

Flow velocity waveforms of the middle cerebral artery are not useful in evaluation of the effects of the indomethacin therapy on the ductus arteriosus in all cases because ductal constriction without tricuspid insufficiency does not influence the pulsatility index of the middle cerebral artery.

In summary, when the middle cerebral artery of a fetus whose mother takes indomethacin is evaluated, it is fundamental to exclude ductal constriction and tricuspid insufficiency because these conditions may affect changes in the Doppler waveforms obtained from the cerebral circulation that could be incorrectly interpreted to represent fetal hypoxia. The decrease of the pulsatility index of the middle cerebral artery that we observed suggests, but does not prove, an increase in blood flow to the brain if indomethacin produces severe ductal constriction. Discontinuation of the therapy is not followed by adverse fetal or neonatal effects.

REFERENCES

1. Zuckerman H, Reiss U, Rubinstein I. Inhibition of human premature labor by indomethacin. Obstet Gynecol 1974; 44:787-92.
2. Niebyl JR, Blake DA, White RD, et al. The inhibition of premature labor with indomethacin. AM J OBSTET GYNECOL 1980;136:1014-9.
3. Cabrol D, Landesman R, Muller J, Uzan M, Sureau C, Saxena BB. Treatment of polyhydramnios with prostaglandin synthetase inhibitor (indomethacin). AM J OBSTET GYNECOL 1987;157:422-6.
4. Kirshon B, Cotton DB. Polyhydramnios associated with a ring chromosome managed with indomethacin. AM J OBSTET GYNECOL 1988;158:1063-4.
5. Huhta JC, Moise KJ, Fisher DJ, et al. Detection and quantitation of constriction of the fetal ductus arteriosus by Doppler echocardiography. Circulation 1987;75:406-12.
6. Moise KJ, Huhta JC, Sharif DS, et al. Indomethacin in the treatment of premature labor: effects on the fetal ductus arteriosus. N Engl J Med 1988;319:327-31.
7. Haller JA, Morgan WW, Rodgers BM, Gengos DG, Margulies SI. Chronic hemodynamic effects of occluding the fetal ductus arteriosus (pathophysiologic and cineradiographic studies in fetal dogs). J Thorac Cardiovasc Surg 1967;54:770-6.
8. Rudolph AM. Distribution and regulation of blood flow in the fetal and neonatal lamb. Circ Res 1985;57:811-21.
9. Mari G, Moise KJ, Deter RL, Kirshon B, Carpenter RJ, Huhta JC. Doppler assessment of the pulsatility index in the cerebral circulation of the human fetus. AM J OBSTET GYNECOL 1989;160:698-703.
10. Gosling RG, King DH. Ultrasound angiology. In: Marons AW, Adamson L, eds. Arteries and veins. Edinburgh: Churchill Livingstone, 1975:61-98.
11. Bayer W. CRC Handbook of tables for probability and statistics. 2nd ed. Cleveland:Chemical Rubber Co, 1968:287.
12. Friedman WR, Printz MP, Kirkpatrick SE, Hoskins EJ. The vasoactivity of the fetal lamb ductus arteriosus studied in utero. Pediatr Res 1983;17:331-7.
13. Levin DL, Millis LJ, Parkey M, Garriott J, Campbell W. Constriction of the fetal ductus arteriosus after administration of indomethacin to the pregnant ewe. J Pediatr 1979;94:647-50.
14. Klein KL, Scott WJ, Clark KE, et al. Indomethacin-placental transfer, cytotoxicity and teratology in the rat. AM J OBSTET GYNECOL 1981;141:448-52.
15. Woo JK, Liang ST, Lo RS, Chan FY. Middle cerebral artery Doppler flow velocity waveforms. Obstet Gynecol 1987;70:613-6.
16. Wladimiroff JW, Tonge HM, Stewart PA. Doppler ultrasound assessment of cerebral blood flow in the human fetus. Br J Obstet Gynaecol 1986;93:471-5.
17. Cohn HE, Sacks EJ, Heymann MA, Rudolph AM. Cardiovascular response to hypoxemia and acidemia in fetal lambs. AM J OBSTET GYNECOL 1974;120:817-24.

Human fetal tricuspid and mitral deceleration time: Changes with normal pregnancy and intrauterine growth retardation

Kathryn L. Reed, MD, Christopher P. Appleton, MD, David J. Sahn, MD, and Caroline F. Anderson, RDMS

Tucson, Arizona, and San Diego, California

Studies in instrumented adults have shown an association between Doppler echocardiography–derived atrioventricular valve deceleration times and ventricular function. To determine how gestational age affects deceleration time in the tricuspid and mitral valve, pulsed Doppler ultrasonographic examinations were performed in 54 normal human fetuses. In addition, 26 fetuses with growth retardation and the absence of end-diastolic Doppler velocities in the umbilical artery were examined. Measurements were obtained from strip chart recordings; between three and five beats were used to obtain an average heart rate, ratio of peak velocity at atrial contraction to peak velocity in early diastole, and deceleration time in early diastole. Deceleration times increased during gestation from 86 ± 27 to 116 ± 18 msec in the tricuspid valve and from 98 ± 25 to 132 ± 21 msec in the mitral valve. Fetuses with growth retardation and the absence of end-diastolic Doppler velocities in the umbilical artery had longer deceleration times than those of normal fetuses ($p < 0.01$). The increase in deceleration time across both atrioventricular valves in normal fetuses may be related to heart rate, an increased rate of ventricular relaxation, or an increase in ventricular compliance. The fetuses with intrauterine growth retardation and the absence of end-diastolic velocities in the umbilical artery have abnormally increased deceleration times; in the abnormal fetus this may be a result of impaired ventricular relaxation or decreased ventricular compliance. (AM J OBSTET GYNECOL 1989;161:1532-5.)

Key words: Doppler echocardiography, human fetuses, cardiac physiology

Studies in instrumented adults have shown an association between Doppler echocardiography–derived mitral and tricuspid flow velocity deceleration times and ventricular function. Mitral deceleration time correlates with the maximal negative rate of fall of pressure, the height of the left ventricular rapid filling wave ($-dP/dt$), pulmonary wedge pressure, left ventricular systolic pressure, and left ventricular end-diastolic pressure.[1] In addition, mitral valve deceleration time correlates with the rate of left ventricular relaxation.[2]

We studied Doppler echocardiography–derived deceleration times across the tricuspid and mitral valves in normal human fetuses and fetuses with intrauterine growth retardation (IUGR) and the absence of end-diastolic velocities in the umbilical artery (AEDV). The study was performed to establish the changes that occur during gestation and the changes that occur in the presence of fetal cardiac dysfunction associated with IUGR. AEDV is associated with IUGR and increased perinatal morbidity and mortality.[3-5] Fetuses with AEDV were found to have alterations in cardiac function as determined by Doppler ultrasonographic measurements of volume flow and mitral valve velocity waveforms.[4]

We hypothesized that inasmuch as fetal cardiac function differs from adult cardiac function, the Doppler echocardiography–derived mitral and tricuspid flow velocity deceleration times in the fetus should vary from normal adult values. Both animal[6] and human[7] studies have shown that cardiac function changes with age; therefore, deceleration time should lengthen with advancing gestation as heart rate decreases and the myocardium matures. In addition, because fetal heart rate is faster, deceleration time may be shorter than in the adult. Finally, changes in deceleration time would be predicted in association with the alterations in cardiac function in fetuses with abnormalities of umbilical artery velocity that accompany presumed increases in placental resistance.

Methods

Fetuses with suspected IUGR or suspected anomalies were subjects of this study. Approval for performance of Doppler ultrasonographic studies of the fetuses was obtained from the Human Subjects Committee, and

From the Departments of Obstetrics and Gynecology and Internal Medicine, Arizona Health Sciences Center, and the Department of Pediatrics (Cardiology), University of California at San Diego.

Supported in part by a grant from the American Heart Association, Arizona affiliate.

Presented in part at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.

Reprint requests: Kathryn L. Reed, MD, Department of Obstetrics and Gynecology, Arizona Health Sciences Center, 1501 N. Campbell Ave., Tucson, AZ 85724.

6/6/15667

Table I. Atrioventricular valve deceleration times in normal fetuses

	Gestational weeks				
	20 to 27 (n = 14)	*28 to 31 (n = 14)*	*32 to 35 (n = 16)*	*36 to 39 (n = 10)*	*p Value*
Tricuspid valve heart rate (beats/min)	144.8 ± 11.6	140.7 ± 8.9	136.1 ± 10.6	132.8 ± 10.4	NS*
Mitral valve heart rate (beats/min)	142.2 ± 11.6	141.4 ± 10.7	137.4 ± 10.9	132.2 ± 15.1	NS
Tricuspid valve A/E ratio	1.45 ± 0.23	1.29 ± 0.08	1.26 ± 0.13	1.20 ± 0.12	<0.01
Mitral valve A/E ratio	1.45 ± 0.29	1.28 ± 0.22	1.18 ± 0.19	1.11 ± 0.17	<0.01
Tricuspid valve deceleration time (msec)	86 ± 27	89 ± 26	115 ± 27	116 ± 18	<0.01
Mitral valve deceleration time (msec)	98 ± 25	103 ± 22	124 ± 23	132 ± 21	<0.01

Values are reported as mean ± SD.
*p Values compare fetuses at 36 to 39 weeks with fetuses at 20 to 27 weeks.

individual mothers gave informed consent. Fetuses underwent two-dimensional examinations for establishment of fetal size and anatomy, location of the placenta, and amount of amniotic fluid. Normally grown fetuses with normal umbilical artery Doppler flow velocity waveforms and without congenital anomalies were subsequently defined as normal. Pulsed Doppler flow velocity tracings were obtained from the tricuspid valve and mitral valve in 54 normal fetuses and 26 fetuses with IUGR and the absence of end-diastolic Doppler velocities in the umbilical artery. Strip chart recordings obtained during the fetal examinations were measured with a digitizing board. Measurements performed included heart rate, peak velocity during early diastole (E), peak velocity during atrial contraction (A), ratio of peak velocity during atrial contraction to peak velocity during early diastole (A/E ratio), and deceleration time. Deceleration time was measured from the peak velocity during early diastole to the time the slope of the deceleration curve intersected the zero line. Values from three cardiac cycles were averaged for each fetus (Fig. 1). For two-dimensional studies, 3.5 and 5.0 MHz transducers were used; 3.5 MHz transducers were used for Doppler ultrasonographic studies.

Gestational age was established with menstrual dates and ultrasonographic measurements. For the purposes of analysis, normal fetuses were divided into four groups: those between 20 and 27 weeks' gestation, 28 to 31 weeks' gestation, 32 to 35 weeks' gestation, and 36 to 39 weeks' gestation. In addition, measurements from the group of fetuses with IUGR and the absence of end-diastolic velocities in the umbilical artery were compared with measurements from the entire group of normal fetuses. Growth retardation was defined as less than the tenth percentile birth weight by institutional standards.

Statistical analysis consisted of comparison of group means with the Student t test, and correlation of values with regression analysis; p values of 0.05 were considered significant.

Table II. Correlations between atrioventricular valve Doppler echocardiographic measurements, heart rate, and gestational age

	R values	*p Values*
Tricuspid valve A/E ratio versus tricuspid valve deceleration time	−0.31	<0.05
Mitral valve A/E ratio versus mitral valve deceleration time	−0.41	<0.01
Tricuspid valve A/E ratio versus heart rate	0.53	<0.001
Mitral valve A/E ratio versus heart rate	0.53	<0.001
Tricuspid valve A/E ratio versus gestational age	−0.56	<0.001
Mitral valve A/E ratio versus gestational age	−0.53	<0.001
Tricuspid valve heart rate versus gestational age	−0.44	<0.01
Mitral valve heart rate versus gestational age	−0.38	<0.01
Tricuspid valve deceleration time versus heart rate	−0.42	<0.01
Mitral valve deceleration time versus heart rate	−0.59	<0.001
Tricuspid valve deceleration time versus gestational age	0.46	<0.001
Mitral valve deceleration time versus gestational age	0.53	<0.001
Tricuspid valve A/E ratio versus mitral valve A/E ratio	0.65	<0.001
Tricuspid valve deceleration time versus mitral valve deceleration time	0.69	<0.001

Results

Changes in heart rate, tricuspid and mitral valve A/E ratios, and tricuspid and mitral valve deceleration times in the four groups of fetuses divided by gestational age are reported in Table I. Correlations between tricuspid and mitral valve A/E ratios, heart rates, gestational age, and deceleration times are shown in Table II. Table III compares measurements from the group of fetuses with IUGR and AEDV with the entire group of normal fetuses. The average gestational age of the group with IUGR and AEDV was 30.8 ± 4.6



Fig. 1. Mitral valve Doppler flow velocity tracing in 34-weeks' gestation fetus with absence of end-diastolic velocity in umbilical artery. Deceleration time is measured from time of peak velocity during early diastole to time of intersection of deceleration slope with zero line. Dots are 0.5 seconds apart in horizontal plane.

Table III. Comparison of normal fetuses and fetuses with IUGR and absence of end-diastolic velocities

	All normal fetuses n = 54	*Fetuses with IUGR and absence of end-diastolic velocities n = 26*	*p Value*
Triscuspid valve heart rate (beats/min)	138.9 ± 11.0	143.3 ± 8.9	NS
Mitral valve heart rate (beats/min)	138.7 ± 12.1	142.6 ± 8.9	NS
Tricuspid valve peak velocity during early diastole (cm/sec)	35.1 ± 6.8	31.0 ± 10.0	NS
Tricuspid valve peak velocity during atrial contraction (cm/sec)	44.9 ± 7.8	39.4 ± 11.3	<0.05
Mitral valve peak velocity during early diastole (cm/sec)	32.4 ± 7.5	27.5 ± 7.8	<0.01
Mitral valve peak velocity during atrial contraction (cm/sec)	39.9 ± 8.8	31.2 ± 10.7	<0.001
Tricuspid valve A/E ratio	1.31 ± 0.17	1.30 ± 0.21	NS
Mitral valve A/E ratio	1.26 ± 0.25 (p = NS)	1.10 ± 0.24 ($p < 0.01$)	<0.01
Tricuspid valve deceleration time (msec)	101 ± 28	119 ± 18	<0.001
Mitral valve deceleration time (msec)	113 ± 26 ($p < 0.05$)	127 ± 17 (p = NS)	<0.01

weeks, compared with the average gestational age of the normal group of 31.1 ± 4.7 weeks (p = NS).

In fetuses with IUGR and AEDV between 20 and 27 weeks' gestation, tricuspid valve deceleration time (138 ± 31 msec, $n = 5$) was longer than normal ($p < 0.01$), and mitral valve deceleration time (127 ± 18 msec, $n = 5$) was longer than normal ($p < 0.02$). Tricuspid valve peak velocity during early-diastolic filling (26.8 ± 3.3 cm/sec) was less than normal ($p < 0.05$), as was tricuspid valve peak velocity during atrial contraction (34.9 ± 9.5 cm/sec, $p < 0.05$). Mitral valve peak velocity during early-diastolic filling was also lower than normal (20.8 ± 4.2 cm/sec, $p < 0.01$), as was mitral valve peak velocity during atrial contraction (24.3 ± 9.9 cm/sec, $p < 0.01$).

Comment

Cardiovascular physiology in the fetus is usually examined with the use of noninvasive methods and this necessarily limits the amount of information obtained.

Information about blood pressure and resistance are only estimated; volume flow can be calculated but has a large component of error.[8] Studies that correlate information derived invasively from the instrumented adult to information derived noninvasively by Doppler echocardiography were extrapolated to the fetus. In a recent study by Appleton and colleagues,[1] left ventricular function was examined in the adult by comparing pressure and flow data with various Doppler echocardiography–derived indices from mitral flow velocity recordings obtained simultaneously. Mitral flow velocity deceleration times were correlated with ventricular diastolic properties including the rate of relaxation of the left ventricle. In general, mitral deceleration time was correlated with the maximal negative rate of fall of pressure ($-dP/dt$), the height of the left ventricular rapid filling wave, pulmonary wedge pressure, left ventricular systolic pressure, and left ventricular end-diastolic pressure. In addition, at relatively normal atrial filling pressures, deceleration time was also related to the time constant of isovolumic relaxation.[1]

In this study of normal fetuses, tricuspid and mitral flow velocity deceleration times were shortened as compared with adults. This may be partly because the adult heart rate averaged 65 beats/min in the study that reported a normal mitral valve deceleration time in adults of 193 ± 23 msec,[1] although the effects of heart rate on deceleration time have been only preliminarily studied. Atrioventricular valve deceleration time increased with gestational age and decreased with A/E ratio and heart rate. Tricuspid and mitral valve A/E ratios increased with heart rate and decreased with deceleration time and gestational age.

Human fetal life can be complicated by the development of growth retardation and later neonatal difficulties. Pulmonary hypertension is a known complication of the chronic intrauterine hypoxia associated with severe growth retardation. Fetuses with the absence of end-diastolic velocities in the umbilical artery were shown to have a high incidence of cardiac chamber dilatation, wall hypertrophy, and pulmonary hypertension in a preliminary study.[5] Perinatal morbidity and mortality were increased in the group of fetuses with the absence of end-diastolic velocity in the umbilical artery,[3-5] and cardiac function has been studied in this group.[4] These studies showed an increase in the relative amount of blood that flows through the right ventricle and pulmonary artery (2.15 : 1), compared with that in the left ventricle and aorta in normal fetuses (1.33 : 1).[4] In addition, a lower mitral valve A/E ratio was also shown, which suggests that changes had occurred in ventricular filling pressures or left ventricular diastolic function. Doppler echocardiographic measurements from fetuses with AEDV, an extreme of umbilical artery velocity abnormality, were therefore used in this report to compare with measurements from normal fetuses.

In fetuses with AEDV, tricuspid and mitral flow velocity deceleration times were longer than normal. This may occur as a result of a slower relaxation time in the ventricles of these fetuses. The mitral valve A/E ratio during early diastole ratio was lower than the tricuspid valve ratio. This is because peak velocities were decreased both during early diastole and during atrial contraction. Both peak velocities may be decreased in response to lower volume flow through the left side of the fetal heart with AEDV.[4]

We conclude that in the normal fetus, Doppler echocardiography–derived mitral and triscupid flow velocity deceleration times are shorter than those in normal adults and increase during gestation. In fetuses with IUGR and AEDV, deceleration time is abnormally prolonged. Whereas these changes may occur in part as a result of a decrease in heart rate, changes in ventricular chamber or myocardial compliance may also explain the lengthening of deceleration time in normal fetuses with advancing gestation. The changes in fetuses with IUGR and AEDV most likely result from a prolongation of ventricular relaxation, perhaps because afterload increases with placental resistance.

REFERENCES

1. Appleton CP, Hatle LK, Popp RL. Relationship of transmitral flow velocity patterns to left ventricular diastolic function: new insights from a combined hemodynamic and Doppler echocardiographic study. J Am Coll Cardiol 1988;12:426-40.
2. Courtois M, Kovacs SJ, Ludbrook PA. Transmitral pressure–flow velocity relation. Circulation 1988;78:661-71.
3. Rochelson B, Schulman H, Farmakides G, et al. The significance of absent end-diastolic velocity in umbilical artery waveforms. AM J OBSTET GYNECOL 1987;156:1213-8.
4. Reed KL, Anderson CF, Shenker L. Changes in intracardiac Doppler blood flow velocities in fetuses with absent end-diastolic flow. AM J OBSTET GYNECOL 1987;157:774-9.
5. Mattice K, Anderson CF, Borjon ND, Reed KL. Neonatal outcome in fetuses with absent end-diastolic Doppler velocities in the umbilical artery. Clin Res 1989;37: 99A.
6. Romero T, Covell J, Friedman WF. A comparison of pressure-volume relations of the fetal, newborn and adult heart. Am J Physiol 1972;222:1285-90.
7. Reed KL, Sahn DJ, Scagnelli S, et al. Doppler echocardiographic studies of diastolic function in the human fetal heart: changes during gestation. J Am Coll Cardiol 1986; 8:391-5.
8. Reed KL, Meijboom EJ, Sahn DJ, et al. Cardiac Doppler flow velocities in human fetuses. Circulation 1986;73: 41-6.

Effect of placental laterality on uterine artery resistance and development of preeclampsia and intrauterine growth retardation

Alexander D. Kofinas, MD, Mary Penry, RN, Melissa Swain, RN, and Christos G. Hatjis, MD

Winston-Salem, North Carolina

We studied 153 pregnant women with normal pregnancies and 147 women with complicated pregnancies (diabetes, hypertensive disorders, and intrauterine growth retardation) to evaluate the association of placental location and the development of preeclampsia, intrauterine growth retardation, and uterine artery resistance. The placental location was determined by real-time ultrasonography, and the uterine artery resistance was determined by continuous-wave Doppler flow velocity waveform analysis. In the presence of preeclampsia or intrauterine growth retardation, up to 75% of the patients had unilaterally located placentas and 25% central placentas, whereas in the absence of these two conditions only 51% of the patients had unilateral and 49% central placentas ($p < 0.02$). In patients with unilateral placentas, the incidence of preeclampsia and intrauterine growth retardation was 2.8-fold and 2.7-fold greater than in patients with central placentas ($p < 0.03$ and $p < 0.01$). Among all patients unilateral placental location was more likely to be associated with abnormal uterine artery flow velocity waveforms than central placental location ($p < 0.001$). We conclude that unilateral placental location may predispose to the development of preeclampsia and intrauterine growth retardation by its effect on uterine artery resistance. (AM J OBSTET GYNECOL 1989;161:1536-9.)

Key words: Placenta, preeclampsia, intrauterine growth retardation, flow velocity waveform analysis

The significance of placental location in the uterine cavity has been studied extensively. Placental location has been found to correlate with fetal position and presentation,[1,2] length of gestation,[3] course of labor,[4] presence of preeclampsia,[5,6] and pregnancy outcome.[7] Several methods have been used to document placental location, including manual exploration of the uterus, soft tissue x-ray films, and isotopic placentography.[1,8-11] In the past two decades ultrasonography has proved to be the safest, easiest, and most accurate method for assessing placental location.[2,12,13]

The relationship between placental location and the development of preeclampsia has been examined previously with controversial results.[5,6] In the two previous reports the placental location was examined with regard to its proximity to the uterine fundus. The two groups used different methods. There has been no study to examine the clinical significance of placental laterality and its association with the development of preeclampsia and intrauterine growth retardation (IUGR). There is a significant association between placental location and uterine artery resistance.[14] It is possible that placental location may influence uterine artery blood flow distribution and predispose the pregnancy to such adverse outcomes as preeclampsia, premature birth, and IUGR. This study was designed to examine the association of placental laterality and uterine artery resistance and the development of preeclampsia and IUGR.

Table I. Patient characteristics according to placental location

	Placenta location		
	Central	*Unilateral*	*p Value*
Maternal age (mean ± SD)	23.9 ± 6.0	23.9 ± 5.3	1.00
Gravidity (mean ± SD)	2.0 ± 1.1	1.9 ± 1.2	0.92
Parity (mean ± SD)	0.6 ± 0.8	0.6 ± 0.8	0.84

Material and methods

A total of 311 patients were enrolled in the study. Eleven were excluded because of insufficient pregnancy outcome information. Of the remaining 300 patients, 153 had normal uncomplicated pregnancies, and 147 patients had pregnancies complicated by diabetes

From the Section on Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Bowman Gray School of Medicine of Wake Forest University.

Presented in part at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.

Reprint requests: Alexander D. Kofinas, MD, Department of Obstetrics and Gynecology, Bowman Gray School of Medicine, 300 South Hawthorne Road, Winston-Salem, NC 27103.

6/6/15651

Table II. Relationship of placental laterality and presence or absence of preeclampsia, IUGR, or both

	Preeclampsia		*IUGR*		*Preeclampsia, IUGR, or both*	
Placental location	*Yes*	*No*	*Yes*	*No*	*Yes*	*No*
Central	9 (26%)	129 (49%)	11 (25%)	127 (49%)	16 (28%)	117 (48%)
Unilateral	25 (74%)	137 (51%)	30 (75%)	132 (51%)	41 (72%)	126 (52%)
χ^2 test	$p < 0.03$		$p < 0.02$		$p < 0.007$	

mellitus ($n = 39$), hypertensive disorders ($n = 69$), and pregnancies suspected of IUGR ($n = 39$). Of the 39 women with diabetes 18 had gestational diabetes and 21 had insulin-dependent diabetes. The normal patients were recruited from a population referred to the ultrasonography unit for pregnancy dating and from the low-risk prenatal clinic. The high-risk patients were recruited from a population referred to the antenatal testing unit for fetal surveillance. All high-risk patients were examined during the third trimester of pregnancy. The majority of the normal patients were also examined during the third trimester, and the remainder were examined between 24 and 28 weeks of gestation. The study was approved by the institution's Clinical Research Practices Committee; all patients gave written informed consent.

Diagnosis and classification of the hypertensive disorders were according to criteria of the American College of Obstetricians and Gynecologists.[15] Growth charts appropriate for our population were used to classify the infants as small for gestational age or appropriate for gestational age.[16]

The placental location was determined by real-time ultrasonography. The method has been previously described.[14] In brief, the placenta was classified as right, left, or central regardless of its anteroposterior and fundal position. For the purpose of analysis the patients with right or left placentas were classified as having unilaterally located placentas. Placental location was evaluated without prior knowledge of the flow velocity waveforms. The interobserver variation in assignment of placental location was 13%.

The flow velocity waveforms were obtained with the mother lying comfortably in a slight left lateral tilt according to method that has been described previously.[14] A 4 MHz continuous-wave Doppler device equipped with a real-time spectrum analyzer was used to obtain the uterine artery flow velocity waveforms. Briefly, two measurements were obtained from each side. The average of the two measurements on each side is classified as right uterine artery flow velocity waveform and left uterine artery flow velocity waveform, respectively. When necessary for comparisons, the mean of the right and left uterine arteries is classified as the uterine artery flow velocity waveforms. The uterine artery flow velocity waveforms were classified as abnormal if they exceeded the 95th percentile of our normal values (systolic/diastolic ratio >2.8 after 24 weeks' gestation). The intraobserver coefficient of variation for the flow velocity waveforms was 8%. Statistical significance of the results was evaluated by the χ^2 test. A value of $p < 0.05$ was considered statistically significant.

Table III. Incidence of preeclampsia and IUGR in study group according to placental location

	Placental location	
	Central	*Unilateral*
Presence of preeclampsia		
Yes	9 (6.5%)	25 (15.4%)
No	129 (93.5%)	137 (84.6%)
	χ^2, $p < 0.03$	
Presence of IUGR		
Yes	11 (8%)	30 (18.5%)
No	127 (92%)	132 (81.5%)
	χ^2, $p < 0.01$	

Results

Data from a total of 300 patients were used for analysis. There was no difference in the incidence of laterality between pregnancies <28 weeks' gestation and those of >28 weeks or between white and nonwhite patients. Maternal characteristics are listed in Table I. The incidence of the different placental locations with regard to laterality in the entire group was as follows: central, 46%; right-sided, 33%; left-sided, 21%. Thus the incidence of unilateral placentation was 54%. The incidence of unilateral placentation in the normal group was not statistically different from the group of high-risk patients (52% vs. 56%). However, when the patients with preeclampsia and/or IUGR were grouped together and compared with the rest of the patients, significant associations and differences were found (Table II).

Patients with unilateral placental location had a 2.8-fold higher increase in the incidence of preeclampsia than patients with centrally located placentas ($p < 0.03$). Similarly, patients with unilateral placental location had a 2.7-fold increase in the incidence of IUGR than patients with centrally located placenta ($p < 0.01$, Table III).

Table IV. Placental laterality according to uterine artery flow velocity wave forms

	Right or left uterine artery		*Mean of both sides*	
Placental location	*Abnormal*	*Normal*	*Abnormal*	*Normal*
Central	20 (27%)	118 (52%)	16 (30%)	122 (49%)
Unilateral	55 (73%)	107 (48%)	37 (70%)	125 (51%)
χ^2 test	$p < 0.001$		$p < 0.02$	

Regardless of which vessel was examined (right or left uterine artery or the mean of the two sides), 70% to 73% of patients with abnormal flow velocity waveforms had unilaterally located placentas (Table IV). In the presence of preeclampsia and abnormal uterine artery flow velocity waveforms ($n = 20$), 16 (80%) of the patients had unilateral placentas and only four (20%) had centrally located placentas. In the presence of IUGR with abnormal uterine artery flow velocity waveforms ($n = 18$), 15 (83%) of the patients had unilateral placentas and only three (17%) had central placentas. In the group of diabetic patients five (13%) developed preeclampsia; four of those five patients (80%) had unilateral placentas and only one (20%) had a central placenta.

Comment

This study provides evidence of the existence of a significant association between placental laterality and the presence of preeclampsia, IUGR, or both. It also appears that the placenta is located laterally in the majority of patients with abnormal uterine artery flow velocity waveforms.

The association of placental location and the development of preeclampsia has been examined twice with contradictory results.[5,6] Both reports examined the placental location with regard to the proximity of the placenta to the fundus. Booth et al.[5] found a significant association between fundal placentas and the presence of preeclampsia; they explained it by Bieniarz's theory. Bieniarz[17] theorized that when the placenta is located high in the uterine cavity, the draining of the uteroplacental circulation might cause a pathologic redistribution of blood in the renal and visceral circulation, resulting in preeclamptic toxemia. In contrast, Little and Friedman[6] failed to find any association between highly implanted placentas and preeclampsia.

Preeclampsia and IUGR are both conditions strongly related to poor placental development and function. Khong et al.[18] showed that poor trophoblastic conversion of the spiral arterioles leads to development of preeclampsia, IUGR, or both conditions. It has been shown in humans that both uterine arteries have a significant number of branches and that each supplies the corresponding uterine side. In some patients arcuate branches of the uterine artery cross over to the other side and create major anastomoses.[19] Although anatomic anastomoses between the two uterine arteries exist, there is no proof that these anastomoses are functional.

When the placenta is laterally located, the uterine artery close to the placenta has lower resistance than the one opposite from it. In patients with centrally located placentas both uterine arteries demonstrate similar resistance.[14,20] It is possible that when the placenta is centrally located, the uteroplacental blood flow needs are met by equal contribution from both uterine arteries. However, when the placenta is laterally located, in the majority of patients the uteroplacental blood flow needs are to be met primarily by one of the uterine arteries, with some contribution by the other uterine artery via collateral circulation. The degree of collateral contribution, however, may not be the same in all patients and deficient contribution may facilitate the development of preeclampsia, IUGR, or both. The existence of major vascular anastomoses in some patients may explain the normal uterine artery flow velocity waveforms and the absence of preeclampsia and IUGR despite the presence of a unilateral placenta.

The pathophysiologic characteristics of preeclampsia are complex and the cause remains unknown. One of the fundamental disturbances in patients with this condition is decreased uteroplacental blood flow. However, whether this is the cause or the result of preeclampsia is yet to be discovered. Our data indicate that the presence of a unilateral placenta and abnormal uterine artery flow velocity waveforms (suggestive of high uterine artery resistance) is strongly associated with preeclampsia. Studies are currently being conducted both in the United States and abroad to evaluate the impact of aspirin in the prevention of preeclampsia. Further studies are necessary to evaluate the sensitivity of placental laterality and abnormal uterine artery flow velocity waveforms as a predictor of the development of preeclampsia. If this proves to be the case, the use of aspirin in those patients who are at increased risk will help prevent or at least ameliorate some of the severe consequences of preeclampsia.

In summary, this study is the first to demonstrate a significant relationship between placental laterality and the presence of preeclampsia, IUGR, or both. There is strong evidence to indicate that patients with unilateral

placentation are more likely to have abnormal uterine artery flow velocity waveforms indicative of high uterine artery resistance. It is speculated that insufficient collateral blood supply in patients with unilateral placentation may facilitate the development of preeclampsia, IUGR, or both. Further study of this relationship is needed to evaluate its sensitivity as a screening tool for patients at risk to develop preeclampsia or IUGR.

REFERENCES

1. Kian LS. The role of the placental site in the aetiology of breech presentation. J Obstet Gynaecol Br Commonw 1963;70:795.
2. Hoogland HJ, deHaan J. Ultrasonographic placental localization with respect to fetal position in utero. Eur J Obstet Gynecol Reprod Biol 1980;11:9.
3. Harris VG. Relation between placental site and length of gestation. Br J Obstet Gynaecol 1975;82:581.
4. Davydov SN, Orlov VM, Samorodinova LA, Khrustalkov SV. Location of placenta and clinical course of labour. Acta Chir Hung 1987;28:3.
5. Booth RT, Wood C, Beard RW, Gibson JRM, Pinkerton JHM. Significance of site of placental attachment in uterus. Br Med J 1962;1:1732.
6. Little WA, Friedman EA. Significance of the placental position. Obstet Gynecol 1964;23:804.
7. Chapman MG, Furness ET, Jones WR, Sheat JH. Significance of the ultrasound location of placental site in early pregnancy. Br J Obstet Gynaecol 1979;86:846.
8. Vrettos AS, Megapanos E, Costamis P, Binopoulos D. Isotopic placentography using Cr^{51}-tagged erythrocytes. Am J Obstet Gynecol 1965;93:957.
9. Davison TW, Ewing KL, Sayat N, Mulla NP, Fergason JL. Liquid crystal thermographic placental location. Obstet Gynecol 1973;42:574.
10. Thompson W, Bell TK, Pinkerton JHM. Location of placenta by means of a colour autoscan technique. Br Med J 1967;4:390.
11. Townsend DE, Swanson LA. Determination of placental location with technetium-99M. Am J Obstet Gynecol 1969;104:560.
12. Kobayashi M, Hellman LM, Fillisti L. Placental localization by ultrasound. Am J Obstet Gynecol 1970;106:279.
13. Badria L, Young GB. Correlation of ultrasonic and soft tissue x-ray placentography in 300 cases. J Clin Ultrasound 1976;4:403.
14. Kofinas AD, Penry M, Greiss FC Jr, Meis PJ, Nelson LH. The effect of placental location on uterine artery flow velocity waveforms. Am J Obstet Gynecol 1988;159:1504.
15. American College of Obstetricians and Gynecologsts. Management of preeclampsia. Washington, DC: American College of Obstetricians and Gynecologists, 1986; ACOG technical bulletin no. 91.
16. Brenner WE, Edelman DA, Hendricks CH. A standard of fetal growth for the United States of America. Am J Obstet Gynecol 1976;126:555.
17. Bieniarz J. The patho-mechanism of late pregnancy toxemia and obstetrical hemorrhages. II. Placental site and venous drainage of the pregnant uterus. Am J Obstet Gynecol 1959;78:385.
18. Khong TY, DeWolf F, Robertson WB, Brosens I. Inadequate maternal vascular response to placentation in pregnancies complicated by pre-eclampsia and by small-for-gestational age infants. Br J Obstet Gynaecol 1986; 93:1049.
19. Itskovitz J, Lindenbaum ES, Brandes JM. Arterial anastomosis in the pregnant human uterus. Obstet Gynecol 1980;55:67.
20. Campbell S, Bewbey S, Cohen-Overbeek T. Investigation of the utero-placental circulation by Doppler ultrasound. Semin Perinatol 1987;11:362.

Ultrasonographic criteria for the prenatal diagnosis of placental chorionicity in twin gestations

Hung N. Winn, MD,[a] Sandro Gabrielli, MD,[a] E. Albert Reece, MD,[a] J. Andres Roberts, MD,[a] Carolyn Salafia, MD,[b] and John C. Hobbins, MD[a]

New Haven, Connecticut

Thirty-two patients with uncomplicated twin pregnancies had ultrasonographic examinations for genetic amniocentesis, confirmation of twinning, or assessment of fetal growth. The dividing membranes between the fetuses were visualized, and the thickness of the membranes was measured. With a thickness of 2 mm used as a cutoff point, the accuracy in predicting monochorionic or dichorionic twinning was 82% and 95%, respectively. Prenatal assessment of these dividing membranes may be helpful in the management of twin gestations. (AM J OBSTET GYNECOL 1989;161:1540-2.)

Key words: Twins, chorion, amnion, ultrasonography, prenatal diagnosis

In the United States, the overall incidence of twinning is about one in 90 pregnancies.[1] Complications from twin pregnancies still contribute significantly to the overall perinatal mortality and morbidity. The incidence of morbidity is affected by the status of the placental chorion and amnion. Since monochorionic placentas almost always have some vascular anastomoses between the two fetal circulations, identification of placental chorion is useful in the management of a twin pregnancy, especially when it is complicated by growth discrepancy, fetal hydrops, or fetal death. Whereas the identification of different fetal genders or two separate placentas indicates dichorionic twinning,[2] differentiation between monochorionic and dichorionic placentas must rely on other parameters when only one placenta or similar genders exist. Qualitative evaluation of the dividing membranes by ultrasonography has been attempted to determine the type of placental chorion.[3] The current study was undertaken, however, to quantitatively establish the sonographic criteria for the prenatal diagnosis of chorion status in twin gestations.

Patients and methods

This study was conducted at the Perinatal Unit of Yale–New Haven Hospital during the period January 1987 to January 1988. Indications for ultrasonographic examination included confirmation of twin gestation, assessment of fetal growth, and genetic amniocentesis. The dividing membranes between the twins were evaluated as part of the ultrasonographic examination. After the membranes were well visualized and delineated, their thickness was measured between the outer borders, perpendicular to the long axis of the membrane segment (Fig. 1), without prior knowledge of fetal gender or number of placentas. The segment of the dividing membranes used was as perpendicular to the ultrasonographic beams as possible to utilize the better axial resolution. The ultrasonographic scans were obtained with the Acuson-128 ultrasonography machine (Acuson, Mountinview, Calif.) with 3.5 and 5 MHz transducers and an axial resolution of about 0.5 mm. Placental chorionic type was confirmed by histologic examination after delivery. The cutoff point of the membrane thickness measured by ultrasonography to differentiate between monochorionic and dichorionic placentas was retrospectively determined on the basis of results of the histologic examinations. Thirty-two twin pregnancies with dividing membranes evaluated both ultrasonographically and histologically formed the basis of this report. In four patients who had longitudinal ultrasonographic examinations of the dividing membranes, only one measurement for each patient was used to determine the cutoff point of membrane thickness (Tables I and II). The difference between the mean membrane thickness was analyzed by the *t* test.

Results

Of 6029 deliveries during the study period, 103 twin pregnancies occurred. Therefore the incidence of twinning was 1.7% among viable pregnancies. Thirty-one percent of the twin gestations were monochorionic and 62.1% were dichorionic. The remaining 6.9% (seven

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology,[a] and the Department of Pathology,[b] Yale University School of Medicine.

Presented at the Thirty-fifth Annual Meeting of the Society for Gynecologic Investigation, Baltimore, Maryland, March 17-20, 1988.

Reprint requests: Hung N. Winn, MD, Department of Obstetrics and Gynecology, Washington University Medical Center, 4911 Barnes Hospital Plaza, St. Louis, MO 63110.

6/6/16235

Table I. Placental chorionicity in twin gestations

Twin type	Membrane thickness (mm) (n = 32)		
	Range	Mean	SD
Monochorionic	1.0-2.0	1.4	±0.3
Dichorionic	1.2-4.0	2.4	±0.7

$p < 0.001$.

Table II. Placental chorionic status in twin gestations ($n = 32$)

Membrane thickness	Placental histology		Total
	Monochorionic	Dichorionic	
<2 mm	9	2	11
≥2 mm	1	20	21
TOTAL	10	22	32

For dichorionic twinning: sensitivity = 90.9%; specificity = 90.0%; positive predictive value = 95.2%; negative predictive value = 81.8%.

pregnancies) consisted of twin pregnancies that did not have histologic placental examinations. These seven twin gestations consisted of five sets of the same gender and two sets of different genders. Among 103 twin pregnancies, only 32 twin pregnancies had both ultrasonographic examinations of the membranes (eight during the second trimester and 24 during the third trimester) and pathologic confirmation. The membrane thickness was essentially unchanged from the second to third trimester in four patients who had serial measurements of the dividing membranes. There was a significant difference in the mean membrane thickness between the monochorionic and dichorionic membranes ($p < 0.001$) (Table I). With a thickness of 2 mm used as a cutoff point, the accuracy in predicting monochorionic and dichorionic membranes was 82% and 95%, respectively (Table II). The interobserver and intraobserver variability of measurements was 15% and 10%, respectively.

Comment

The overall incidence and distribution of monochorionic and dichorionic twin gestations in our patient populations were comparable to those previously reported. The incidence of twinning in the United States ranges from 1% to 2% with dichorionic placentations being present in two of three twin gestations.[1, 4] Determination of the type of placental chorion is helpful in the management of twin gestations. Vascular communications between the two fetal circulations occur almost exclusively in monochorionic twinning, and almost all monochorionic placentas have some degree of vascular anastomoses.[5, 6] Clinically significant twin-twin transfusion occurs in about 20% to 30% of monochorionic twinning.[7] Therefore monochorionic twinning requires more intense surveillance of fetal growth and well-being. Although growth discrepancy may occur in both monochorionic and dichorionic twinning, the diagnosis of twin-to-twin transfusion, which has a more omnious prognosis, should be strongly considered in monochorionic gestations. Furthermore, when fetal death occurs, the surviving twin with the monochorionic placenta may suffer thromboembolic complications resulting in multicystic encephalomalacia,[8] disseminated intravascular coagulation, and infarction of other visceral organs.[9] As a result, selective feticide of one twin would be ill-advised in the case of monochorionic twinning if one twin should have a congenital anomaly. In general, there is a high discordancy rate for congenital anomalies between twin pairs.[10] Identification of monochorionic placentas, however, indicates monozygotic twinning, which usually has identical chromosomal anomalies.

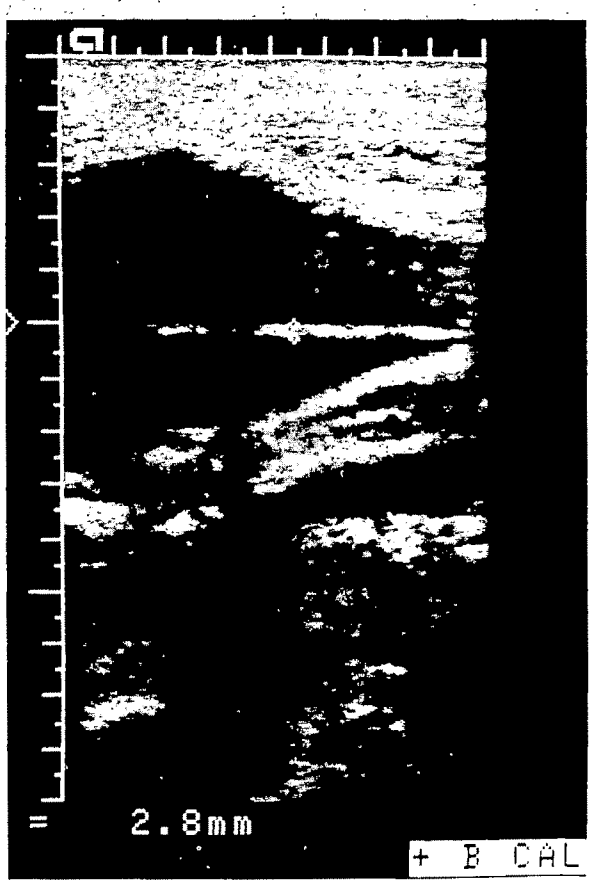


Fig. 1. The thickness of the dividing membranes was measured between the outer borders (+). The segment chosen was perpendicular to the ultrasonography beams.

Histologically, the dichorionic dividing membranes are thicker than the monochorionic membranes, because the former contain two layers of chorion and two layers of amnion whereas the latter contain only two layers of amnion. Ultrasonography has been used recently for the prenatal diagnosis of placental chorion status, which is based on the difference in thickness between the two types of membranes. Qualitative evaluation of the membranes by inspection has been reported with a high degree of accuracy.[3] This method, however, is applicable when the membrane thickness is at the extremes of measurement and depends, to a great extent, on the ultrasonographer's experience. We have found that there was a significant difference in mean membrane thickness between monochorionic and dichorionic placentas, and often the difference in membrane thickness is <1 mm and may not be accurately assessed by simple inspection. Our results also demonstrated that the membranes of monochorionic gestations are measurable.

In conclusion, the determination of the types of placental chorionic type is helpful in the management of twin gestations. Ultrasonography has permitted prenatal diagnosis of monochorionic and dichorionic twinning with a high degree of accuracy by identifying the number of placentas and fetal gender and measuring the membrane thickness. It should be emphasized that these measurements should be done only with high-resolution ultrasonography machines and that, even with quantitation of the membrane thickness, the two types of chorionicity cannot always be distinguished. A prospective study is being conducted to confirm these findings.

REFERENCES

1. Powers WF. Twin pregnancy: complications and treatment. Obstet Gynecol 1973;42:795.
2. Mahony BS, Filly RA, Callen PW. Amnionicity and chorionicity in twin pregnancies: prediction using ultrasound. Radiology 1985;155:205-9.
3. Barss VA, Benacerraf BR, Frigoletto FD. Ultrasonographic determination of chorion type in twin gestation. Obstet Gynecol 1985;66:779-82.
4. Hertzberg BS, Kurtz AB, Choi HY, et al. Significance of membrane thickness in the sonographic evaluation of twin gestations. AJR 1987;148:151-3.
5. Benirschke K, Chung KK. Multiple pregnancy (first of two parts). N Engl J Med 1973;288:1276-84.
6. Robertson EG, Neer KJ. Placental injection studies in twin gestations. AM J OBSTET GYNECOL 1983;147:170-4.
7. Tan KL, Tan R, Tan SH, Tan AM. The twin transfusion syndrome. Clinical observations on 35 affected pairs. Clin Pediatr 1979;18:111-4.
8. Yoshioka H, Kadomoto Y, Mino M, et al. Multicystic encephalomalacia in liveborn twins with a stillborn macerated co-twin. J Pediatr 1979;95:798-800.
9. Moore CM, McAdams AJ, Sutherland J. Intrauterine disseminated intravascular coagulation. A syndrome of multiple pregnancy with a dead twin fetus. J Pediatr 1969; 74:523-8.
10. Benirschke K, Kim CK. Multiple pregnancy (second of two parts). N Engl J Med 1973;288:1329-36.

Esterase activity in second- and third-trimester amniotic fluid: An indicator of chorioamnionitis

Iffath Abbasi Hoskins, MD, Joseph Katz, PhD, Steven A. Ordorica, MD, and Bruce K. Young, MD

New York, New York

Accurate and rapid diagnosis of chorioamnionitis poses a major diagnostic dilemma. We previously reported that leukocyte esterase activity in amniotic fluid, as measured by dipstick assay, could be used as an aid in the diagnosis of chorioamnionitis. This study examines the effectiveness of an in vitro spectrophotometric assay of esterase activity in amniotic fluid. We define baseline levels of esterase activity in uninfected amniotic fluid and demonstrate a quantative increase when infection is present. Fifty-seven amniotic fluid samples obtained at second- and third-trimester amniocenteses were divided into three parts, one for culture and two for a comparison of esterase activitites by the dipstick and spectrophotometric methods. In this study, the spectrophotometric assay, because of its higher specificity and sensitivity in the determination of elevated esterase activity, was shown to be more reliable for predicting chorioamnionitis than either the dipstick or culture method. (Am J Obstet Gynecol 1989;161:1543-5.)

Key words: Leukocyte esterase, chorioamnionitis screening, in vitro assay

Chorioamnionitis plays a major role in perinatal morbidity and mortality. One of the basic problems in its diagnosis is the lack of uniformity and accuracy of the criteria on which this diagnosis is made. The clinical diagnosis is usually based on a syndrome of fever, maternal or fetal tachycardia, uterine tenderness, foul odor of the amniotic fluid, and peripheral blood leukocytosis, but there is uncertainty regarding early diagnosis and laboratory confirmation.[1] Since leukocytes are known to be released in response to infections, examination of the amniotic fluid for their presence may prove to be useful in the evaluation of chorioamnionitis. Neutrophilic leukocytes contain several esterases that are not present in serum, urine, or vaginal secretions.[2,3] * We have previously described the reliability of leukocyte esterase activity in the rapid detection of urinary tract infections in obstetric patients[4] and for the detection of chorioamnionitis in term pregnancies uncomplicated by other diseases.[5] In the present study, we assessed the use of an in vitro assay to establish baseline levels of esterase activity in second- and third-trimester amniotic fluid samples and to demonstrate any alterations of these levels in chorioamnionitis.

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, New York University Medical Center.

Presented in part at the Thirty-fifth Annual Meeting of the Society for Gynecologic Investigation, Baltimore, Maryland, March 17-20, 1988.

Reprint requests: Iffath Abbasi Hoskins, MD, Department of Obstetrics and Gynecology, NYU-Bellevue Hospital Center, 450 First Ave., Room 9 North 1, New York, NY 10016

**Chemstrip 9 Biodynamics, package insert.*

6/6/15954

Table I. Relationship between leukocyte esterase activity and other parameters used to diagnose chorioamnionitis

	Uninfected		*Infected*	
	Pos.	*Neg.*	*Pos.*	*Neg.*
Amniotic fluid				
Culture	2	37	14	4
Gram's stain	2	37	15	3
Leukocyte esterase activity				
Dipstick assay	2	37	17	1
In vitro assay	0	39	18	0

Material and methods

Sterile specimens of amniotic fluid were obtained from 57 antepartum patients who had undergone amniocenteses during the second and third trimesters for genetic studies, for rhesus isoimmunization, for fetal lung maturity studies, or to rule out chorioamnionitis. Thirty-nine samples were obtained from uninfected patients along with 18 samples from women who had chorioamnionitis as determined by the presence of at least two of the clinical criteria used for its diagnosis, including maternal pyrexia (temperature >37.8° C), leukocytosis (white blood cell count >12,000/mm^3), uterine tenderness, and fetal tachycardia (heart rate >160 beats per minute). Each sample was divided into three parts: one for the in vitro leukocyte esterase assay, one for detection of leukocyte esterase activity by dipstick, and one for Gram's stain and aerobic and anaerobic cultures.

Table II. Reliability of parameters used to detect chorioamnionitis

	Sensitivity (%)	*Specificity (%)*	*Predictive values*	
			Positive	*Negative*
Amniotic fluid				
Culture	78	95	90	80
Gram's stain	83	95	93	88
Leukocyte esterase activity				
Dipstick assay	94	95	97	90
In vitro assay	100	100	100	100

For in vitro leukocyte esterase assay, known amounts (10 λ) of amniotic fluid were added to the solutions containing 20 λ of a 5 μmol/L solution of *p*-nitrophenyl hexanoate in dimethyl sulfoxide and 1 ml of buffer (0.1 mol/L phosphate, pH 7.4). Activity was calculated from the initial slopes of the change in absorbance at 405 nm as a function of time. One unit of activity was defined as a change of 1 absorbance unit at 405 nm/min. Each sample was then centrifuged at 2000 rpm for 10 minutes and the assay was repeated.

Aliquots of the same amniotic fluid samples were tested for leukocyte esterase activity by immersion of the dipstick (Chemstrip 9, Boehringer-Mannheim Diagnostics, Bio-Dynamics Division) in the fluid and comparison of the color reaction with the color chart after 1 minute.

Gram's stain test was performed with commercial reagents (crystal violet, safranine, Gram's iodine; Difco Laboratories, Detroit) under standard conditions.[6] The presence of white blood cells and microorganisms was noted, and a semiquantitative assessment was made after examination of 20 high-power fields. A positive Gram's stain was defined as one in which there were any microorganisms per high-power field. The amniotic fluid aliquots destined for cultures were collected in plastic-capped syringes and transported to the laboratory immediately after collection for inoculation into the culture media.

Positive cultures were defined as those containing one or more types of organisms in excess of 10^4 cfu/ml. The leukocyte esterase activity test results were then compared with the results obtained from Gram's stains and aerobic and anaerobic cultures.

Results

Table I represents the relationship between leukocyte esterase activity, as measured by dipstick and in vitro assay, and results of Gram's stain culture in both the uninfected and infected samples of amniotic fluid. There were 39 uninfected samples for analysis. Twenty-one of these were obtained during the second trimester after amniocenteses for genetic indications. Eighteen were obtained during the third trimester—4 for Rh sensitization and 14 for studies of lung maturity. No differences were found between the Rh-sensitized and normal samples. Two samples from the third trimester showed positive cultures with *Escherichia coli* organisms $>10^4$ cfu/ml. Both of these samples were also positive on Gram's stain analysis and showed leukocyte esterase activity, as measured by dipstick. However, when the in vitro enzyme assay was performed, the leukocyte esterase activity was within the ranges we had defined as baseline normal (5.8 and 6.3 enzyme activity units) for uninfected samples (Fig. 1).

There were 18 infected samples of amniotic fluid obtained from women with chorioamnionitis. All of these samples were obtained during the third trimesters from patients who had undergone amniocenteses to rule out chorioamnionitis (spontaneous rupture of membranes, 8; preterm labor, 10). Fourteen of these women had positive amniotic fluid cultures, with organism counts $>10^4$ cfu/ml (*E. coli*, 10; group B streptococci 2; *Enterobacteriaceae* 2). However, if a positive culture was defined as one containing organisms $>10^2$ cfu/ml, then all 18 samples were found to be positive (*E. coli*, 13; group B streptococci, 2; *Enterobacteriaceae*, 3). Only 15 of these samples were positive by the Gram's stain test, and the organisms revealed by this test were similar to those revealed by cultures. Seventeen of the 18 infected specimens were positive by leukocyte esterase dipstick assay, but only 15 of these showed evidence of bacteria on Gram's stain analysis. The two samples with positive leukocyte esterase activity revealed by dipstick but negative Gram stains showed varying numbers of white blood cells on the slides. The one sample that tested negative by dipstick assay grew organisms $>10^4$ cfu/ml *(Enterobacteriaceae)*. When the in vitro enzyme assay was performed, the observed esterase activity was severalfold higher (range, 17.8 to 18.2 enzyme activity units) than that of the uninfected normal samples (Fig. 1). When the uninfected samples were centrifuged and assayed by the in vitro leukocyte esterase method, there was a severalfold increase in observed esterase activity (range, 14.6 to 17.2 enzyme activity units) to levels similar to those seen in the infected samples. This increase was found to be due to cell disruption and lysis that allowed the release of azurophilic, esterase-containing granules in the leukocytes.

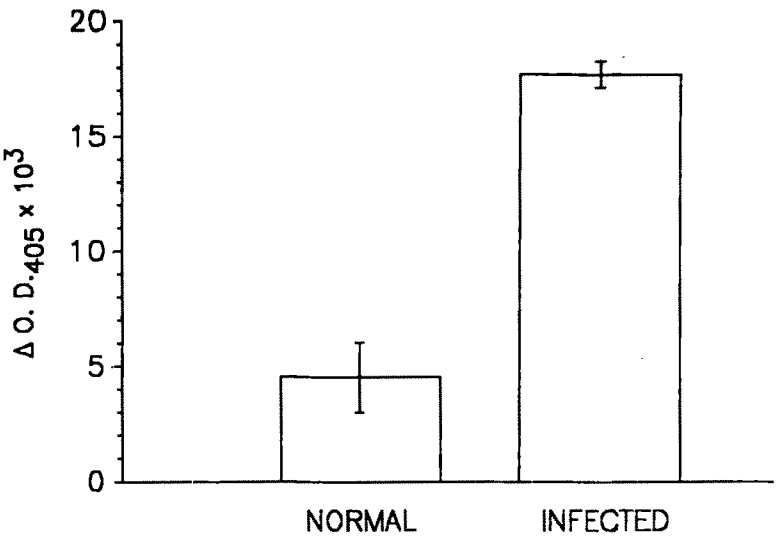


Fig. 1. Esterase activity in normal and infected samples of centrifuged amniotic fluid.

Table II shows the sensitivity, specificity, and predictive values of the various parameters used in the comparison of leukocyte esterase activity by the dipstick and the in vitro methods. In our study, the in vitro assay had a 100% sensitivity and a 100% specificity for chorioamnionitis, whereas the leukocyte esterase dipstick had a sensitivity of 94% and a specificity of 95%. The cultures of these samples had shown a sensitivity of only 78% and a specificity of 95%.

Comment

Acute chorioamnionitis remains a serious infection for the mother and her baby. Despite the seriousness of this condition, there are major problems associated with its diagnosis. At present it is impossible to determine what constitutes an optimal diagnostic finding, and attempts to improve our ability to diagnose intrapartum infections have met with little success. To identify an optimal screening test for chorioamnionitis, we reported previously[5] on the dipstick method of measuring leukocyte esterase activity as an inexpensive and reliable (sensitivity 91%, specificity 95%) test for diagnosing chorioamnionitis in term patients in the absence of significant numbers ($<10^4$ cfu/ml) of organisms on culture. However, the results of the dipstick assay, like those of the aerobic and anerobic cultures, can sometimes be misleading or equivocal. When that is the case, in vitro spectrophotometric assay of esterase activity may be more useful in identifying infected amniotic fluid samples and eliminating any confusion, since this test has both a sensitivity and a specificity much higher (100% each) than those of the other tests.

Thus a simple, rapid, and inexpensive regimen for the evaluation and diagnosis of chorioamnionitis could involve the use of the dipstick assay of esterase activity as an initial screening test (before culture results have been obtained), with in vitro spectrophotometric assay as a backup for samples that yield equivocal or negative results by the dipstick method. The use of this test may serve to eliminate the need for any additional testing or aid management decisions in situations in which waiting for culture results is not feasible.

REFERENCES

1. Gibbs RS, Castillo MS, Rodgers PJ. Management of acute chorioamnionitis. Am J Obstet Gynecol 1980;136:709.
2. Rindler-Ludwig R, Schmazl F, Braunsteiner H. Esterases in human neutrophilic granulocytes: Evidence for their protease nature. Br J Haematol 1974;27:57.
3. Li CY, Lam KW, Yam LT. Esterases in human leukocytes. J Histochem Biochem 1973;21:1.
4. Abbasi IA, Hess LW, Johnson TRB, et al. Leukocyte esterase activity in the rapid detection of urinary tract and lower genital tract infections in obstetric patients. Am J Perinatol 1985;2:311.
5. Hoskins I, Johnson TRB, Winkel CA. Leukocyte esterase activity in human amniotic fluid for the rapid detection of chorioamnionitis. Am J Obstet Gynecol 1987;157:730.
6. Hendrickson DA. Reagents and stains. In: Lennette EH, Balows A, Hausler WJ, Shadomy HJ, eds. Manual of clinical microbiology. 4th ed. Washington DC: American Society for Microbiology, 1985:1093.

Rapid chromosome analysis with the use of spontaneously dividing cells derived from umbilical cord blood (fetal and neonatal)

Robert E. Tipton, MD, Avirachan T. Tharapel, PhD, Hua-Hua T. Chang, BS, Joe Leigh Simpson, MD, and Sherman Elias, MD

Memphis, Tennessee

An accurate and reproducible method to obtain high-quality metaphases within 24 hours has been developed. Preparations can be derived from cord blood samples obtained either at delivery or by percutaneous umbilical blood sampling. Mitogens are not required. Rapid cytogenetic analysis can facilitate management of pregnancies charcterized by malformations or intrauterine growth retardation. (AM J OBSTET GYNECOL 1989;161:1546-8.)

Key words: Rapid karyotyping, prenatal cytogenetic diagnosis, fetal anomalies, percutaneous umbilical blood sampling

Rapid chromosome analysis (within 24 hours) would provide timely diagnostic information that could alter pregnancy management in instances in which the fetus is found to have ultrasonographic evidence of malformations or intrauterine growth retardation. A rapid diagnostic method also would facilitate management of anomalous or growth retarded neonates. Until recently the only available method to obtain rapid chromosome analysis used immature cells from bone marrow aspirated from iliac crest or tibia.[1,2] In 1987 Garnham and Sutherland[3] reported that spontaneously dividing cells present in newborn umbilical cord blood could be used for rapid chromosome analysis. Although later confirmed by Day et al.,[4] the quality of these chromosomal preparations was not adequate to allow identification of subtle structural chromosomal abnormalities. Moreover, these initial studies had not ascertained whether spontaneously dividing cells were present in fetal blood. Thus it was not known whether blood samples obtained by percutaneous umbilical blood sampling could provide rapid chromosomal analysis. In this study we sought to develop methods to reliably obtain high-quality metaphases from spontaneously dividing cells present in cord blood.

Table I. Specimens studied

Gestational age (wk)	*No. of cases (N = 63)*	*Type of specimen**
22	1	Percutaneous umbilical blood sample
27	1	Percutaneous umbilical blood sample
29	1	Newborn cord blood
30	2	Newborn cord blood
31	2	Newborn cord blood
31	1	Effusion*
32	1	Newborn cord blood
32	1	Percutaneous umbilical blood sample (47,XX,+18)
33	1	Newborn cord blood
34	2	Newborn cord blood
35	2	Newborn cord blood
36	2	Newborn cord blood
37	0	—
38	7	Newborn cord blood
39	4	Newborn cord blood
40	31	Newborn cord blood
41	1	Newborn cord blood
42	3	Newborn cord blood

*Bloody pleural fluid obtained by intrauterine thoracocentesis.

Material and methods

Umbilical cord blood samples were obtained at delivery from 59 phenotypically normal newborns (gestational ages 29 to 42 weeks). Percutaneous umbilical blood sampling was performed on three fetuses, all with malformations and growth retardation (22, 27, and 32 weeks' gestation). In addition, we processed a sample of bloody pleural effusion obtained by intrauterine thoracocentesis from a fetus with chylothorax at 31

From the Departments of Pediatrics and Obstetrics and Gynecology, The University of Tennessee.

Supported in part by the National Institute of Child Health Development Grant No. HD22968, United States Public Health Service contract N01-HD-8-2904, the United States Agency for International Development Project BR-US-0004, University Physician's Foundation, and a University of Tennessee Chair of Excellence (J. L. S.).

Presented at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.

Reprint requests: Robert E. Tipton, MD, 843 Jefferson, Memphis, TN 38163.

6/6/15655

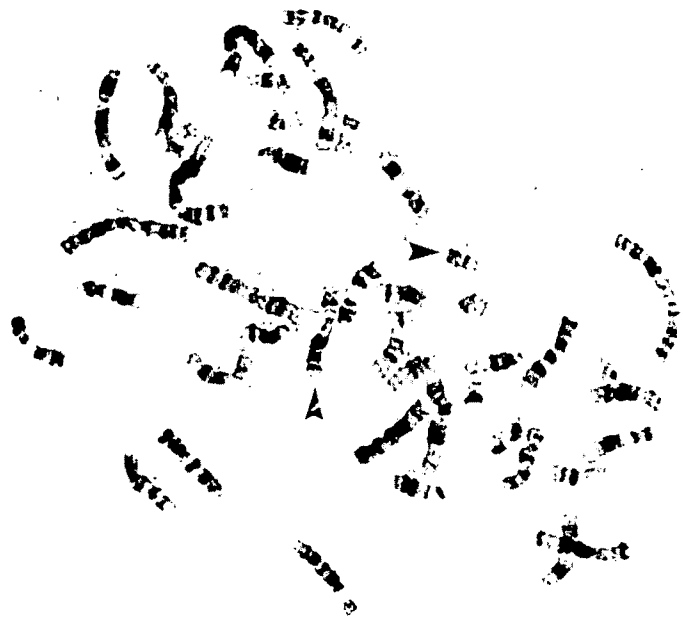


Fig. 1. Metaphase spread from cord blood of term male infant. *Arrows* point to X and Y chromosomes.

weeks' gestation. Each of the 59 newborn cord blood specimens (2 to 10 ml) were collected in Vacutainers that contained sodium heparin and were transported to the cytogenetics laboratory within 1 to 2 hours. Percutaneous umbilical blood samples and pleural effusion fluid were collected in heparinized syringes.

Cultures that contained 1 ml heparinized blood were incubated 18 hours in 9 ml modified Eagle's minimum essential medium (Irvine Scientific, Santa Ana, Calif.) supplemented with 15% Chang medium (Hana Biologics, Alameda, Calif.), 15% fetal bovine serum, and final concentrations of 0.8% L-glutamine, 100 μg/ml streptomycin, and 10 IU/ml penicillin. No mitogens were added. Cultures were incubated at 37° C for 17 hours and then were treated with 0.5 μg/ml actinomycin D and 0.2 μg/ml demecolcine (Colcemid). After incubation for 1 additional hour, cultures were centrifuged for 8 minutes at 1000 rpm. The supernatant was discarded and the cells were washed twice in 10 ml Hank's balanced salt solution. This supernatant was discarded and the cell pellet was resuspended at room temperature for 2 hours in 10 ml Ohnuki's hypotonic solution, consisting of 0.055 mol/L potassium chloride, sodium nitrate, and sodium acetate (ratio 10:5:2).[5, 6] After 2 hours of hypotonic treatment, 2 ml of 3:1 methanol-glacial acetic acid mixture was added. The cell suspension was mixed and centrifuged for 8 minutes at 1000 rpm. The supernatant was again discarded, the cells were fixed twice more for 10 minutes each, and then they were centrifuged again. With a Pasteur pipette, four to six drops of diluted cell suspension was dropped from a distance of 30 to 60 cm onto precleaned, wet, chilled slides. Slides were dried on a hot plate for 1 to 2 minutes at 60° C. Chromosomal preparations were stained (trypsin-Giemsa). The bloody pleural effusion was processed in a similar fashion to that of the blood specimens, except that 5 ml of the effusion was incubated with 5 ml of culture medium. For each case at least five metaphases were counted and two karyotypes prepared. To confirm the accuracy of our results, conventional 72-hour cultures were initiated for the first 20 cases with the mitogens phytohemagglutinin and pokeweed. At least five metaphases were analyzed from each of these 20 cultures. No attempt was made to determine the exact mitotic index.

Results

Metaphases readily allowing diagnoses were obtained in all 63 cases: 59 neonatal cord blood specimens, three

percutaneous umbilical blood samples, and one bloody fetal pleural effusion (Table I). Numbers of well-banded metaphases in the unstimulated preparations averaged 5 to 10 per slide, lower than our usual number for routine blood cytogenetics but easily sufficient to allow diagnosis. Moreover, the quality of the chromosome preparations was adequate to diagnose structural abnormalities. Banding resolution approximated 400 to 450 bands (Fig. 1). Hypomodal cells, a reflection of metaphase spreads broken in preparation, were minimal. There were no diagnostic discrepancies in the 20 cases in which both conventional cytogenetic analysis and the newly described method were used. Thus it seems there is no reason to doubt the accuracy of the method we used. No relationship between the gestational age and the numbers of dividing cells was apparent.

Comment

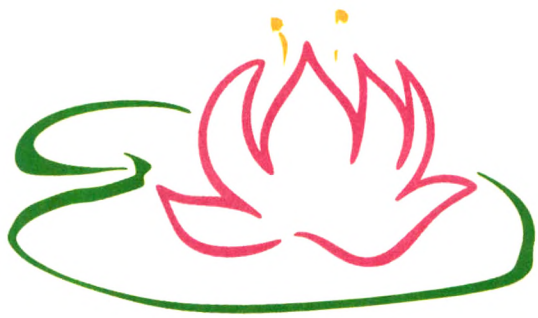
The method we describe allows cytogenetic diagnosis within 24 hours of specimen collection. Spontaneously dividing cells were detected not only in newborn umbilical cord blood samples (gestational ages 29 to 42 weeks) but also in fetal blood samples (gestational ages 22, 27, and 32 weeks). Overnight incubation consistently provided well-banded metaphases suitable for analysis, thus offering a marked improvement over earlier methods.[3, 4] The quality of the preparations is consistent (400 to 450 band resolution) to allow detection of chromosomal rearrangements such as deletions, duplications, or translocations. If percutaneous umbilical blood sample is available, the chromosomal complement of the fetus can be established more rapidly than with conventional laboratory methods, which facilitates pregnancy-management decisions. If fetal anomalies or intrauterine growth retardation are detected at birth, processing a blood specimen from the umbilical cord by our method provides a noninvasive method to determine chromosomal status. This would be especially useful in preterm infants, whose blood volume is correspondingly small.

The origin of these spontaneously dividing cells is uncertain. Our finding of spontaneously dividing cells in percutaneous umbilical blood samples as early as 22 weeks' gestation casts doubt over previous claims that such cells were present in cord blood as result of spillover of cells from bone marrow because of anoxic stresses.[3, 4] One of the percutaneous umbilical blood samples in our series was obtained on a a fetus possibly undergoing anoxic stress, as manifested by acidotic cord blood pH and poor biophysical profile score. However, there was no reason to suspect anoxia in the other two fetuses from which percutaneous umbilical cord blood sampling was obtained, nor to believe that anoxia was frequent in the 59 neonates who had undergone normal deliveries.

REFERENCES

1. Francke U, Brown MG, Jones KL. Immediate chromosome diagnosis on bone marrow cells: an aid to management of the malformed newborn infant. J Pediatr 1979;94:289-92.
2. Page BM, Coulter JBS. Bone marrow aspiration for chromosome analysis in newborn. Br Med J 1978;1:1455-6.
3. Garnham I, Sutherland GR. Rapid karyotyping of neonates on the basis of data from cord blood. Am J Hum Genet 1987;41:668-70.
4. Day DW, Jalal SM, Sinclair-Worley L, Kukolich M, Freeman M, Sakakini J. Direct chromosome analysis from neonatal cord blood. Am J Med Genet 1988;31:943-6.
5. Ohnuki Y. Structure of chromosomes. I. Morphological studies of the spiral structure of human somatic chromosomes. Chromosoma 1968;25:402-28.
6. Rybak J, Tharapel A, Robinett S, Garcia M, Mankinen C, Freeman M. A simple reproducible method for prometaphase chromosome analysis. Hum Genet 1982;60:328-33.

Finally, a discovery to end vaginal dryness



New

REPLENS™

Replenishes vaginal moisture

Vaginal moisture that lasts and lasts

Mucoadhesive delivery provides ongoing moisturization

Until now, the millions of women with vaginal dryness could ease their discomfort only temporarily. But today, new REPLENS ends dryness by replenishing vaginal moisture. REPLENS is delivered through mucoadhesion, a system that holds moisture in place. The primary ingredient, polycarbophil, is not absorbed. By design it is able to hydrate underlying vaginal tissue to provide lasting vaginal moisture and continuous lubrication.



Lasting moisture—clinically proven to end discomfort and painful intercourse

In double-blind clinical studies, REPLENS has been proven to be more effective than the most widely used lubricant for promoting lasting moisturization.[1] REPLENS increases mucosal thickness, elasticity, and turgor, thereby ending painful intercourse and vaginal discomfort. And when compared with K-Y® jelly, both patients and their spouses preferred REPLENS.

Three-day-a-week regimen ensures full-time comfort

When used three times a week, REPLENS provides vaginal moisture every minute of every day. Unlike other lubricants, women don't need to reapply before intercourse—encouraging spontaneity and avoiding embarrassment.

For your patients over 40 and some younger, restore a natural feeling

Menopausal women, nursing mothers, and any of more than 25 million women who suffer from vaginal dryness will appreciate your recommendation of REPLENS. When you replenish vaginal moisture, you bring back a sense of youth and confidence that many women thought they had lost forever.

K-Y is a registered trademark of Johnson & Johnson Products.



New

REPLENS™

Replenishes vaginal moisture

new comfort for more than 25 million women

Introducing

REPLENS™

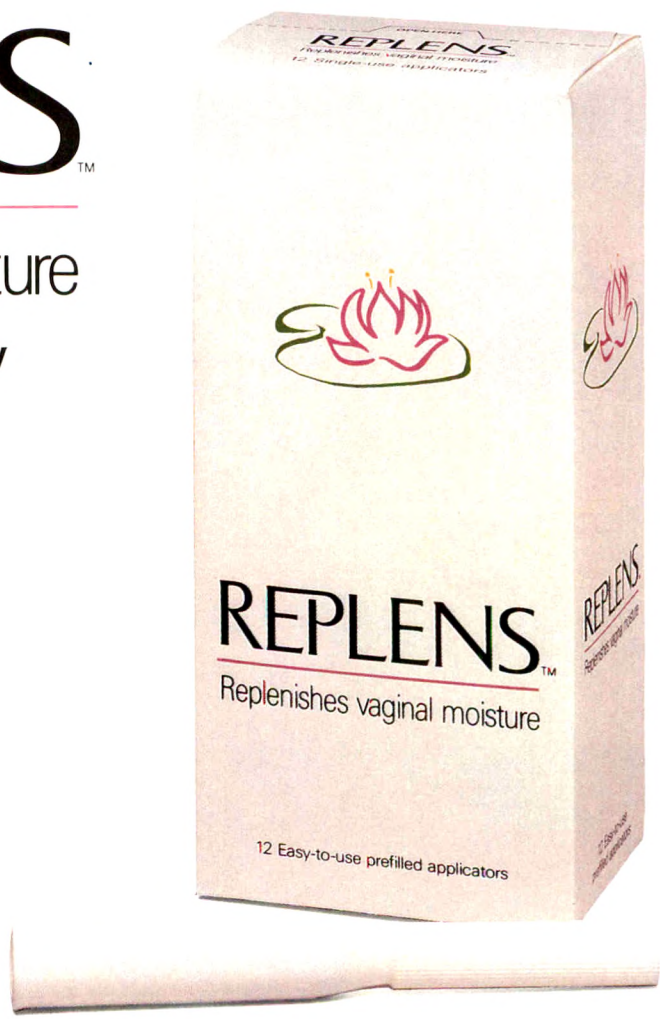
Replenishes vaginal moisture

Vaginal moisture that's back to stay

- Mucoadhesive delivery provides lasting moisturization, continuous lubrication
- Clinically proven relief of dryness, discomfort, and painful intercourse[1]
- For menopausal women, new mothers, and all women with vaginal dryness
- Three-time-a-week regimen ensures full-time comfort
- Available in boxes of 12 single-use prefilled applicators

Use REPLENS Vaginal Gel three times a week at bedtime.

1. Data on file, Columbia Laboratories Inc.



Columbia Laboratories, Inc.
Miami, FL 33169

© 1989, Columbia Laboratories Inc.
Printed in USA RE-1138 (11/89)

Effects of fetal sex and dexamethasone on preterm maternal serum concentrations of human chorionic gonadotropin, progesterone, estrone, estradiol, and estriol

Ray V. Haning, Jr., MD,[a, b] Luis B. Curet, MD,[b] W. Kenneth Poole, PhD,[c] Lynn M. Boehnlein, BS,[b] Donna L. Kuzma, BS,[b] and Sandra M. Meier, BS[b]

Providence, Rhode Island, Madison, Wisconsin, and Research Triangle Park, North Carolina

The serum human chorionic gonadotropin concentration for the 43 female-bearing gestations was significantly higher (geometric mean 15,603 mIU/ml; 95% confidence interval 12,337 to 19,733) than that for 26 male-bearing pregnancies (geometric mean 8902 mIU/ml; 95% confidence interval 6288 to 12,596), $p = 0.0087$. The mean gestational age was 32 ± 2.4 weeks (mean ± SD). There was no significant difference between male- and female-bearing pregnancies for progesterone, cortisol, estrone, estradiol, estriol, or gestational age. In 19 placebo-treated pregnancies and 14 dexamethasone-treated pregnancies with a mean gestational age of 31.5 ± 2.7 weeks, there was no significant change from the baseline value observed for either maternal serum human chorionic gonadotropin level or maternal serum progesterone level at 42 hours after institution of treatment. In the dexamethasone-treated subjects the geometric mean was reduced to 20% of baseline for cortisol; 42% of baseline for estrone; 30% of baseline for estradiol; and 41% of baseline for estriol (all $p < 0.001$). (AM J OBSTET GYNECOL 1989;161:1549-53.)

Key words: Pregnancy, sex difference, hCG, progesterone, estrogens, dexamethasone

There are two lines of evidence to indicate the secretion of human chorionic gonadotropin (hCG) by the placenta is partially under fetal endocrine control. First, unlike human placental lactogen and pregnancy-specific β_1-glycoprotein, which are also secreted by the syncytiotrophoblast and which rise progressively in relationship to the rise in trophoblastic mass, hCG rises exponentially to a peak at 8 to 10 weeks, then falls to a plateau for the remainder of pregnancy.[1] Second, unlike the other hormones secreted by the syncytiotrophoblast, which have similar concentrations in both male- and female-bearing pregnancies, the concentration of hCG in maternal peripheral blood[2-4] and placental tissue[5, 6] is higher at term in female-bearing pregnancies than in male-bearing pregnancies even though no difference has been demonstrated earlier in pregnancy until this investigation. Studies from our laboratory have demonstrated statistical evidence for an inhibitory effect of the fetal adrenal gland on in vivo secretion of hCG[7] and in vitro evidence that addition of dehydroepiandrosterone sulfate to cultures of term human placental explants could inhibit the stimulation of hCG secretion produced by gonadotropin-releasing hormone.[8] These two observations led us to investigate whether administration of dexamethasone to pregnant women would produce stimulation of hCG secretion by suppressing the fetal adrenal. The residual samples from the completed double-blind prospective trial of dexamethasone versus placebo in premature labor[9] offered a unique chance to investigate both the effect of the sex of the fetus and the effect of administration of dexamethasone on secretion of hCG, progesterone, estrone, estradiol, and estriol in vivo before term in a double-blind randomized study.

From the Department of Obstetrics and Gynecology, Brown University and Women and Infants Hospital, Providence, the Department of Obstetrics and Gynecology, University of Wisconsin, Madison, and Research Triangle Institute, Research Triangle Park.

This investigation was supported in part by a grant from the National Institute of Child Health and Human Development (HD20071) and contracts NO1-HR-6-2948, 2949, 2950, 2951, 2952, and 2953 with the National Heart, Lung, and Blood Institute, National Institutes of Health, United States Department of Health and Human Services.

Presented in part at the Thirty-fifth Annual Meeting of the Society for Gynecologic Investigation, Baltimore, Maryland, March 17-20, 1988.

Reprint requests: Ray V. Haning, Jr., MD, Department of Obstetrics and Gynecology, Women and Infants Hospital, 101 Dudley St., Providence, RI 02905.

6/6/16238

Material and methods

Human subjects. The process of informed consent and patient selection for the dexamethasone study has been described.[9] Briefly, a total of 7893 patients from the five participating centers were screened; 347 of them received placebo and 349 received dexamethasone. Subjects received 5 mg of dexamethasone phosphate every 12 hours intramuscularly or a placebo in a similar vehicle for up to 4 doses (a total of 20 mg of active drug). Since the first dose was administered at time "zero," the last of the four doses was administered at time "36 hours." The sample at "42 hours" used for our posttreatment level was thus drawn 6 hours after the last dose of dexamethasone, a time when the concentration of maternal serum dexamethasone had be-

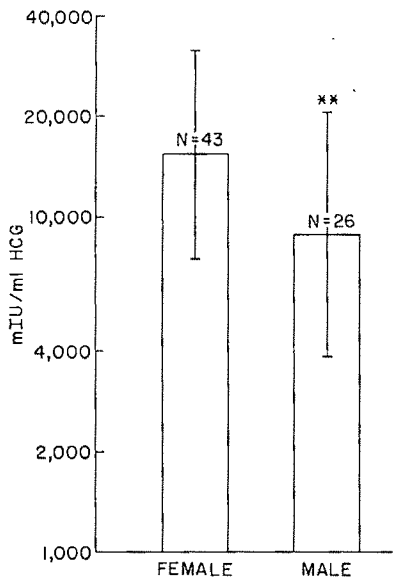


Fig. 1. Effect of fetal sex on maternal serum concentration of hCG determined in baseline samples. Data shown are mean and SD calculated on log-transformed data (note log scale). *Double asterisk,* $p < 0.01$.

gun to fall but the concentrations of maternal serum cortisol and estradiol were still suppressed.[9] We obtained access to the residual samples from the study after completion of all planned analyses and based our sample selection on the volumes of the residual baseline and paired residual 42-hour sample without knowledge of the mode of treatment, the outcome of the pregnancy, the sex of the infant, or any other laboratory or clinical information. After completion of the analyses for hCG and progesterone, the data on the mode of treatment, sex of the infant, and other clinical variables were requested along with the values previously determined in the selected samples for cortisol, estrone, estradiol, and estriol.[9]

Chemical analyses. hCG was determined by direct double-antibody radioimmunoassay with an antibody produced in this laboratory as previously described.[7] An hCG standard (Calbiochem, La Jolla, Calif.) calibrated against the World Health Organization second international standard was used. The cross-reaction of this antibody (H1-9/2/76) with human luteinizing hormone was 0.16%. Between- and within-assay variations were 3.8% and 3.7%, respectively. The assay sensitivity was 0.5 mIU/ml.[7] Progesterone was determined by radioimmunoassay without extraction with the Pantex Immuno-direct progesterone iodine 125 kit (Pantex, Santa Monica, Calif.) provided without charge by the manufacturers. The cross-reactivity was 6.5% with 20α-hydroxy-pregn-4-ene-3-one, 1.5% with deoxycorticosterone, and 0.18% with 17α-hydroxyprogesterone. Aldosterone, androstenedione, androsterone, dehydroepiandrosterone, dihydrotestosterone, 17α- and 17β-estradiol, estriol, estrone, etiocholanolone, and testosterone all had values <0.1%. Cholesterol, corticosterone, cortisol, 11-deoxycortisol, 17-hydroxypregnenolone, pregnenolone, and pregnenolone sulfate had values <0.01%. Between- and within-assay variation were 5.7% and 5.3%, respectively, with a 50 ng/ml serum pool run in each assay with the actual samples. The assay sensitivity was 0.07 ng/ml, and the lowest standard used was 0.5 ng/ml.

Statistical analysis. The data were analyzed by one-way analysis of variance, Student's *t* test, and the paired *t* test with the Minitab statistical program[10] used after log transformation to correct for the log-normal distribution of the data.

Results

Effect of sex of fetus on hormone concentrations in baseline samples. Samples from 69 single pregnancies with sufficient volume for determination of hCG and progesterone were analyzed. Missing data resulted in decreases in the number of data points available for certain variables. There were 43 female-bearing and 26 male-bearing pregnancies determined by the sex of the infant at delivery. The mean gestational ages for these pregnancies were 32.1 ± 2.4 and 31.8 ± 2.3 weeks, respectively (mean ± SD). This difference was not statistically significant ($p > 0.5$). The mean gestational age for all 69 pregnancies was 32.0 ± 2.4 weeks. The range of gestational ages was 27 to 37 weeks, and the median gestational age was 32 weeks. The sex of the infant and data for cortisol, estrone, estradiol, and estriol previously determined on these samples were collated with the data for hCG and progesterone obtained in our laboratory. The baseline samples were analyzed for effect of the sex of the fetus by Student's *t* test. The serum hCG concentration for the female-bearing gestations was significantly higher (geometric mean 15,603 mIU/ml; 95% confidence interval 12,337 to 19,733) than that for male-bearing pregnancies (geometric mean 8902 mIU/ml; 95% confidence interval 6288 to 12,596), $p = 0.0087$ (Fig. 1). However, there was no significant difference between male- and female-bearing pregnancies for progesterone, cortisol, estrone, estradiol, or estriol (data not shown).

Effect of treatment on maternal serum hCG and progesterone. To eliminate all subjects who gave birth before the time that the 42-hour sample was obtained, all subjects who gave birth <3 days after the study was begun were eliminated from all statistical analyses involving the 42-hour samples. There remained 19 placebo-treated pregnancies (13 female-bearing and 6 male-bearing) and 14 dexamethasone-treated pregnancies (9 female-bearing and 5 male-bearing) available for analysis of treatment effects. The mean gestational age of these 33 pregnancies was 31.5 ± 2.7 weeks (mean ± SD) with a median age of 32 weeks and a range of 28 to 37 weeks. The combined data for both sexes for maternal serum hCG and progesterone levels in the baseline placebo-, baseline dexamethasone-, 42-hour

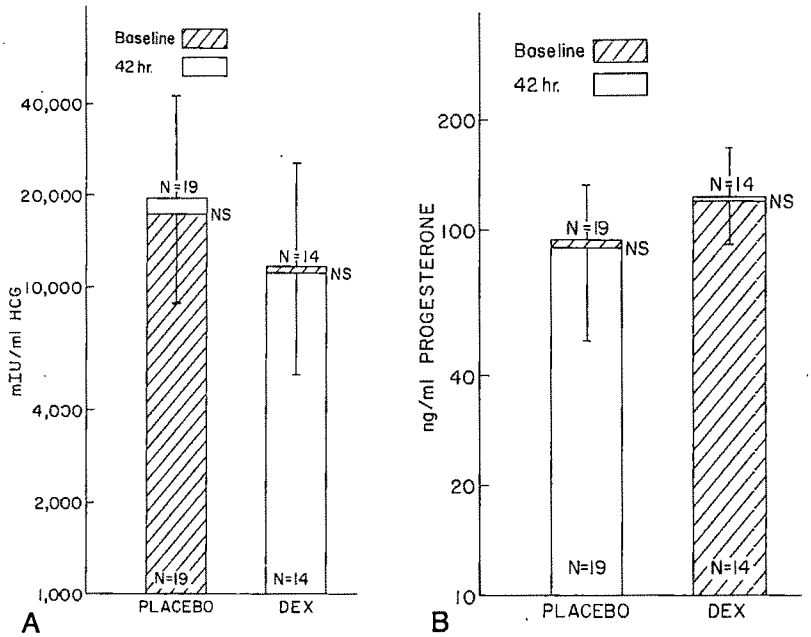


Fig. 2. Maternal hCG **(A)** and progesterone **(B)** concentrations determined in baseline placebo-, baseline dexamethasone-, 42-hour placebo-, and 42-hour dexamethasone-treated subjects. Data shown are mean and SD calculated on log-transformed data (note log scale). Test for significance of difference between baseline and 42-hour samples in placebo- and dexamethasone *(DEX)*–treated subjects was calculated from paired *t* test, with each subject serving as her own control (*NS*, not significant).

placebo-, and 42-hour dexamethasone-treated subjects are presented in Fig. 2, *A* and *B*, respectively. To test for an effect of placebo or dexamethasone treatment we used the pairing between baseline and the 42-hour posttreatment samples to evaluate the paired differences (42-hour sample minus baseline) in the log transformed data, with each subject serving as her own control. There was no significant change from the baseline value observed in either the placebo-treated subjects or the dexamethasone-treated subjects for either maternal serum hCG or maternal serum progesterone level. There was no effect of fetal sex or of administration of antibiotics, ethanol, or isoxsuprine on the paired differences for maternal serum hCG or progesterone concentrations (data not shown).

Effect of treatment on maternal serum cortisol, estrone, estradiol, and estriol. Data analysis was restricted to the same groups of patients used above for analysis of hCG and progesterone concentrations. Analysis of the log transformed data for maternal serum cortisol, estrone, estradiol, and estriol by paired *t* test with each patient serving as her own control showed that there was no significant decrease from the baseline value for cortisol, estrone, estradiol, or estriol in the placebo-treated subjects. When each 42-hour value was expressed as a percent of baseline in the dexamethasone-treated subjects, the geometric mean was 20% for cortisol (95% confidence interval 14% to 28%; $p < 0.0001$), 42% for estrone (95% confidence interval 33% to 53%; $p < 0.0001$), 30% for estradiol (95% confidence interval 22% to 40%; $p < 0.0001$), and 41% for estriol (95% confidence interval 31% to 53%, $p < 0.0001$). There was no effect of fetal sex or of administration of antibiotics, ethanol, or isoxsuprine on the paired differences for maternal serum cortisol, estrone, estradiol, or estriol concentrations (data not shown).

Comment

The hormones investigated here can be divided into those known to be dependent on adrenocorticotropic hormone and those not known to be dependent on adrenocorticotropic hormone. Both cortisol and dehydroepiandrosterone sulfate are secreted almost entirely by the maternal or fetal adrenal, and both are dependent on adrenocorticotropic hormone. Since dehydroepiandrosterone sulfate and 16α-hydroxydehydroepiandrosterone sulfate (derived from dehydroepiandrosterone sulfate by 16α-hydroxylation in the liver or adrenal) are the principal precursors for estrone and for estradiol and estriol, respectively, in pregnancy, the administration of dexamethasone was expected to suppress the 42-hour values for cortisol, estrone, estradiol, and estriol by inhibiting both maternal and fetal adrenocorticotropic hormone secretion.[9, 11] On the other hand, since neither progesterone nor hCG is dependent on adrenal substrates, no effect of either placebo or dexamethasone administration would be anticipated on their secretion. One of the reasons that the current study was undertaken was the observation by Haning et al.[8] that administration of dehydroepiandrosterone sulfate to placental ex-

plants in vitro had inhibited the stimulatory effect of gonadotropin-releasing hormone on hCG secretion. Also, statistical analysis of the concentrations of hCG, estrogens, and progesterone in the maternal peripheral blood and the estimated masses of the fetal adrenal and the placental trophoblast had provided evidence of an inhibitory effect of the fetal adrenal gland on secretion of hCG.[7] We reasoned that if there were no direct action of dexamethasone itself on hCG secretion, administration of dexamethasone to pregnant women would be expected to stimulate hCG production by reducing the concentration of dehydroepiandrosterone sulfate in both maternal and fetal compartments.

The duration of our period of testing for an effect of dexamethasone administration on secretion of hCG was limited by the design of the original study, a multicenter, double-blind trial of dexamethasone versus placebo completed many years before we obtained access to the samples. The 42-hour time interval was selected for analysis because it was the last sample before the adrenal gland could be expected to recover from the suppressive effect of dexamethasone, on the basis of data for maternal serum cortisol and estradiol.[9] We found no evidence for an effect of dexamethasone on hCG secretion under the conditions of this study. Both direct inhibitory[12] and direct stimulatory[13] in vitro effects of glucocorticoids on hCG have been reported. Likewise, in experiments performed in this laboratory, even direct addition of 2 mmol/L dibutyryl cyclic adenosine monophosphate to placental explants in vitro has a delayed effect with the maximal result not achieved until day 6 in placentas from term pregnancies.[14] There was a relatively smaller effect of dibutyryl cyclic adenosine monophosphate in placental tissue obtained from 12- to 17-week pregnancies, and a statistically significant effect of 2 mmol/L dibutyryl cyclic adenosine monophosphate administration on hCG secretion could not be demonstrated until days 5 and 6, even though a trend was evident as early as day 2.[14] Thus it is possible that a longer period of treatment with dexamethasone would have produced an effect on the concentration of maternal serum hCG. It is also possible that a direct effect of dexamethasone on the placenta could have obscured an effect produced by reduction in the concentration of circulating dehydroepiandrosterone sulfate. However, it is obvious that we have not provided any evidence for the anticipated stimulatory effect on hCG secretion of reducing the concentration of dehydroepiandrosterone sulfate in the present study.

Likewise, Grimshaw et al.[15] have demonstrated that dehydroepiandrosterone, estrone, androstenedione, and testosterone could all inhibit the conversion of pregnenolone to progesterone in vitro. However, the present study demonstrated no significant effect of dexamethasone on the concentration of progesterone in the maternal peripheral blood even though the dexamethasone produced decreases in estrone, estradiol, and estriol to be 42%, 30%, and 41% of baseline, respectively.

The sex of the fetus was demonstrated to modulate the maternal serum concentration of hCG in the present study. There was no demonstrable effect of fetal sex on maternal serum progesterone, cortisol, estrone, estradiol, or estriol concentration. Previous studies also have failed to demonstrate an effect of fetal sex on the maternal peripheral blood concentration of progesterone.[2] Higher concentrations of maternal serum hCG in female-bearing pregnancies than in male-bearing pregnancies have been reported at term by others,[2-4] and the placentas from female infants are known to have a higher content of hCG per unit weight of tissue at term[5,6] and to secrete more hCG in vitro per unit weight of tissue,[14] when compared with those from male infants. However, the reason for this phenomenon still awaits elucidation. The mean gestational age of the pregnancies in this study was 32 weeks. To our knowledge, this is the first study to demonstrate a statistically significant effect of sex on the concentration of maternal serum hCG this early in pregnancy. It is possible that the observed difference in the maternal peripheral blood hCG concentration between female-bearing and male-bearing pregnancies in the last trimester will provide a clue to understanding the mechanism modulating hCG in early pregnancy as well.

REFERENCES

1. Braunstein GD, Rasor JL, Engvall E, Wade ME. Interrelationships of human chorionic gonadotropin, human placental lactogen, and pregnancy-specific β_1-glycoprotein throughout normal human gestation. Am J Obstet Gynecol 1980;138:1205-13.
2. Broditsky RS, Reyes FI, Winter JSD, Faiman C. Serum human chorionic gonadotropin and progesterone patterns in the last trimester of pregnancy: relationship to fetal sex. Am J Obstet Gynecol 1975;121:238-41.
3. Penny R, Olambiwonnu NO, Frasier SD. Measurement of human chorionic gonadotropin (HCG) concentrations in paired maternal and cord sera using an assay specific for the beta subunit of HCG. Pediatrics 1976;58:110-4.
4. Danzer H, Braunstein GD, Rasor J, Forsythe A, Wade ME. Maternal serum human chorionic gonadotropin concentrations and fetal sex prediction. Fertil Steril 1980;34:336-40.
5. Hobson B, Wide L. Chorionic gonadotrophin in the human placenta in relation to the sex of the foetus at term. J Endocrinol 1974;60:75-80.
6. Wide L, Hobson B. Relationship between the sex of the foetus and the amount of human chorionic gonadotrophin in placentae from the 10th to the 20th week. J Endocrinol 1974;61:75-81.
7. Haning RV Jr, Choi L, Curet LB, et al. Interrelationships among human chorionic gonadotropin (hCG), 17β-estradiol, progesterone, and estriol in maternal serum: evidence for an inhibitory effect of the fetal adrenal on secretion of hCG. J Clin Endocrinol Metab 1983;56:1188-94.
8. Haning RV Jr, Choi L, Kiggens AJ, Kuzma DL, Summerville JW. Effects of dibutyryl adenosine 3′,5′-monophosphate, luteinizing hormone-releasing hormone, and aromatase inhibitor on simultaneous outputs of progesterone, 17β-estradiol, and human chorionic go-

nadotropin by term placental explants. J Clin Endocrinol Metab 1982;55:213-8.
9. Collaborative Group on Antenatal Steroid Therapy. Effect of antenatal dexamethasone administration on the prevention of respiratory distress syndrome. AM J OBSTET GYNECOL 1981;141:276-87.
10. Ryan BF, Joiner BL, Ryan TA Jr. Minitab handbook. 2nd ed. Boston: Duxbury Press, 1985:206.
11. Kauppila A, Tuimala R, Ylikorkala O, Reinila M, Ylostalo P. Placental steroid synthesis from DHEAS during dexamethasone therapy. Obstet Gynecol 1979;54:39-42.
12. Toshiaki M, Chou JY. Regulation of human chorionic gonadotropin synthesis in cultured human placental cells by glucocorticoids. Endocrinology 1981;109:888-92.
13. Wilson EA, Jawad MJ. Stimulation of human chorionic gonadotropin secretion by glucocorticoids. AM J OBSTET GYNECOL 1982;142:344-9.
14. Haning RV Jr, Breault PH, Desilva MV, Hackett RJ. Effects of fetal sex, stage of gestation, dibutyryl cyclic adenosine monophosphate, gonadotropin-releasing hormone on secretion of human chorionic gonadotropin by placental explants in vitro. AM J OBSTET GYNECOL 1988; 159:1332-7.
15. Grimshaw RN, Mitchell BF, Challis JG. Steroid modulation of pregnenolone to progesterone conversion by human placental cells in vitro. AM J OBSTET GYNECOL 1983;145:234-8.

High glucose levels decrease proliferation of cultured human fetal cells from placenta

D. Michael Nelson, MD, PhD, and Edward M. Curran, MS
St. Louis, Missouri

We used the placenta as a source of undifferentiated cells to study the effect high glucose levels can have on human fetal cell proliferation in vitro. Cells were subcultured in a modified minimum essential medium with 10% fetal bovine serum containing either 5.5 mmol/L (100 mg/dl) D-glucose (control), 11 mmol/L (200 mg/dl) D-glucose, or 22 mmol/L (400 mg/dl) D-glucose. Cells grown in mannitol-containing media were used as controls for osmolality. After 3 and 7 days' growth in different media, the labeling index was determined by autoradiographic analysis, and cell numbers were determined with a Coulter counter. The labeling indices for cells grown 3 days in 11 or 22 mmol/L D-glucose were 89% ($p < 0.002$) and 84% ($p < 0.001$), respectively, of control cells grown in 5.5 mmol/L D-glucose. After 7 days' growth, the labeling indices of cells grown in 11 or 22 mmol/L D-glucose were 84% ($p < 0.002$) and 70% ($p < 0.001$), respectively, of cells grown in 5.5 mmol/L D-glucose media. There was a significant decrease in the number of cells present at both 3 and 7 days in cultures grown in 22 mmol/L D-glucose compared with control. We conclude that a few day's exposure to high glucose levels can have an effect on proliferation of human placental cells in vitro. We suggest that a glucose effect on proliferation of other cells derived from the products of conception might be one mechanism contributing to abnormal development in some pregnancies of diabetic women. (AM J OBSTET GYNECOL 1989;161:1553-8.)

Key words: High glucose, cell proliferation, placenta

Studies of pregnant diabetic women who are hyperglycemic during organogenesis[1] and those who have increased glycosylated hemoglobin levels in the first trimester of pregnancy[2] suggest that elevated glucose levels adversely affect the developing conceptus. Hyperglycemia in pregnant diabetic women increases the risk for spontaneous abortion,[3, 4] anomaly development,[2, 5] and growth delay in the first trimester.[6] The mechanisms by which elevated glucose levels adversely affect the products of conception during morphogenesis are unknown. We have used the placenta as a source of rapidly proliferating, undifferentiated cells to study the effect of high glucose concentrations on proliferation of cultured human cells derived from the products of conception.

From the Department of Obstetrics and Gynecology, The Jewish Hospital of St. Louis and Washington University School of Medicine.
Supported by grants from the Jewish Hospital of St. Louis, the Faye Beth and Charles S. Baer Endowment Fund, and National Institute of Child Health and Human Development grant HD-22913.
Presented at the Thirty-fourth Annual Meeting of the Society for Gynecologic Investigation, Atlanta, Georgia, March 18-21, 1987.
Reprint requests: D. Michael Nelson, MD, PhD, Department of Obstetrics and Gynecology, Washington University School of Medicine, The Jewish Hospital of St. Louis, 216 S. Kingshighway, St. Louis, MO 63110.
6/6/15962

Material and methods

Cell cultures. Placentas were obtained from normal term pregnancies in women who had negative results of screening for gestational diabetes. All deliveries were by repeat cesarean section with patients under epidural anesthesia after hydration with 15 ml/kg of lactated Ringer's fluid.

Primary cell cultures were obtained according to a previously published method.[7] Briefly, villous tissue was

rinsed with a modified Eagle's minimum essential medium and treated 10 minutes at 37° C with the enzyme dispase (Collaborative Research, Inc., Lexington, Mass.); cells in the supernatant were harvested. Most cells stained cytochemically for cytokeratin intermediate filaments indicating they were trophoblastic cells, but 8% of the cells were vimentin positive and Factor VIII negative.[7] The latter population was an undifferentiated stromal cell that rapidly proliferated during long-term culture without density limitation after reaching confluence. These cells were grown in a humidified 5% carbon dioxide in air atmosphere at 37° C in modified Eagle's minimum essential medium with 100 U/ml penicillin G, 100 μg/ml streptomycin sulfate, 0.25 μg/ml Fungizone, and 10% (vol/vol) chemically defined fetal bovine serum (Hyclone, Logan, Utah) containing an average of 7.3 μU/ml insulin and 102 μg/ml of lactic acid.

Labeling index. Cells from the fourth passage of four primary cultures were collected after detachment with 0.01% (wt/vol) trypsin (type XII from bovine pancreas, Sigma Chemical Co., St. Louis), and viability was >95% after exposure to trypan blue. Cells were subcultured on slide flasks (Nunc, Schaumburg, Ill.) or Petri dishes (Corning Glass Works, Corning, N.Y.) at 2.0×10^4 cells per 21 cm^2 in 4 ml modified Eagle's minimum essential medium with 10% fetal bovine serum (vol/vol) containing 5.5 mmol/L (100 mg/dl) D-glucose and antibiotic-antimycotic solution. After 24 hours, the cells were rinsed with fresh medium to eliminate unattached cells, and quadruplicate slide flasks or Petri dishes were established for each of the differential treatments: modified Eagle's minimum essential medium plus 10% fetal bovine serum containing either 5.5 mmol/L (100 mg/dl) D-glucose (control media), 11 mmol/L (200 mg/dl) D-glucose, 22 mmol/L (400 mg/dl) D-glucose, 5.5 mmol/L D-glucose plus 5.5 mmol/L mannitol, or 5.5 mmol/L D-glucose plus 16.5 mmol/L mannitol. Media osmolality and glucose levels were determined before and after incubation with cells. Triplicate slide flasks from each of the five media treatments were rinsed with modified Eagle's minimum essential medium after 3 or 7 days of growth, incubated in 2 ml modified Eagle's minimum essential medium containing 0.5 μCi/ml [^{3}H]thymidine (15.7 Ci/mmol/L; New England Nuclear, Boston) for 3 hours at 37° C, rinsed twice with ice-cold modified Eagle's minimum essential medium, and either fixed in situ with methanol or detached and embedded in plastic as a cell pellet for autoradiographic analysis as previously described.[8] The rinse procedure was established to remove unincorporated [^{3}H]thymidine. In the first of the four labeling index experiments, autoradiographic analysis of cells grown 3 or 7 days in the different media was done after fixation in situ on their slide flasks.

The cells grown 3 days were easily evaluated, but the higher density of cells present after 7 days' growth made some areas of the slide flask difficult to analyze in this experiment. Thus cells from day 7 in the subsequent three experiments were detached from their slide flasks and embedded in plastic as a cell pellet for autoradiographic analysis. The number of fields of nuclei available for analysis (and thus the total number of nuclei present) was less for the experiments done with plastic sections than for the three done with in situ fixed specimens.

An observer, who was blinded to the experimental conditions, examined a series of equidistant fields of each autoradiogram, recording the total number of nuclei and nuclei with ≥5 overlying grains. The labeling index is the number of labeled nuclei divided by the total number of nuclei counted.

Cell numbers during growth in high-glucose media. Cells from four different primary cultures were subcultured onto 32 Petri dishes at 2.0×10^4 cells per 60 mm dish (21 cm^2) containing 4 ml modified Eagle's minimum essential medium with 10% fetal bovine serum, 5.5 mmol/L D-glucose, and antibiotic-antimycotic solution. After 24 hours to allow cell attachment, medium in each of eight dishes was changed in a blinded manner to medium containing either 5.5, 11, or 22 mmol/L D-glucose. Cells cultured in 5.5 mmol/L D-glucose plus 16.5 mmol/L mannitol provided the control for osmolality. Media were replaced again at 2, 4, and 6 days. On the third or seventh day after the differential treatment conditions were established, medium from each dish was collected and assessed for the presence of detached, nonviable cells. Attached cells in the same dishes were detached with trypsin, assessed for viability by trypan blue exclusion, and counted in a ZBF model Coulter counter equipped with a 100 μm aperture.

Statistical analysis. The number of labeled and unlabeled nuclei in the control and experimental treatments was compared by χ^2 analysis. The differences in number of cells in control cultures and cultures exposed to differential treatments were evaluated by analysis of variance for individual experiments and by the Wilcoxon signed-rank test for the series, since the cell numbers for different placentas were not normally distributed populations. Values are reported as mean ± SD. A computer software package was used for analysis (Abstat, Anderson Bell, Parker, Colo.) with $p < 0.01$ for significance because multiple comparisons were made.

Results

Characteristics of media and cells. Osmolality was 294 ± 2 (mean ± SD) mmol/kg in media containing 5.5 mmol/L D-glucose, 300 ± 3 (mean ± SD) mmol/kg in media containing 11.0 mmol/L D-glucose,

Table I. Labeling index of cells grown 3 days in normal or high-glucose media

Medium (mmol/L)		*Total nuclei counted*	*Labeling index*	*Difference from control labeling index*
Glucose	*Mannitol*			
5.5 (control)	0	6576	0.1863 ± 0.0378	–
11.0	0	7359	0.1650 ± 0.0337	−11%*†
22.0	0	6618	0.1561 ± 0.0366	−16%‡§‖
5.5	5.5	7233	0.1752 ± 0.0334	−6%
5.5	16.5	7377	0.1803 ± 0.0429	−3%

Cells were subcultured at 2.0×10^4 cells per 21 cm^2 of culture surface, grown 3 days in normal or high-glucose media, exposed to [^{3}H]-thymidine, and processed in situ for autoradiographic analysis by an observer blinded to treatment conditions as described in Material and methods. The labeling index is the ratio of labeled nuclei divided by total nuclei counted. Results are mean (± SD) from $n = 12$ in four experiments.

*$p < 0.002$, compared with control.

†$p < 0.02$, compared with 16.5 mmol/L mannitol.

‡$p < 0.001$, compared with control.

§$p < 0.004$, compared with 5.5 mmol/L mannitol.

‖$p < 0.001$, compared with 16.5 mmol/L mannitol.

Table II. Labeling index of cells grown 7 days in normal or high-glucose media

Medium (mmol/L)		*Total nuclei counted*	*Labeling index*	*Difference from control labeling index*
Glucose	*Mannitol*			
5.5 (control)	0	4368	0.1694 ± 0.0162	–
11.0	0	3598	0.1431 ± 0.0295	−16%*
22.0	0	2284	0.1191 ± 0.0211	−30%†‡
5.5	5.5	2404	0.1481 ± 0.0153	−13%§
5.5	16.5	3104	0.1453 ± 0.0116	−14%‖

Cells were subcultured at 2.0×10^4 cells per 21 cm^2, grown 7 days in normal or high-glucose media, exposed to [^{3}H]-thymidine, and processed for autoradiographic analysis by an observer blinded to treatment conditions as described in Material and methods. The labeling index is the ratio of labeled nuclei divided by total nuclei counted. Results are mean (±SD) from $n = 12$ in four experiments.

*$p < 0.002$, compared with control.

†$p < 0.0001$, compared with control.

‡$p < 0.005$, compared with 11 mmol/L glucose, 5.5 mmol/L mannitol, and 16.5 mmol/L mannitol.

§$p < 0.025$, compared with control.

‖$p < 0.004$, compared with control.

300 ± 2 (mean ± SD) mmol/kg in media containing 5.5 mmol/L D-glucose and 5.5 mmol/L mannitol, 312 ± 2 (mean ± SD) mmol/kg in media containing 22.0 mmol/L D-glucose, and 311 ± 3 (mean ± SD) mmol/kg in media containing 5.5 mmol/L D-glucose and 16.5 mmol/L mannitol. No morphologic differences could be detected by phase-contrast microscopy in the placental cell cultures grown in different media. Glucose concentrations remained within 4% of the initial glucose level of the media. Cell viability by trypan blue exclusion remained at >95% for all cultures.

Labeling index. Background grain counts on control slides were ≤1/500 μm. Preliminary experiments established that the use of ≥5 grains overlying a nucleus clearly differentiated labeled from unlabeled nuclei. In addition, unlabeled cells had background grain counts, thereby excluding dexoyribonucleic acid repair as a significant contributor to [^{3}H]thymidine incorporation.

An alteration in cell proliferation in cultures with high glucose levels was identified by studying the labeling index of cells grown in control medium with 5.5 mmol/L D-glucose compared with cells grown in media containing either 11 or 22 mmol/L D-glucose. Parallel cultures of cells grown in the presence of mannitol were used to control for the change in osmolality that accompanied the high-glucose media. The labeling indices of cells grown with the high glucose levels were significantly lower after 3 days (Table I) and 7 days (Table II) in culture than those of control cells grown in 5.5 mmol/L D-glucose medium without mannitol. The effect was greater after 7 days' growth in high levels of glucose than after 3 days' exposure, and the effect of 22 mmol/L D-glucose was greater than the effect of 11 mmol/L D-glucose. The lower labeling index for cells grown 3 days in the high-glucose conditions could not be solely attributed to the higher osmolality of the media since no significant difference in labeling index was present in cells grown 3 days in

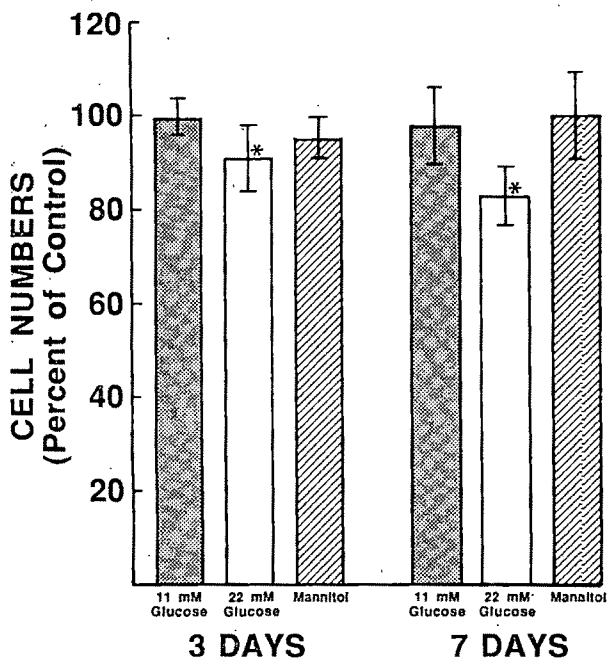


Fig. 1. Differences in cell numbers in cultures grown 3 or 7 days in hyperglycemic or mannitol-containing (16.5 mmol/L) media compared with control media with 5.5 mmol/L glucose. Each percentage is the mean ± SD calculated from $n = 16$ from four experiments. *Asterisk*, $p < 0.01$ compared with control.

mannitol-containing media when compared with cells grown in 5.5 mmol/L D-glucose. However, a part of the decrease in the labeling index observed in cells grown 7 days in the high-glucose conditions was attributable to a change in osmolality since the labeling index of cells grown in mannitol-containing media for 7 days also had a labeling index that was significantly lower than that of control cells.

Cell numbers during growth in high-glucose media. Cells to be exposed to the different treatments were initially grown 24 hours after subculture in modified Eagle's minimum essential medium with 10% fetal bovine serum containing 5.5 mmol/L D-glucose. Medium was then replaced with one of the study media. This approach avoided any effect of high glucose level or high osmolality on plating efficiency. The number of cells in cultures grown 3 or 7 days in 11 mmol/L D-glucose was not significantly different from the number of cells in the control medium with 5.5 mmol/L D-glucose (Fig. 1). However, there was a significant ($p < 0.01$) decrease in the number of cells present in cultures grown 3 or 7 days in medium with 22 mmol/L D-glucose when compared with those in control medium (Fig. 1). The average number of cells present in control media after 3 or 7 days' growth was 6.21 ± 1.3 (mean ± SD) × 10^5 and 4.20 ± 1.10 (mean ± SD) × 10^6, respectively ($n = 16$ from four experiments). The numbers of cells in the 22 mmol/L D-glucose cultures ranged between 1.0% and 25.0% lower than control after 3 days' growth, with an average decrease of 9.7%. The decrease in numbers of cells after 7 days ranged between 4.7% and 26.8% for the 22 mmol/L D-glucose cultures compared with control, with an average decrease of 17.8%. There was no difference in the number of cells in cultures grown in the presence of 16.5 mmol/L mannitol compared with control cultures grown in 5.5 mmol/L D-glucose (Fig. 1).

The number of detached, nonviable cells present in the media at the time of media changes was <500 cells in all cultures. Viability of attached cells by trypan blue exclusion remained at >95% in all cultures with no differences attributable to the growth medium.

Comment

These data indicate that proliferation of cultured human fetal cells from term placentas is altered by short-term exposure to high glucose concentrations. The effect on the labeling index was dependent on both the glucose concentration and the time of exposure to the high glucose levels. The glucose caused a decrease in number of cells present in the cultures exposed to the 22 mmol/L (400 mg/dl) D-glucose concentration when compared with those in control cultures with 5.5 mmol/L (100 mg/dl) D-glucose. Whether the antiproliferative effect of glucose resulted from an alteration of the cell cycle, the growth fraction, or both remains to be determined. We conclude that a few days' exposure to high glucose levels can have an effect on proliferation of human placental cells in vitro. We suggest that a glucose effect on proliferation of other cells derived from the products of conception might be one mechanism contributing to abnormal development in some pregnancies of diabetic women.

Average blood glucose levels for nondiabetic pregnant patients are in the range of 5.5 mmol/L (100 mg/dl) D-glucose.[9, 10] We selected 11 mmol/L (200 mg/dl) as an average high glucose level and 22 mmol/L (400 mg/dl) as an extreme glucose level that realistically can be observed in some diabetic women during pregnancy.

Malformations characteristic of offspring from diabetic women are established by the seventh week of gestation.[11] The specific insult that causes malformations is not organ specific since a variety of organ systems are affected by anomalies.[5] To expect that a complex problem such as anomaly development will be explained by a single mechanism is unrealistic.[12, 13] However, cell proliferation is a prerequisite for normal development throughout pregnancy, and small changes in cell replication activity caused by a teratogenic agent during critical stages of fetal development could affect morphogenesis. We selected the labor

intensive approach of autoradiographic analysis of [^{3}H]thymidine incorporation as the best method to identify small but significant differences in cell proliferation activity.[14] Using autoradiographic analysis, we also avoided two criticisms raised when [^{3}H]thymidine is used for biochemical studies of cell proliferation[14]: Our results cannot be due to glucose-induced alterations in [^{3}H]thymidine transport by the cells because a change in thymidine pools would change only the density of labeling of a cell, not the number of cells labeled; and our results are not due to a glucose-induced increase in deoxyribonucleic acid repair[15] since this would appear as a generalized increase in radioactive background in all cells.

Experimental studies of anomaly development in pregnancies complicated by diabetes mellitus have generally used animal models.[16-24] Animal models offer the advantage of in vivo study of environmental influences that might affect growth and differentiation of embryos, but caution is required when extrapolating results in animals to answer questions about human development. Our cell system is not proposed to be a model of human embryogenesis. The novelty of our cell model resides in the use of undifferentiated, rapidly proliferating human cells that are derived from the products of conception to study the effect high glucose levels can have on cell proliferation in a controlled environment. The disadvantages of our model include the use of term cells instead of first-trimester cells and the use of placental cells instead of cells from the embryo. We therefore recognize that our conclusion that high glucose levels affect the proliferation of cultured human fetal cells from term placentas may not apply to human fetal cells obtained from the embryo in the first trimester where factors regulating growth and development may be very different. However, our study is the first to show that a high glucose level itself has a direct effect on cell proliferation in a human fetal cell system that is not solely due to a change in osmolality. The only other report to use human fetal cells to study glucose effects on cell proliferation used umbilical vein cells.[25] Cells cultured in 20 mmol/L D-glucose or 20 mmol/L mannitol were delayed in reaching confluence when compared with cells grown in 5 mmol/L D-glucose. This was due in part to a decrease in the mitotic index and in part to excess cellular death. These results were not specific to the high glucose levels but were related to the change in osmolality occurring in both the high-glucose and mannitol-containing media.

Cell death did not influence our results since viability as measured by trypan blue exclusion was the same in all cultures and the number of detached, nonviable cells was negligible. We also found a labeling index lower than that in control cultures in cells grown 3 or 7 days in 11 mmol/L D-glucose or 7 days in mannitol. However, the number of cells in these cultures were not significantly different from control. The growth of our cells was not density limited, and growth beyond 7 days in culture resulted in marked cell piling. This phenomenon created irreversible cell clumping and damage when we attempted to detach cells to determine their numbers at later time points beyond 7 days in culture. We were thus not able to determine if the number of cells in the 11 mmol/L D-glucose or mannitol-containing media would become significantly lower than those in control after growth beyond 7 days.

A derangement in maternal metabolism is a causative factor for developmental abnormalities in the offspring of diabetic women.[12, 13] Hyperglycemia is the marker of decompensated diabetes most easily assessed clinically and most commonly associated with anomaly development.[1, 2] Associated derangements of maternal metabolism implicated in anomaly development include hyperketonemia,[19] somatomedin inhibitors,[20] alterations of glycolysis,[21] hypoglycemia,[22] alterations of trace metals,[23] and enhanced synthesis of hyaluronic acid.[24] Recent studies suggest a role for arachidonic acid,[16, 17] myoinositol,[26] and glycolytic substrates[21] for anomaly development in experimental animal diabetes. Ely[27] proposed that hyperglycemia may inhibit mitosis by lowering intracellular ascorbic acid levels while raising plasma dehydroascorbic acid levels. The molecular mediators involved in the antiproliferative effect we associated with high glucose levels are unknown, but further studies using our cell system should help to identify such mediators.

We are grateful to Dr. Ronald C. Strickler and Dr. Donna R. Farmer for their helpful reviews.

REFERENCES

1. Fuhrmann K, Reiher H, Semmler K, Fischer F, Fischer M, Glockner E. Prevention of congenital malformations in infants of insulin-dependent diabetic mothers. Diabetes Care 1983;6:219-23.
2. Miller E, Hare JW, Cloherty JP, et al. Elevated maternal hemoglobin A_{1c} in early pregnancy and major congenital anomalies in infants of diabetic mothers. N Engl J Med 1981;304:1331-4.
3. Mills JL, Simpson JL, Driscoll SG, et al. Incidence of spontaneous abortion among normal women and insulin-dependent women whose pregnancies were identified within 21 days of conception. N Engl J Med 1988; 319:1617-23.
4. Miodovnik M, Mimouni F, Tsang RC, Ammar E, Kaplan L, Siddiqi TA. Glycemic control and spontaneous abortion in insulin-dependent diabetic women. Obstet Gynecol 1986;68:366-9.
5. Kucera J. Rate and type of congenital anomalies among offspring of diabetic women. J Reprod Med 1971;7:61-70.
6. Pedersen JF, Molsted-Pedersen L. Early fetal growth delay detected by ultrasound marks increased risk of congenital malformation in diabetic pregnancy. Br Med J 1981; 283:269-71.
7. Nelson DM, Walsh SW. Thromboxane and prostacyclin

production by different compartments of the human placental villus. J Clin Endocrinol Metab 1989;68:676-83.
8. Nelson DM, Enders AC, King BF. Cytological events involved in protein synthesis in cellular and syncytial trophoblast of human placenta. An electron microscope autoradiographic study of ^{3}H-leucine incorporation. J Cell Biol 1978;76:400-17.
9. Cousins L, Rigg L, Hollingsworth D, Brink G, Aurand J, Yen SSC. The 24-hour excursion and diurnal rhythm of glucose, insulin, and C-peptide in normal pregnancy. Am J Obstet Gynecol 1980;136:483-8.
10. Gillmer MDG, Beard RW, Brooke FM, Oakley NW. Carbohydrate metabolism in pregnancy. Part 1. Diurnal plasma glucose profile in normal and diabetic women. Br Med J 1975;3:399-402.
11. Mills JL, Baker L, Goldman AS. Malformations in infants of diabetic mothers occur before the seventh gestational week. Implications for treatment. Diabetes. 1979;28: 292-3.
12. Sadler JW, Hunter ES, Wynn RE, Phillips LS. Evidence for multifactorial origin of diabetes induced embryopathies. Diabetes 1989;38:70-4.
13. Naftolin F, Diamond MP, Pinter E, Reece EA, Sanyal MK. A hypothesis concerning the general basis of organogenetic congenital anomalies. Am J Obstet Gynecol 1987; 157:1-4.
14. Wright N, Alison M. Methodology in epithelial cell kinetics. In: Wright N, Alison M, eds. The biology of epithelial cell populations. London: Clarendon Press, 1984: 97-202.
15. Lorenzi M, Montisano DF, Toledo S, Barrieux A. High glucose induces DNA damage in cultured human endothelial cells. J Clin Invest 1986;77:322-5.
16. Reece EA, Pinter E, Leranth CZ, et al. Ultrastructural analysis of malformations of the embryonic neural axis induced by *in vitro* hyperglycemic conditions. Teratology 1985;32:363-73.
17. Pinter E, Reece EA, Leranth CZ, et al. Arachidonic acid prevents hyperglycemia-associated yolk sac damage and embryopathy. Am J Obstet Gynecol 1986;155:691-702.
18. Sadler TW, Horton WE Jr. Effects of maternal diabetes on early embryogenesis: the role of insulin and insulin therapy. Diabetes 1983;32:1070-4.
19. Horton WE Jr, Sadler TW. Effects of maternal diabetes on early embryogenesis. Alterations in morphogenesis produced by the ketone body, β-hydroxybutyrate. Diabetes 1983;32:610-6.
20. Balkan W, Phillips LS, Goldstein S, Sadler TW. Potential role of somatomedin inhibitors in the production of diabetic embryopathies. Teratology 1988;37:271-82.
21. Freinkel N, Lewis NJ, Akazawa S, Roth SI, Gorman L. The honeybee syndrome—implications of the teratogenicity of mannose in rat-embryo culture. N Engl J Med 1984;310:223-30.
22. Buchanan TA, Schemmer JK, Freinkel N. Embryotoxic effects of brief maternal insulin-hypoglycemia during organogenesis in the rat. J Clin Invest 1986;78:643-9.
23. Uriu-Hare JY, Stern JS, Reaven GM, Keen CL. The effect of maternal diabetes on trace element status and fetal development in the rat. Diabetes 1985;34:1031-40.
24. Slourish M, Morris GM. Glycosaminoglycan synthesis in rat embryos during the formation of the primary mesenchyme and neural folds. Dev Biol 1985;57:75-86.
25. Lorenzi M, Cagliero E, Toledo S. Glucose toxicity for human endothelial cells in culture. Delayed replication, disturbed cell cycle, and accelerated death. Diabetes 1985; 34:621-7.
26. Sussman I, Matschinsky FM. Diabetes affects sorbitol and myo-inositol levels of neuroectodermal tissue during embryogenesis in rat. Diabetes 1988;37:974-81.
27. Ely JTA. Hyperglycemia and major congenital anomalies. N Engl J Med 1981;305:833.

Stimuli for fetal swallowing: Systemic factors

Michael G. Ross, MD, Dan J. Sherman, MD, M. Gore Ervin, PhD, Linda Day, and James Humme

Torrance and Los Angeles, California, and Tel Aviv, Israel

Although the fetal gastrointestinal tract is believed to be a major site of amniotic fluid absorption, there is little information with regard to the acute regulation of fetal swallowing. A model for the study of ovine fetal swallowing was developed to incorporate electromyogams, an esophageal flow probe, and a computer data acquisition and analysis program. The fetal swallowing responses to two primary thirst stimuli, plasma hyperosmolality and angiotensin II, were studied. On alternate days, chronically prepared fetal lambs (131 ± 2 days) received an intravenous infusion of angiotensin II (100 ng/kg per minute) or bolus injections (3 ml) of 0.15 and 3.97 mol/L saline solution. In response to the angiotensin II infusion, fetal systolic (49.3 to 64.7 mm Hg; $p < 0.05$) and diastolic (31.1 to 40.5 mm Hg; $p < 0.05$) blood pressures significantly increased. However, fetal swallowing did not change from basal rates of 0.85 swallows per minute and a net esophageal flow of 0.98 ml/min. In response to the injection of 3.97 mol/L saline solution, fetal plasma osmolality increased (292 to 306 mOsm; $p < 0.05$) and subsequently decreased to 300 mOsm at 15 minutes after the injection. Within 1 minute after injection of the hypertonic saline solution, fetal swallowing activity (6.0 swallows per minute; $p < 0.05$) and net esophageal flow (2.4 ml/min; $p < 0.05$) significantly increased. Swallowing returned to basal values within 5 minutes after the injection. The data indicate that ovine fetal responses to osmolar thirst challenges are intact at 130 days' gestation. Fetal swallowing and thus amniotic fluid volume may be affected by fetal responses to in utero stimuli. (AM J OBSTET GYNECOL 1989;161:1559-65.)

Key words: Ovine, thirst, gastrointestinal tract

Swallowing may be of great importance to the developing fetus. Both the ovine and human fetuses have been estimated to swallow 500 to 1000 ml per day of amniotic fluid and likely additional volumes of pulmonary and salivary secretions.[1-7] Accordingly, alterations in amniotic fluid volume have been attributed to abnormal swallowing in human fetuses[8, 9] and a direct relationship between fetal swallowing and amniotic fluid volume has been shown in animal models.[10-12] Furthermore, fetal swallowing of amniotic fluid may be essential for normal gastrointestinal development and maturation.[13] Yet, the regulation of fetal thirst and swallowing is largely unexplored.

In the adult mammal there appear to be two stimuli for primary (thirst-mediated) drinking, one arising from cellular dehydration and the other from extracellular volume depletion.[14] Cellular dehydration, or perhaps cell shrinkage, is sensed centrally in areas near the preoptic nucleus of the hypothalamus and the tissue surrounding the anteroventral portion of the third ventricle.[15] These parapreoptic neural regions may respond to either plasma or cerebrospinal fluid osmolality changes, mediated in part by the cellular permeability to the osmotic agent. Notably, these regions are in close proximity to hypothalamic nuclei synthesizing arginine vasopressin. Extracellular thirst stimulation appears to be mediated principally by neuroendocrine factors. In particular angiotensin II, secreted in response to perceived intravascular volume depletion, may act either systemically or centrally to stimulate thirst.[15]

The ontogeny of the physiologic and endocrine mechanisms that regulate swallowing has received only limited study, primarily in the newborn rat.[17] The fetus develops in an aquatic environment and thus requires only reflex or "secondary" drinking for the ingestion of fluid. It is possible that "primary" drinking mechanisms do not develop in utero. However, previous studies indicate a significant in utero maturation process of endocrine systems that mediate renal antidiuresis, the unconscious counterpart to thirst.[18] Thus it is likely that thirst mechanisms also mature. To initiate studies of the regulation of fetal "thirst," we developed a model for the quantification of swallowing activity and volume in the ovine fetus. Fetal responses to two primary systemic thirst stimuli, plasma hyperosmolality and angiotensin II, were studied. The results suggest an intact "thirst" mechanism in the late gestation ovine fetus.

From the Departments of Obstetrics and Gynecology and Pediatrics, Harbor–University of California at Los Angeles Medical Center, Torrance, the Cedars Sinai Hospital, University of California at Los Angeles School of Medicine, Los Angeles, and Assaf-Harafe Medical Center, Tel Aviv.

Supported in part by Grant Nos. HD00744 and HD 06335 from the National Institute of Child Health and Human Development and Grant No. 5-590 from the March of Dimes Birth Defects Foundation.

Presented in part at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.

Reprint requests: Michael G. Ross, MD, Harbor–UCLA Medical Center, RB1, 1124 W. Carson St., Torrance, CA 90502.

6/6/16242

Animals and methods

Six chronically instrumented Western crossbred ewes with singleton fetuses were studied. Guidelines for the care and use of animals approved by Harbor–University of California at Los Angeles Medical Center were followed in all studies. Five experiments were performed to quantify the swallowing response to angiotensin II, and four experiments studied the fetal swallowing activity in response to plasma hyperosmolality. Three animals had both studies performed, with the sequence of the angiotensin II or hypertonic saline solution study chosen at random. The mean gestational age for both studies was 131 ± 2 days. Animals were housed indoors in individual steel study cages and acclimated to a lighting regimen of 12-hour light and dark periods. Both food (alfalfa pellets) and water were available as desired, except for the withholding of food during the 24-hour period immediately preceding surgery.

Anesthesia for surgery was induced by an intramuscular injection of ketamine hydrochloride (800 mg) plus atropine sulfate (300 mg) and was subsequently maintained by a continuous intravenous infusion of ketamine (600 mg/hour) plus locally applied lidocaine (Xylocaine) (1%) for all maternal and fetal incisions. The uterus was exposed by a midline abdominal incision and a small hysterotomy was performed to provide access to a single fetal hind limb. Polyethylene catheters (inner diameter, 1.0 mm; outer diameter, 1.8 mm) were placed in a fetal dorsal hind limb vein and artery and threaded to the inferior vena cava and aorta, respectively. Before hysterotomy closure, an amniotic fluid catheter (Corometrics Medical Systems, Wallingford, Conn.) was sutured to the distal tip of the fetal hind limb.

A second hysterotomy was made over the fetal neck and a 3 cm vertical incision was made in the fetal nuchal midline, extending cephalad from the thyroid cartilage. The skin was gently clamped to the uterus and the pharyngeal muscles were identified. Two wires (AS632, Cooner Wire, Chatsworth, Calif.) were inserted into one thyrohyoid muscle and a third ground wire was placed in the adjacent subcutaneous tissues. The nuchal esophagus was exposed and similarly prepared with bipolar electromyogram and ground electrodes. The skin incision and uterus were closed. The fetal thorax was positioned, the uterus was incised, and the fetal left hemithorax was entered via the eighth intercostal space. The peritoneum overlying the esophagus was incised for a distance of 2 cm cephalad to the diaphragm. Electromyogram electrode wires were placed on the thoracic esophagus and a 4 mm ultrasonographic flow probe (Transonic System Inc., Ithaca, N.Y.) was placed loosely around the esophagus, distal to the electromyogram wires. The fetal chest was closed in layers, the uterus and maternal incisions were repaired, and the catheters and electrodes were externally accessed via a maternal flank incision. Catheters also were placed in the maternal inferior vena cava and abdominal aorta via the femoral vein and artery in one leg. Fetal vascular catheters were maintained patent by filling the catheter dead space (1 ml) with 1000 U/ml sodium heparin; catheters were sealed with sterile plastic caps. Maternal vascular catheters were filled with heparinized saline solution (10 U/ml) and both fetal and maternal catheters were flushed daily. All animals were allowed a minimum of 5 days for postoperative recovery during which time the fetuses received intravenous infusions of methicillin sodium (100 mg) twice daily. Methicillin sodium (400 mg) and chloramphenicol (500 mg) also were administered into the maternal vein and amniotic cavity. After the studies, the animals were killed and the flow probe was removed from the fetal esophagus. There was no evidence of obstruction or constriction of the esophagus in any fetus.

Experimental protocol

Angiotensin infusion. All experiments were performed on unanesthetized ewes standing in the same individual cages in which they were maintained. During an initial 60-minute control period (time −60 to 0) fetal swallowing activity was continuously monitored. At 0 minutes, a fetal intravenous infusion of angiotensin II (100 ng/kg per minute) in 0.9% saline solution was administered at a rate of 0.05 ml/kg per minute and maintained for 60 minutes. The infusion was preceeded by a 1 ml bolus injection of angiotensin II (20 ng/kg). Infusion concentrations were determined on the basis of estimated fetal body weight (FBW) from the formula FBW (kg) = [0.096 × gestational age (days)] − 9.2228, as described by Robillard and Weitzman.[18]

At 60 minutes the angiotensin II was discontinued and the animals were monitored for an additional 60-minute recovery period (time 60 to 120 minutes). Fetal arterial blood samples (2.5 ml), obtained at −30 and 0 minutes of the control period, 15, 30, and 60 minutes of the angiotensin II infusion, and 75, 90, and 120 minutes of the recovery period were collected into iced tubes that contained 10 μl lithium heparin per milliliter of blood and were immediately centrifuged. Osmolality was measured in aliquots of the prepared plasma. An additional 0.5 ml of blood was collected into heparinized 1 ml syringes for immediate measurement of pH, P_{O_2}, and P_{CO_2}. The total volume of fetal blood withdrawn at each sampling was immediately replaced with an equal volume of heparinized maternal blood withdrawn before the study. Maternal arterial blood samples were drawn at −30, 0, 60, and 120 minutes for

measurement of hematocrit, pH, P_{O_2}, P_{CO_2}, and plasma osmolality. Fetal arterial blood pressure and heart rate were monitored continuously by means of a Beckman R-612 physiologic recorder (Beckman, Palo Alto, Calif.) with a Statham P23 transducer (Gould Inc., Oxnard, Calif.). Amniotic fluid pressure was subtracted from fetal arterial pressure.

Hypertonic saline solution infusion

On a different day, four of the animals were studied to determine the effect of acute plasma hyperosmolality. During an initial 60-minute control period (time −60 to 0 minutes) fetal swallowing activity was continuously monitored. Basal maternal and fetal blood samples (3.0 ml) were drawn at −30 and 0 minutes for hematocrit, pH, P_{O_2}, P_{CO_2}, plasma osmolality, and plasma arginine vasopressin as described above. At time 0, an intravenous bolus (3 ml) of isotonic (0.15 mol/L) saline solution was injected into the fetus. Repeat fetal blood samples were drawn at 1, 5, 10, and 15 minutes after the injection. At 15 minutes, an intravenous bolus of 3.97 mol/L saline solution was injected into the fetus and identical blood sampling was performed at 16, 20, 25, and 30 minutes. Preliminary studies had indicated that fetal plasma osmolality remained elevated for >30 minutes after injection of hypertonic saline solution. To avoid the potential effect of persistent plasma hyperosmolality on the response to isotonic saline solution, the injection of 0.15 mol/L saline solution was performed before the injection of 3.97 mol/L saline solution in all studies. Injections were not administered during spontaneous bouts of swallowing. In such cases, the injection was delayed until there was a return to basal activity. Repeat maternal blood samples were drawn at 15 and 30 minutes.

Analytical methods

Plasma arginine vasopressin levels were measured by radioimmunoassay of 1 ml plasma samples extracted on Sep-Pak (Waters Associates, Milford Mass.) columns. The extraction procedure of LaRochelle et al.[19] was modified to include 0.1% trifluoroacetic acid (in place of 4% acetic acid) and sample elution was accomplished with 2 ml of 50% methanol in 0.1% trifluoroacetic acid. Synthetic arginine vasopressin (obtained from Bachem, Torrance, Calif.) was used as the standard. The arginine vasopressin radioimmunoassay used in our laboratory is sensitive to 0.8 pg of arginine vasopressin per milliliter of plasma (0.16 pg/tube). The intra-assay and interassay coefficients of variation are 6% and 9%, respectively.

Blood pH, P_{O_2}, and P_{CO_2} values were measured at 39° C with a BM 33 MK 2-PHM 72 MK2 acid base analyzer system (Radiometer, Copenhagen, Denmark). Plasma osmolality was measured by freezing point depression on a digimatic osmometer (model 3MO, Advanced Instruments, Needham Heights, Mass.).

All electromyogram signals were directed into a Beckman R612 physiologic recorder with a Beckman voltage-pressure/pulse coupler (type 9853A). A common ground was formed from each of the electromyogram ground wires. The electrode impedence in muscle tissue was 1 to 15 kOhms at 1 kHz. The raw signal ranged from 0.1 to 1 mV. Amplifier impedence was 2×10^6 ohms, sensitivity range was 0.5 to 5 V/mm, and the common mode rejection ratio was 100 dB at 60 Hz. A low cut-off 5.3 Hz filter was present on the preamplifier with a narrow band (60 Hz) filter on the amplifier. A "leaky" integrator (moving average) with a variable time constant was inserted in series after preamplication of the raw electromyogram signal, which served to rectify and integrate the electromyogram signal. The integrated signal was further amplified (×100) and the output was plotted on the chart recorder and directed in parallel to both an FM tape recorder (Tandberg Instrument Recorder Series 100, Tandbergs Radiofabrikk, Oslo, Norway) and a microcomputer. With the use of an analog to digital–converter and Asyst (MacMillan Software Company, N.Y.) software, the integrated electromyograph signals were digitized at 50 samples per second per channel. Flowmeter signals (milliliters per minute) were processed without electronic integration and were similarly stored on the hard disc.

Flowmeter signals were displayed after the study. An analysis program calculated mean and SD of periods of zero flow (absent swallowing). With the use of mean and 3 SDs to represent the range of baseline noise, the program determined psoitive and negative thresholds for flow analysis and subsequently identified all positive (cranial to caudal) and negative (caudal to cranial) flows. Each flow was mathematically integrated by the computer, yielding actual volume per swallow. Net esophageal flow was calculated as positive flow minus negative flow. Similarly, electromyograph patterns were displayed and threshold voltages were selected for discrimination of noise versus signal. The analysis program scanned each channel denoting the time of onset, maximum amplitude, and the duration of each "spike" (single channel). "Swallows" were defined as a sequence of spikes from the thyrohyoid through the thoracic esophagus within time limitations determined for each fetus by analysis of wave form progression (Fig. 1).

The transit-time ultrasonographic flowmeter (T101, Transonic Systems Inc., Ithaca, N.Y.) produces linear bidirectional flow signals over a range of ±500 ml. The transit-time flowmeter is unaffected by vessel dimensions or misalignment and does not require a constric-

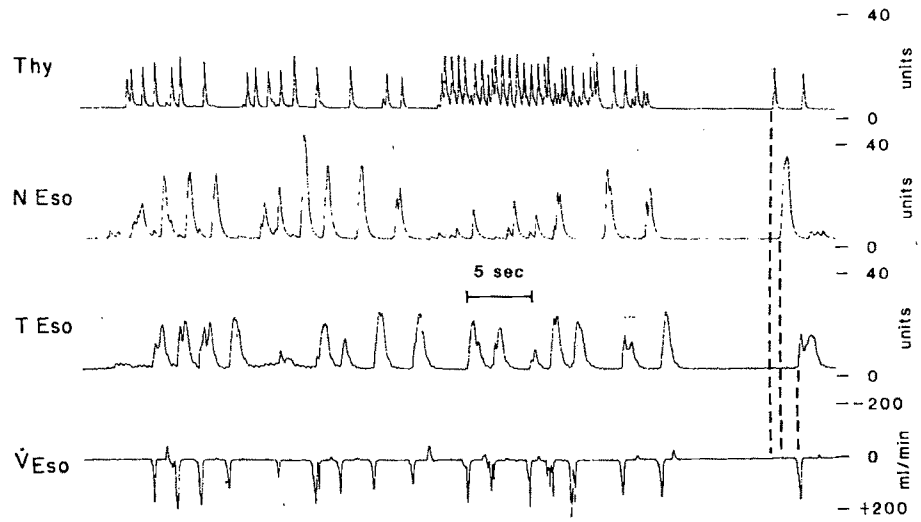


Fig. 1. Fetal swallowing demonstrating progression of electromyogram activity from thyrohyoid *(Thy)* to nuchal esophagus *(N Eso)* and thoracic esophagus *(T Eso)* associated with esophageal flow *(V Eso)*.

Table I. Mean (±SEM) fetal arterial values during control (60 to 0 minutes), angiotensin II (0 to 6 minutes), and recovery (60 to 120 minutes) periods ($n = 5$)

	Time (minutes)							
	−30	*0*	*15*	*30*	*60*	*75*	*90*	*120*
Hematocrit (%)	30.6 ± 1.6	30.4 ± 1.5	31.5 ± 1.4	32.4 ± 1.3*	32.3 ± 1.1*	32.4 ± 1.4*	30.7 ± 1.4	30.3 ± 1.2
pH	7.36 ± 0.01		7.34 ± 0.01		7.33 ± 0.02			7.35 ± 0.03
P_{O_2} (mm Hg)	17.6 ± 1.8		17.4 ± 1.4		14.8 ± 1.9			16.4 ± 2.1
P_{CO_2} (mm Hg)	44.0 ± 1.0		44.6 ± 1.8		43.8 ± 1.2			42.0 ± 2.2
Blood pressure (mm Hg)								
Systolic	49.5 ± 3.7	49.1 ± 3.5	65.4 ± 5.4*	64.8 ± 5.3*	63.8 ± 5.3*	48.6 ± 3.6	50.4 ± 3.2	49.0 ± 2.5
Diastolic	31.7 ± 3.5	30.5 ± 3.0	39.8 ± 4.2*	40.6 ± 4.4*	41.2 ± 5.0*	32.2 ± 3.1	33.2 ± 1.7	32.0 ± 1.4
Heart rate (beats/min)	185 ± 21	175 ± 15	179 ± 17	196 ± 17	204 ± 17	235 ± 14*	201 ± 15	184 ± 12
Osmolality (mOsm)	294 ± 3	294 ± 3	294 ± 3	295 ± 3	295 ± 2	295 ± 2	292 ± 2	293 ± 3

*$p < 0.05$ Versus basal values.

tive fit to the vessel. Flowmeter calibration was confirmed in vitro and in vivo. All flow probes are factory precalibrated. On receipt, an additional bench calibration with a variable infusion pump setup and collection of outflow confirmed the factory calibration. During chronic implantation, the flowmeter confirms zero and full-scale flow calibration. Furthermore, a zero-baseline adjustment allows for correction for minimal baseline noise.

After computer analysis the reduced data are presented as swallows or volume swallowed per unit of time. All values are expressed as the mean ± SEM. Differences over time were assessed by one-way analysis of variance with repeated measures. Differences within groups were determined with Dunnetts test. Maternal samples were analyzed by the Student paired t test to compare the mean of basal values with the mean of the values at the conclusion of the studies.

Results

Angiotensin infusion. During the infusion of angiotensin II to the fetus there was a significant increase in fetal systolic (49.3 ± 3.6 to 64.7 ± 5.2 mm Hg) and diastolic (31.1 ± 3.1 to 40.5 ± 4.5 mm Hg) blood pressures, which returned to basal values during the recovery period (49.3 ± 3 mm Hg, 32.5 ± 2.0 mm Hg, respectively) (Table I). Fetal heart rate did not change during the infusion of angiotensin II but did increase significantly 15 minutes after the conclusion of the infusion. Fetal hematocrit increased significantly during the angiotensin II infusion (30.5% ± 1.6% to 32.4% ± 1.3%) and returned to basal levels during the recovery period. Fetal arterial pH, P_{O_2}, P_{CO_2}, and plasma osmolality did not change during the study (Table I).

Fetal swallowing activity averaged 0.85 ± 0.21 swallows per minute and esophageal flow was 0.98 ± 0.64 ml/min during the 60-minute control period.

Table II. Mean (±SEM) fetal arterial values in response to isotonic and hypertonic saline solution infusions* ($n = 4$)

	Time (minutes)			
	−30	*0*	*15*	*30*
Hematocrit (%)	30.3 ± 2.0	30.0 ± 1.9	29.3 ± 1.9	28.6 ± 1.5
pH	7.33 ± 0.01	7.35 ± 0.02	7.35 ± 0.02	7.35 ± 0.02
P_{O_2} (mm Hg)	17.8 ± 1.8	18.1 ± 2.1	17.0 ± 1.4	16.3 ± 2.4
P_{CO_2} (mm Hg)	47.0 ± 2.7	47.3 ± 1.9	45.3 ± 0.9	45.0 ± 0.7
Blood pressure (mm Hg)				
Systolic	56.3 ± 4.7	55.7 ± 7.8	55.2 ± 6.1	56.7 ± 9.8
Diastolic	37.3 ± 4.9	38.6 ± 5.5	37.2 ± 4.4	38.6 ± 6.7
Heart rate (beats/min)	182 ± 13	172 ± 4	177 ± 3	198 ± 2

*0.15 mol/L Saline solution administered after 0-minute sample; 3.97 mol/L saline solution administered after 15-minute sample.

In response to the angiotensin II infusion, swallowing activity (0.62 ± 0.15 swallows per minute) and flow (0.40 ± 0.06 ml/min) did not change significantly. Swallowing remained stable during the recovery period (0.99 ± 0.25 swallows per minute, 0.50 ± 0.26 ml/min). In response to the fetal angiotensin II infusion, maternal mean blood pressure (86 ± 1 mm Hg), heart rate (135 ± 8 beats/min), hematocrit (28.5 ± 1.9%), pH (7.48 ± 0.01), P_{O_2} (99.4 ± 6.7 mm Hg), P_{CO_2} (30.6 ± 0.5 mm Hg), and plasma osmolality (297 ± 3 mOsm) did not change.

Hypertonic saline solution administration. Throughout the hypertonic saline solution study, fetal hematocrit, pH, P_{O_2}, P_{CO_2}, heart rate, and systolic and diastolic blood pressure values did not change (Table II). Fetal plasma osmolality (292 ± 3 mOsm) did not change significantly after the 0.15 mol/L sodium chloride injection (Fig. 2). However, 1 minute after the 3.97 mol/L sodium chloride bolus, fetal plasma osmolality significantly increased to 306 ± 5 mOsm, and subsequently decreased to 300 ± 3 mOsm by 15 minutes after the injection. Fetal plasma levels of arginine vasopressin did not change in response to the 0.15 mol/L saline solution bolus. There was a small but nonsignificant increase in plasma arginine vasopressin ($p = 0.058$) in response to the 3.97 mol/L saline solution bolus (Fig. 2).

In response to the 0.15 mol/L saline solution bolus, fetal swallowing activity (0.81 ± 0.18 swallows per minute) or volume (0.46 ± 0.20 ml/min) did not change. However, fetal swallowing activity significantly increased (6.0 ± 1.7 swallows per minute; $p < 0.05$) and swallowed volume increased fivefold (2.4 ± 1.0 ml/min; $p < 0.05$) at 1 minute after the 3.97 mol/L saline solution injection. Both swallowing activity and volume returned to basal values 5 minutes after the injection (Fig. 2).

Maternal hematocrit (29.0% ± 3.3%), pH (7.46 ± 0.01), P_{O_2} (98.4 ± 4.3 mm Hg), P_{CO_2} (34.3 ± 2.3 mm Hg), and plasma osmolality (294 ± 3 mOsm) did not change in response to the fetal 0.15 mol/L and 3.97 mol/L injections of saline solution.

Comment

Human fetuses have been estimated to swallow up to 500 ml/day of amniotic fluid, in addition to significant volumes of lung fluid and salivary secretions.[6,7] Similarly, ovine fetuses swallow up to 1 L per day near term.[2-4] As fetal swallowing appears to be a primary route of amniotic fluid reabsorption, an interruption of normal swallowing in human fetuses or animal models may result in polyhydramnios.[8-12] Fetal swallowing also has been shown to have significant effects on gastrointestinal development. In the absence of swallowing, the maturation of gastric and intestinal tissue is retarded in fetal sheep[20] and rabbits.[13] Intragastric infusions of amniotic fluid, though not saline solution, normalized the maturation. In addition, decreased somatic growth may be a result of inhibited swallowing in either human[9] or rabbit fetuses.[13]

To permit quantification and analysis of fetal swallowing, we developed a model that combines electromyograms, an esophageal flow probe, and a computer data acquisition and analysis program. In pilot studies we showed that fetal esophageal electromyogram activity parallels intraesophageal pressure waveforms during swallowing. Furthermore, in the newborn lamb we have confirmed that isolated thyrohyoid electromyogram activity is associated with licking or sucking, whereas true drinking demonstrates electromyograph progression to the thoracic esophagus. Extrapolation from the basal fetal swallowing rates before the infusion of angiotensin II or saline solution shows that fetuses swallowed between 660 and 1400 ml per day, volumes consistent with previous determinations.[2-4] Prolonged recordings of swallowing activity in our laboratory have

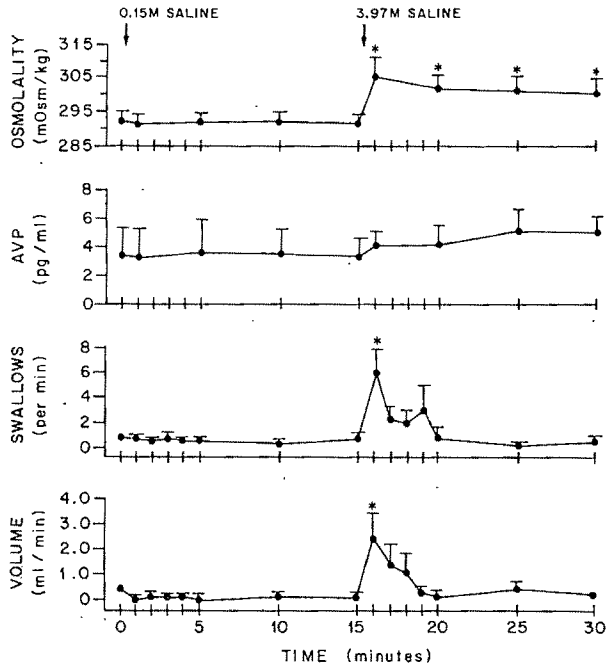


Fig. 2. Fetal plasma osmolality, plasma arginine vasopressin, electromyographic swallows, and net esophageal volume flow in response to intravenous injection of 0.15 or 3.97 mol/L saline solution. *$p < 0.05$ Versus time zero (mean control period value).

confirmed this range of daily volumes (unpublished observations).

Adult mammals display thirst behavior in response to two primary systemic stimuli: plasma hyperosmolality and extracellular volume depletion.[14] Although controversial, it is believed that central or systemic angiotensin II contributes to swallowing stimulation during the latter.[16] However, it is unknown whether the fetus exhibits thirst behavior in response to these or other stimuli.

This study indicates that fetal swallowing is stimulated in response to acute increases in plasma osmolality. At 1 minute after the hypertonic saline solution bolus, fetal plasma osmolality increased by 14 mOsm. Within 5 minutes, plasma osmolality had decreased to within 10 mOsm of basal values. Stimulation of swallowing was observed for 5 minutes, with the maximum effect occurring at 1 minute. Despite the continued plasma hyperosmolality, swallowing returned to basal levels within 5 minutes. Thresholds for thirst sensation in the adult generally occur in response to 2% to 3% (6 to 9 mOsm) increases in plasma osmolality.[21] However, the fetal arginine vasopressin secretory response to increased plasma osmolality is suppressed relative to the newborn or adult.[22] If the assumption is made that the fetal plasma osmolality threshold for stimulation of thirst also is less sensitive than that in the adult, one explanation for the results might be that the osmotic stimulus was above the fetal thirst threshold only during the first 5 minutes after administration of the hypertonic saline solution. This may explain the lack of a significant increase in fetal plasma arginine vasopressin. Alternatively, termination of swallowing may have occurred in response to liquid entering the pharynx or upper gastrointestinal tract.[23] Notably, maternal and thus fetal plasma osmolality may increase to this extent in response to diet, moderate exercise, or dehydration.[21]

During the fetal angiotensin II infusions, fetal arterial blood pressure significantly increased. In response to an identical angiotensin II infusion dose (100 ng/kg per minute), Robillard et al.[25] measured fetal plasma angiotensin II levels of approximately 400 pg/ml. This angiotensin II level is well above the proposed plasma angiotensin II dipsogenic threshold of 200 pg/ml.[26] However, in the present study fetal swallowing activity did not change in response to intravenous angiotensin II infusion. Controversy persists as to the physiologic role of angiotensin II in the regulation of thirst. Exogenous intravenous angiotensin II infusions have been shown to both stimulate[27,28] or have no effect[29] on thirst in adult rats and dogs. Intracarotid angiotensin II, although not intravenous angiotensin II, induces water drinking in adult sheep.[30] Although some studies administered pharmacologic dosages of angiotensin II, others have shown thirst stimulation with physiologic plasma angiotensin II concentrations. However, the lack of an effect of angiotensin II in other studies has raised questions of its importance in thirst regulation. Robinson and Evered[29] have noted that the physiologic conditions in which thirst may be stimulated by renin-angiotensin (dehydration, extracellular volume depletion) generally occur during periods of near or below normal mean arterial pressure. These investigators have proposed and experimentally supported the hypothesis that the failure of intravenous angiotensin II infusions to stimulate thirst in some studies may be a result of the increased blood pressure induced by angiotensin II in the normovolemic state. Thus the hypertension induced in the current study may have inhibited the stimulatory effect of increased plasma angiotensin II on swallowing. Alternatively, central angiotensin II rather than plasma angiotensin II has been proposed to be the actual dipsogen.[16] In adult sheep, angiotensin II injection into the third ventricle consistently stimulated drinking.[30] Thus the ovine fetus may be more likely to respond to central, rather than peripheral, angiotensin II.

Studies of the ontogeny of thirst behavior generally have been limited to neonatal rat and dog pups. However these investigations have inherent difficulties including the stimulation of water intake before weaning and the differentiation of stimuli for food versus water.

More recent studies[17] have circumvented these obstacles and shown the maturation of responses to thirst challenges in the newborn suckling rat: cellular dehydration at the age of 2 days, hypovolemia at 4 days, and β-adrenergic activation at 6 days. Although no responses to thirst challenges were noted in 1-day-old rats, it is possible that stresses associated with birth and neonatal transition inhibited the responses. Alternatively, the neonatal development of swallowing in the rat may be consistent with the altricial development of this species.

The current data represent the first report of the stimulation of fetal swallowing confirmed by direct measurement; the ovine fetal response to osmolar thirst challenge is intact at 130 days' gestation. Thus increased fetal plasma osmolality in response to maternal plasma hypertonicity or dehydration may increase fetal swallowing. The increased resorption of amniotic fluid by swallowing coupled with a decrease in urine production[21] potentially may alter the volume and composition of amniotic fluid. The precise role of fetal thirst and the effects of stimulated or suppressed swallowing remain to be determined.

REFERENCES

1. Harding R, Sigger JN, Poore ER, Johnson P. Ingestion in fetal sheep and its relation to sleep states and breathing movements. Q J Exp Physiol 1984;69:477-86.
2. Tomada S, Brace RA, Longo LD. Amniotic fluid volume and fetal swallowing rate in sheep. Am J Physiol 1985; 249:R133-8.
3. Bradley RM, Mistretta CM. Swallowing in fetal sheep. Science 1973;179:1016-7.
4. Harding S, Bocking AD, Sigger JN, Wickman PJD. Composition and volume of fluid swallowed by fetal sheep. Q J Exp Physiol 1984;69:487-95.
5. Abramovich DR, Garden A, Jandial L, Page KR. Fetal swallowing and voiding in relation to hydramnios. Obstet Gynecol 1979;54:15-20.
6. Pritchard JA. Fetal swallowing and amniotic fluid volume. Obstet Gynecol 1966;28:606-16.
7. Gitlin D, Kumate J, Morales C, Noriega L, Arevalo N. The turnover of amniotic fluid protein in the human conceptus. Am J Obstet Gynecol 1972;113:632-45.
8. Barkin SZ, Pretorius DH, Beckett MK, Manchester DK, Nelson TR, Manco-Johnson MD. Severe polyhydramnios: incidence of anomalies. AJR 1987;148:155-9.
9. Pierro AR, Cozzi G, Colarossi IM, Irving AM, Pierce J, Lister J. Does fetal gut obstruction cause hydramnios and growth retardation? J Pediatr Surg 1987;22:454-7.
10. Plentl AA. Intrauterine operations on Rhesus monkeys. N Engl Obstet Gynecol Soc 1964;18:75-90.
11. Minei LJ, Suzuki K. Role of fetal deglutition and micturation in the production and turnover of amniotic fluid in monkey. Obstet Gynecol 1976;48:177-81.
12. Fink A. Ein tierexperimenteller Bietrag zur Entstehung des hydramnios ztschr Geburtxh U. Gynakologe 1954;140:18-21.
13. Mulvihill SJ, Stone M, Fonkalsrud E, Debas H. Trophic effect of amniotic fluid on fetal gastrointestinal development. J Surg Res 1986;40;291-6.
14. Rolls BJ, Rolls ET. The control of drinking. Br Med Bull 1981;37:127-30.
15. Thrasher TN, Brown CJ, Keil LC, Ramsay DJ. Thirst and vasopressin release in the dog: an osmoreceptor or sodium receptor mechanism. Am J Physiol 1980;238: R333-9.
16. Stricker EM. The renin-angiotensin system and thirst: a reevaluation. II. Drinking elicited in rats by caval ligation or isoproterenol. J Comp Physiol Psychol 1977;91:1220-1231.
17. Wirth JB, Epstein AN. Ontogeny of thirst in the infant rat. Am J Physiol 1976;230:188-98.
18. Robillard JE, Weitzman RE. Developmental aspects of the fetal renal response to exogenous arginine vasopressin. Am J Physiol 1980;238:F407-14.
19. LaRochelle ET, North WG, Stern P. A new extraction of arginine vasopressin from blood: the use of octadecasilylsilica. Pflugers Arch 1980;387:79-81.
20. Trahair JF, Harding R, Bocking AD, Silver M, Robinson PM. The role of ingestion in the development of the small intestine in fetal sheep. Q J Exp Physiol 1986;71:99-104.
21. Wood RJ, Rolls ET, Rolls BJ. Physiological mechanisms for thirst in the nonhuman primate. Am J Physiol 1982;242:R423-8.
22. Leake RD, Weitzman RE, Effros RM, Siegal SR, Fisher DA. Maternal fetal osmolar homeostasis: fetal posterior pituitary autonomy. Pediatr Res 1979;13:841-4.
23. Bott E, Denton DA, Weller S. Water drinking in sheep with oesophageal fistulae. J Physiol (Lond) 1965;176:323-36.
24. Ross MG, Sherman DJ, Ervin MG, Castro R, Humme J. Maternal dehydration-rehydration: fetal plasma and urinary responses. Am J Physiol 1988;255:E674-9.
25. Robillard JE, Gomez A, VanOrdern D, Smith FC. Comparison of the adrenal and renal responses to angiotensin II in fetal lambs and adult sheep. Circ Res 1982;50: 140-7.
26. Johnson AJ, Mann JFE, Rascher W, Johnson JK, Ganten D. Plasma angiotensin II concentrations and experimentally induced thirst. Am J Physiol 1981;240:R229-34.
27. Fitzsimons JT, Simons BJ. The effect on drinking in the rat of intravenous infusion of angiotensin, given alone or in combination with other stimuli of thirst. J Physiol (Lond) 1969;203:45-57.
28. Fitzsimons JT, Kucharczyk J, Richard G. Systemic angiotensin-induced drinking in the dog: a physiological phenomenon. J Physiol (Lond) 1978;276:435-48.
29. Robinson MM, Evered MD. Pressor action of intravenous angiotensin II reduces drinking response in rats. Am J Physiol 1987;252:R754-9.
30. Abraham SF, Baker RM, Blaine EH, Denton DA, McKinley MJ. Water drinking induced in sheep by angiotensin—a physiological or pharmalogical effect? J Comp Physiol Psychol 1975;88:503-18.

Effect of whole-body pH changes on thoracic duct lymph flow in anesthetized sheep

Guillermo J. Valenzuela, MD, Hugh W. Forbes, and Justo G. Alonso, MD
Loma Linda, California

Physiologic alterations of pH in vitro produce alterations of the oncotic pressure of both plasma and interstitial proteins, the effect being more marked in the latter. Therefore we postulated that by changing an animal's pH we could produce alterations in fluid distribution and affect the whole-body lymph flow rate. To test this hypothesis, we infused seven acutely nephrectomized anesthetized sheep with 2% body weight/volume isosmotic lactated Ringer's solution and bicarbonate (200 mEq) after a 30-minute control period. The fluid was infused over a 10-minute period with 20-minute recovery intervals. To another group of seven ewes, we infused the same volume of lactated Ringer's solution and hydrochloric acid (40 mEq). Throughout the experiment, we continuously measured arterial and venous pressures and lymph flow rate. Every 10 minutes we obtained samples for arterial pH, for hematocrit, and for plasma and lymph protein anc osmolality. The infusion of bicarbonate was associated with a comparatively lower peak lymph flow rate (383% over baseline compared with 757% for acid infusion). Also the lymph flow rate after acid infusion started to increase ~5 minutes after the beginning of the infusion, as compared with ~10 minutes after the base infusion. Acid infusion did not increase arterial pressure, in contrast to the other infusions. The changes in pH obtained with the infusions were insignificant for the Ringer's infusion, +0.17 for the base, and −0.16 for the acid. The plasma/lymph protein concentration ratios at the end of the infusion were no different for acid or base: 112.4% ± 4.4% and 101.9% ± 5% (difference from control, mean ± SEM) respectively ($p > 0.1$). In conclusion, minimal alterations of pH produced alterations in the cardiovascular and lymph flow rate responses to fluid challenge in anesthetized animals. (Am J Obstet Gynecol 1989;161:1566-70.)

Key words: Changes of pH, fluid distribution, lymph flow

The distribution of fluid between the intravascular and interstitial systems follows Starling's equation. Hydrostatic intravascular and interstitial oncotic pressures drive fluid into the interstitial space, while interstitial hydrostatic pressure and plasma oncotic pressure try to maintain the fluid intravascularly. In the interstitium, hyaluronic acid is organized in a mesh impregnated with water, which gives the interstitium a gel structure with a negatively charged surface.[1] Therefore it is possible to postulate that even small changes in an animal's pH could affect the Donnan equilibrium in the interstitial gel, thus affecting the oncotic pressure differences across the capillaries and between the interstitial gel and the free fluid. Will and Brace[2] demonstrated in vitro that increases in pH can increase the plasma oncotic pressure in a linear fashion. This phenomenon could be relevant to a better understanding of maternal fluid retention during pregnancy and in the fetus, since pH is different from that in the nonpregnant state in both instances. To test the hypothesis that pH changes would affect the whole-body lymph flow rate, we performed a series of infusions in anesthetized animals, using fluid with normal pH, alkaline pH, and acidic pH, and measured the response of the systemic vasculature and lymph flow.

From the Department of Obstetrics and Gynecology and the Division of Perinatal Biology, Loma Linda University.
This work was supported by the Loma Linda University Obstetrics and Gynecology Departmental Research Fund.
Presented in part at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.
Reprint requests: Guillermo J Valenzuela, MD, 780 E Gilbert St., Department of Obstetrics and Gynecology, San Bernardino Medical Center, San Bernardino, CA 92404.
5/6/16240

Material and methods

A total of 14 ewes was obtained from local breeders and femoral artery and vein, left thoracic duct, and jugular vein catheters were placed with the animals under general anesthesia, as described in detail elsewhere.[3] The renal circulation was tied off bilaterally through a flank incision, and splenectomy was performed at the same time. The arterial and venous pressures were recorded continuously and stored every 1 minute for later analysis with an on-line data-acquisition system as described by Dale et al.[4] The lymph flow was drained to a weighed vial 30 cm below heart level; the lymph flow rate was obtained from the weight differential and stored every 1 minute. In chronic preparations we have demonstrated that changes in the negative pressure of the lymph catheter

(between 0 to −30 cm) do not affect lymph flow rate.[5] The animals were allowed to breathe spontaneously during the experiments to avoid any effect of mechanical ventilation on lymph flow rate. We attempted only moderate changes in pH, because of concern about the direct cardiovascular effects of the base or acid infusions. We also wanted to minimize any possible effect of respiratory rate while still affecting in a measurable manner the circulating pH. The animals underwent splenectomy because of the large red blood cell reservoir represented by the spleen in the sheep.

Experimental protocol. The experiments were initiated 1 hour after the end of surgery, with the animals receiving oxygen (3 L/min), maintained in a state of light anesthesia (0.5% to 1% halothane), and breathing spontaneously. In seven animals the experiment consisted of a 30-minute control period during which baseline measurements of arterial and venous pressures and thoracic duct lymph flow rates were obtained. Lymph was returned continuously to the animal through a neck vein at the lymph flow rate. At the end of the control period, lactated Ringer's solution was infused (2% body weight/volume) over a 10-minute period. Then, after an interval of 20 minutes, we administered sodium bicarbonate (200 mEq) as isosmotic solution (prepared for each animal), over the same time interval (10 minutes) and in the same total volume as the lactated Ringer's solution (2% of body weight). In another seven ewes we infused lactated Ringer's solution and then acid (in the same volume as described above). Arterial samples for pH, plasma osmolality, and hematocrit were obtained every 10 minutes throughout the experiment. Lymph samples for osmolality and protein concentration were also obtained every 10 minutes. Protein concentration was determined with a hand-held refractometer (American Optical). Samples of blood were transported in a capped syringe, and blood gas values were determined immediately.

Vascular and interstitial fluid compliance calculations. Vascular compliance was obtained by estimating the changes in intravascular volume from the changes in hematocrit and venous pressure. An index of the changes in the interstitial fluid compliance was obtained from the changes in interstitial fluid volume, assuming that the changes in interstitial hydrostatic pressure were similar on both sides of the capillaries. Since a known amount of fluid was infused and the ewes were nephrectomized and splenectomized, the changes in intravascular volume could be estimated and the fluid transferred to the interstitial space thus calculated.

Data analysis. Arterial and venous pressures were averaged for the control period (initial 30 minutes), and for the 10 minutes thereafter during the infusions. The changes with time for each group were analyzed by analysis of variance.

The arterial and venous pressures, lymph flow rate, and heart rate for the base and acid groups were compared for the period of the last 5 minutes of the infusion together with the first 5 minutes of the recovery period after the end of the infusion. The acid and base infusion comparisons of lymph to plasma protein concentration ratio, hematocrit, arterial and venous pressures, and heart rate were calculated by assessing the change (in percent over baseline) at the end of the infusion; these results were compared by unpaired t test. The excess lymph over baseline after the base or acid infusion was calculated by measuring the area over baseline and expressed as a ratio of the control period area and compared by t test.

Results

The control values for the 14 ewes (average ± SEM) are presented in Table I. The lymph flow responses to blood volume expansion (expressed as percent of baseline) for the two different groups are presented in Fig. 1. We present the data as the percent change over baseline to correct for interanimal variation. During the infusion of lactated Ringer's solution both group of animals responded similarly ($p > 0.1$); however, the response to the second infusion was higher for the acid ($p < 0.05$). For the sequence lactated Ringer's solution to base, the peak lymph flow after the base infusion occurred at 23 minutes after the beginning of the infusion (383% of control); for the lactated Ringer's solution to acid sequence, the peak after acid occurred at 21 minutes (782% of control). For the latter infusion, the lymph flow rate started to increase at 5 minutes after the beginning of the infusion, compared with after 10 minutes for either lactated Ringer's solution or base.

Fig. 2 shows the arterial pressure response to the fluid infusion. The acid infusion was characterized by absence of an increase in arterial pressure when compared with the response to either lactated Ringer's solution or base ($p < 0.05$).

In Table I, the changes in arterial and venous pressures, heart rate, hematocrit, and pH are presented. The values for the pressures and the lymph flow rate represent the interval 5 minutes before and 5 minutes after the end of the infusion. Osmolality did not change throughout the experiment.

We could not detect a significant change in the estimated blood volume, vascular compliance, or interstitial compliance by t test or Sign test.

The lymph/protein ratio was 101% ± 5% (percent over baseline) at the end of the base infusion and 109.4% ± 4.4% for the acid infusion, a nonsignificant difference ($p > 0.1$). The ratio for excess lymph flow area was 277% for the acid infusion as compared with 120% for the base infusion. Plasma and lymph osmo-

Table I. Control values for 14 ewes

Variable	*Control*		*Infusion*		*% of Baseline*	
	Acid	*Base*	*Acid*	*Base*	*Acid*	*Base*
Arterial pressure (mm Hg)	91.2 ± 5.0	100.5 ± 6.5	84.6 ± 1.5	114.9 ± 2.3	90.1 ± 4.1*†	115.8 ± 5.1*
Venous pressure (mm Hg)	6.7 ± 1.3	7.9 ± 0.7	8.4 ± 0.5	11.1 ± 0.4*	140.1 ± 5.9*	168.5 ± 9.9*
Lymph flow rate (ml/min)	0.8 ± 0.2	2.03 ± 0.28	2.7 ± 0.2*	5.6 ± 0.4*	144.5 ± 21.4*†	96.5 ± 2.4*
Heart rate (beats/min)	139.6 ± 7.6	136.3 ± 11.7	187.6 ± 9.0*†	136.8 ± 4.2	475.7 ± 119.1*†	302.6 ± 27*†
Hematocrit (%)	32.00 ± 2.3	38.03 ± 2.45	29.43 ± 2.8	25.62 ± 2.5	77.0 ± 3.6*	81.9 ± 6.7*
Lymph/plasma protein ratio	0.78 ± 0.01	0.57 ± 0.2	0.5 ± 0.04	0.36 ± 0.06	112.4 ± 4.4	101.9 ± 5
pH	7.16 ± 0.05	7.18 ± 0.04	7.04 ± 0.02*†	7.33 ± 0.06*†	—	—

Control is the average ± SEM for the initial 30 minutes. Infusion is the average ± SEM during the infusion of base or acid. Percent of baseline is the average ± SEM (expressed as percent) for the period 5 minutes before and 5 minutes after the end of infusion.

*Significantly different from control ($p < 0.05$).

†Significantly different from alternative treatment ($p < 0.05$).

lality changes also were not significantly different throughout the experiments when compared within one group or between groups ($p > 0.1$).

There was minimal correlation ($r = 0.28$) between pH and baseline lymph flow.

Comment

Fluid balance is maintained within narrow margins in healthy animals, and the distribution of fluid between the intravascular and interstitial spaces plays an important role in avoiding major swings in intravascular volume during fluid ingestion. The fluid distribution across the capillaries depends on Starling's equation of fluid distribution.[6] Proteins are electrically charged molecules, and an important fraction of the oncotic pressure that they generate derives from the covalently bound ions (Donnan equilibrium). Theoretically, a change in whole-body pH can alter protein electric charge, thus affecting the oncotic pressure. This was confirmed experimentally in vitro by Will and Brace,[2] who demonstrated that pH changes within the physiologic range can alter both plasma and interstitial oncotic pressure.

To our knowledge this is the first attempt to study the effect of pH changes on the whole-body lymph flow rate. The study of this phenomenon is relevant to the understanding of fluid distribution in some pathologic states associated with acidosis or alkalosis and to a better understanding of the fluid distribution in cardiovascular or septic shock, the treatment of which requires the infusion of large amounts of fluids. It also is relevant in some physiologic states such as pregnancy, when there is a normal increase in the arterial pH.[7]

An important finding was the rate at which lymph flow started to increase after the fluid infusion. In several previous works of our own,[8,9] the lymph flow rate started to increase an average of 12 minutes after the beginning of fluid infusion, a value not different from the values obtained here for the lactated Ringer's solution or base infusion. However, the acid infusion produced an increase in the lymph flow rate much sooner. There are several possibilities to explain this finding. One is an alteration of capillary permeability as a secondary effect of the infusion of acid; however, there were no differences in the lymph-protein ratio with either acid or base. Another alternative is that the acid produced a generalized vasodilatation, as evidenced by the decrease in arterial pressure,[10] and the area of interchange increased with the resulting increase in lymph flow; however, the response is too rapid to be attributed only to alterations in interchange area. A third possibility is that the acid infusion produced a release of hormones that could affect capillary permeability or lymphatic contractility directly; we did not address this question in the present experimental protocol.

Another interesting aspect of the lymph flow response to solutions with varying pH levels is the different quantitative response obtained. For instance, that the infusion of base was associated with a lower peak lymph flow rate and a lower total lymph flow response when compared with acid infusion is of interest. There are several possible explanations for the differences in the lymph flow response. One is that the lower lymph flow rate is partially due to a longer intravascular retention of the fluid administered; however, there were no differences in the degree of blood volume expansion with the different infusions. The other possibility is a water inhibition of the gel matrix, which is a secondary effect of an increase in the oncotic

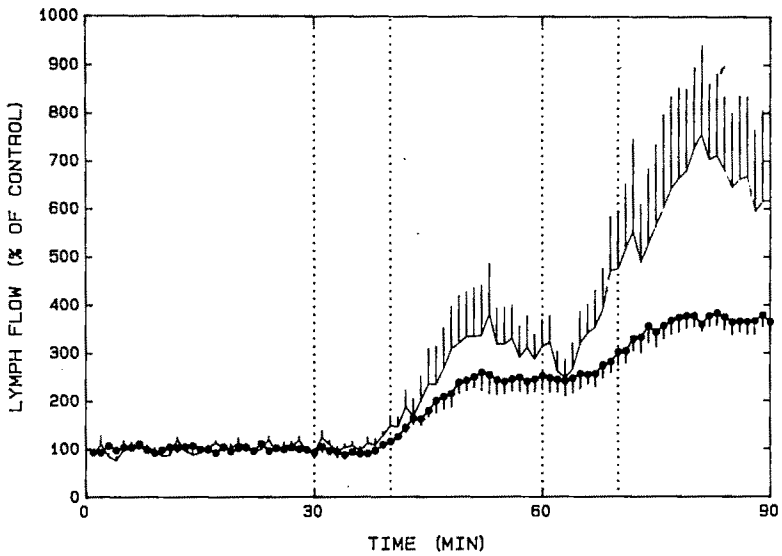


Fig. 1. Lymph flow rate (expressed as percent over baseline) is presented as —— for the sequence lactated Ringer's solution to acid. The sequence lactated Ringer's solution to base is represented as ●—●. *Vertical dotted lines* represent the times of infusion. The sequence with acid first was associated with a significantly higher lymph flow ($p < 0.05$).

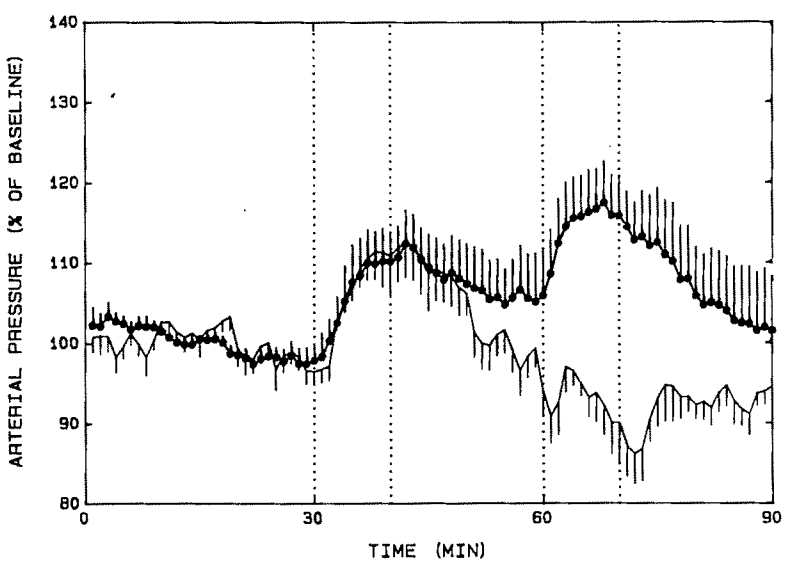


Fig. 2. Arterial pressure (expressed as percent over baseline) is presented as —— for the sequence lactated Ringer's solution to acid and as ●—● for lactated Ringer's solution to base. The arterial response was significantly lower for acid infusion ($p < 0.05$).

pressure inside the gel matrix during the bicarbonate infusion. This later interpretation is compatible with the finding of Will and Brace[2] of a greater increase in the oncotic pressure with increasing pH. We postulate that during the acid infusion the fluid is not incorporated into the gel; therefore smaller changes in pressure produce larger increases in lymph flow. Another factor that may play a role in the responses obtained is the renal ligature; Harake and Power[11] demonstrated a decrease in baseline lymph flow after the ligation of the renal circulation in a group of nonpregnant ewes in a preparation similar to this.

The components of Starling's equation seem not to be changed during the base infusion. There were no changes in the lymph/plasma protein concentration ratio; also there was no difference between the average venous pressures at the end of either base or acid infusion, suggesting that the interstitial fluid pressure changes were not different. It is therefore necessary to consider the possibility that here are factors beyond the

interchange at the capillary level that may affect the distribution of fluid in the whole animal.

Some of the cardiovascular and lymphatic responses in this group of animals may be altered because of an acidotic condition of the animal at the beginning of the experiment; however, our goal in the present study was to observe cardiovascular and lymph flow changes after pH changes. We think that since the pH of most of the proteins is far from physiologic, the effects of base and acid infusions will differ because these infusions are affecting the proteins at different portions of the dissociation curve.

The present results also showed a significant lack of arterial pressure increase during the acid volume infusion, accompanied by a modest increase in venous pressure, higher than during lactated Ringer's solution but lower than during base infusion. These results are in agreement with those changes described for acid infusion by Bygdeman[12] in cats and by Sharpey-Schafer et al.[13] in humans.

In conclusion, a mild change in baseline pH produced a significant change in the lymph and arterial pressure responses to fluid infusion. This study suggests that physiologic changes of pH during a state of cardiovascular shock may play an important role in third-space fluid retention and thus may have important therapeutic implications. Also it can have a significant role in explaining the fluid retention of pregnancy. The next step is to assess the role of pH changes in cardiovascular adaptations and fluid distribution of chronically catheterized animals with normal kidney function.

We thank L. Buyak, C. W. Hewitt, and J. I. Valenzuela for expert technical assistance.

REFERENCES

1. Guyton AC, Taylor AE, Granger HJ. Circulatory physiology. II. Dynamics and control of the body fluids. Philadelphia: WB Saunders, 1975:59-60.
2. Will BR, Brace RA. Physiological effects of pH changes on colloid osmotic pressures. Am J Physiol 1985;248 (Heart Circ Physiol 17):H890-3.
3. Valenzuela GJ, Hewitt CW, Graham AD. Angiotensin II infusion increases thoracic duct lymph flow in chronically catheterized sheep. Am J Physiol 1987;252(Regul Integ Comp Physiol 21):R853-8.
4. Dale PS, Ducsay CA, Gilbert RA, et al. A microcomputer program for real-time data acquisition in the perinatal physiology laboratory. J Dev Physiol [In press].
5. Valenzuela GJ, Kim S. Effect of acute estrogen administration on lymph flow vs. outflow pressure relation. Am J Physiol [In press].
6. Renkin EM. Some consequences of capillary permeability to macromolecules: Starling's hypothesis reconsidered. Am J Physiol 1986;250(Heart Circ Physiol 19):H706-10.
7. Pritchard JA, MacDonald PC, Gant NF, eds. In: Williams' obstetrics. 17th ed. New York: Appleton-Century-Crofts, 1985.
8. Valenzuela GJ, Woods LL, Brace RA. Thoracic duct lymph flow in pregnant sheep and response to blood volume expansion. Am J Physiol 1986;250(Regul Integ Comp Physiol 19):R1095-8.
9. Valenzuela GJ, Hewitt CW, Kramer YD, et al. Effects of sustained lymph drainage on cardiovascular function and thoracic duct lymph in sheep. Am J Physiol 1989;256 (Regul Integ Comp Physiol 25):R867-74.
10. Davis MJ. Microvascular control of capillary pressure during increases in local arterial and venous pressure. Am J Physiol 1988;254(Heart Circ Physiol 23):H772-84.
11. Harake B, Power GG. Thoracic duct lymph flow: a comparative study in newborn and adult sheep. J Dev Physiol 1986;8:87-95.
12. Bygdeman S. Vascular reactivity in cats during changes in the acid-base balance of the blood. Acta Physiol Scand 1963;61(suppl 222):2-66.
13. Sharpey-Schafer EP, Semple SJG, Halls RW, Howarth S. Venous constriction after exercise; its relation to acid-base changes in venous blood. Clin Sci 1965;29:397-406.

Does furosemide alter the hemodynamic response to rapid intravascular transfusion of the anemic fetal lamb?

David H. Chestnut, MD, Kenneth L. Pollack, MD, Carl P. Weiner, MD, Jean E. Robillard, MD, Christine S. Thompson, and Craig S. DeBruyn

Iowa City, Iowa

The purpose of this study was to define the hemodynamic response to rapid intravascular transfusion of the anemic fetal lamb and to determine whether furosemide alters that response. Sixteen experiments were performed in nine chronically instrumented gravid ewes between 0.8 and 0.9 of timed gestation. On day 1 of each experiment, each fetus was subjected to hemorrhage (40 ml/kg of estimated fetal weight) over 1 hour. On day 2, plasma was withdrawn from the stored fetal blood until the hematocrit was approximately 70%, and the packed red blood cells were returned to the fetus intravenously over 10 minutes. Each fetus received either furosemide (2 mg/kg)or control saline solution intravenously at time zero and again at 5 minutes. The order of experiments was randomly determined for each animal. Hemorrhage resulted in a similar decrease in fetal hematocrit in the two groups. The mean ± SEM fetal hematocrit before hemorrhage was 38 ± 3% in the furosemide group (n = 8) and 36 ± 2% in the control group (n = 8). On day 2, the mean ± SEM fetal hematocrit before transfusion was 28 ± 2% in the furosemide group and 25 ± 1% in the control group. There was no significant difference between groups in the fetal hemodynamic response to transfusion. At the end of the transfusion, the fetal central venous pressure had increased from 4.9 ± 0.5 to 6.2 ± 0.5 mm Hg in the furosemide group (p = 0.01) and from 3.9 ± 0.2 to 5.8 ± 0.3 mm Hg in the control group (p = 0.0001). Fetal mean arterial pressure increased from 42 ± 1 to 50 ± 1 mm Hg in the furosemide group (p = 0.0001) and from 40 ± 1 to 46 ± 1 mm Hg in the control group (p = 0.0007). Fetal heart rate decreased from 187 ± 2 to 169 ± 5 beats/min in the furosemide group (p = 0.004) and from 188 ± 4 to 170 ± 5 beats/min in the control group (p = 0.0008). Transfusion did not significantly change fetal pH in either group. At 120 minutes, the fetal Po_2 had increased from 17 ± 1 to 19 ± 1 mm Hg in the furosemide group (p = 0.03) and from 19 ± 1 to 21 ± 2 mm Hg in the control group (p = 0.05). We conclude that rapid transfusion of the anemic fetal lamb resulted in modest increases in fetal central venous pressure and mean arterial pressure. Second, furosemide did not significantly alter the hemodynamic response to rapid transfusion. (AM J OBSTET GYNECOL 1989;161:1571-5.)

Key words: Anemia, fetal lamb, furosemide, transfusion

Direct intravascular transfusion of packed red blood cells has supplanted intraperitoneal transfusion for treatment of fetal hemolytic disease in many centers.[1-6] The method is empiric. Some practitioners perform exchange transfusion to avoid an abrupt increase in fetal intravascular volume.[4,5] In contrast, others have performed rapid intravascular transfusion without exchange.[3,6] There are few data on the fetal hemodynamic response to intravascular transfusion. We hypothesized that furosemide might facilitate fetal adaptation to the abrupt increase in fetal intravascular volume by dilating the capacitance vessels.[6,7] The purpose of this study was to define the hemodynamic response to rapid intravascular transfusion of the anemic fetal lamb and to determine whether furosemide alters that response.

Table I. Fetal hematocrit measurements before hemorrhage and before transfusion

	Furosemide (n = 8)	*Control (n = 8)*
Day 1, before hemorrhage	38% ± 3%	36% ± 2%
Day 2, before transfusion	28% ± 2%	25% ± 1%

All values are expressed as mean ± SEM.

Material and methods

The protocol was approved by the University of Iowa Animal Care Committee. Mixed-breed ewes were obtained from a commercial breeder at approximately 118 days of timed gestation (term = 145 days). Each animal fasted for 36 hours before surgery. At 120 days' gestation, thiopental sodium 600 to 750 mg was given intravenously to induce general anesthesia.

From the Departments of Anesthesia, Obstetrics and Gynecology, and Pediatrics, University of Iowa College of Medicine.

Supported in part by a grant from the American Society of Anesthesiologists.

Presented in part at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.

Reprints not available.

6/6/15663

Table II. Baseline maternal and fetal hemodynamic, blood gas, and acid-base measurements before transfusion on day 2

	Furosemide (n = 8)	*Control (n = 8)*
Maternal		
Heart rate (beats/min)	104 ± 5	108 ± 3
Mean arterial pressure (mm Hg)	89 ± 7	91 ± 6
Arterial pH	7.48 ± .01	7.47 ± .01
Arterial P_{O_2} (mm Hg)	109 ± 5	109 ± 4
Arterial P_{CO_2} (mm Hg)	35 ± 1	36 ± 1
Hematocrit (%)	30 ± 2	30 ± 2
Fetal		
Heart rate (beats/min)	187 ± 2	188 ± 4
Mean arterial pressure (mm Hg)	42 ± 1	40 ± 1
Central venous pressure (mm Hg)	4.9 ± 0.5	3.9 ± 0.2
Arterial pH	7.32 ± .01	7.29 ± .03
Arterial P_{O_2} (mm Hg)	17 ± 1	19 ± 1
Arterial P_{CO_2} (mm Hg)	51 ± 1	50 ± 1

Maternal and fetal arterial blood samples were obtained from the maternal and fetal descending aorta, respectively. Blood gas and acid-base values were corrected for temperature.

All values are expressed as mean ± SEM.

Anesthesia was maintained with halothane 1%, nitrous oxide 50%, and oxygen 50%. Laparotomy and hysterotomy were performed with sterile technique, and catheters (polyethylene-90) were placed in the fetal descending aorta and inferior vena cava, via each femoral artery and vein, and in the amniotic cavity. Catheters (polyethylene-240) were then placed in the maternal descending aorta and inferior vena cava via the left mammary artery and vein, respectively. All catheters were tunneled subcutaneously and exteriorized through a small incision in the left flank.

After surgery each animal was kept in an approved cage in a restricted area, fed a balanced diet, and allowed a recovery period ≥5 days before experimentation. Procaine penicillin G (500,000 U) and dihydrostreptomycin (625 mg, Combiotic, Pfizer, New York) were given to the mother before surgery and daily for 3 days after surgery. Gentamicin (80 mg) was given to the mother on the day of each experiment, and gentamicin (40 mg) was given by means of the amniotic catheter during surgery and on the day of each experiment.

Each experiment was done with the animal standing unrestrained within an approved transport cart. One hour was allowed for the animal to acclimate to the laboratory environment. On day 1 of each experiment, each fetus was subjected to hemorrhage (40 ml/kg estimated fetal weight) over 1 hour. The blood was withdrawn in four 10-minute intervals with a constant-rate withdrawal pump. Fetal blood was replaced with an equal volume of normal saline solution. The estimated fetal weight was calculated according to this formula:[8] Estimated fetal weight in kg = (0.096 × gestational age in days) − 9.223. After hemorrhage, the ewe was returned to the Animal Care Unit. Fetal blood was refrigerated overnight in citrate phosphate dextrose adenine (CPDA-1) anticoagulant.

On day 2, the animal returned to the laboratory and again was allowed 1 hour to acclimate to the laboratory environment. The stored fetal blood was warmed to room temperature, and plasma was withdrawn until the hematocrit was approximately 70%. At time zero the packed red blood cells (approximately 20 ml/kg) were returned to the fetus intravenously over 10 minutes. Each fetus received either 2 mg/kg of furosemide (Elkins-Sinn, Cherry Hill, N.J.) or control saline solution intravenously, at time zero and again at 5 minutes. (This furosemide regimen is identical to the regimen used for transfusion of human fetuses in our hospital.) The volume of furosemide or control saline solution was approximately 0.5 ml for each fetus. Maternal and fetal hemodynamic measurements were continued through 120 minutes after the start of transfusion.

Each animal was allowed to rest ≥24 hours before the experimental sequence was repeated. The order of experiments was determined randomly for each animal. We intended to perform both experiments in each animal. However, catheter occlusion precluded the performance of a second experiment in two of the animals. Therefore, 16 experiments were performed in nine animals. (Both experiments were performed in seven animals, and one experiment was performed in each of the other two animals.)

Maternal and fetal arterial hematocrit, blood gas, and acid-base values were determined at baseline and at 15, 60, and 120 minutes after the start of transfusion. Blood gas and acid-base values were determined with a Instrumentation Laboratory 1302 blood gas analyzer (Leighton, Mass.). All values were corrected for temperature. Fetal blood was replaced by an equal volume of normal saline solution.

Maternal and fetal systemic arterial pressures and amniotic fluid pressure were recorded at 10 second intervals by means of a customized physiologic data acquisition system (Coulbourn Instruments, Lehigh Valley, Pa.) interfaced to an AST 286 premium computer (Irvine, Calif.). Fetal arterial and central venous pressures were corrected by subtracting the simultaneous intraamniotic pressure. Maternal and fetal mean arterial pressures were calculated arithmetically. Maternal and fetal heart rates were calculated from maternal and fetal arterial waveforms.

Statistical analysis was by three-factor analysis of variance, followed by t tests for individual measurements. A p value <0.05 was considered significant.

Results

On day 1, estimated fetal weight was 2.78 ± 0.09 kg in the furosemide group and 2.77 ± 0.05 kg in the

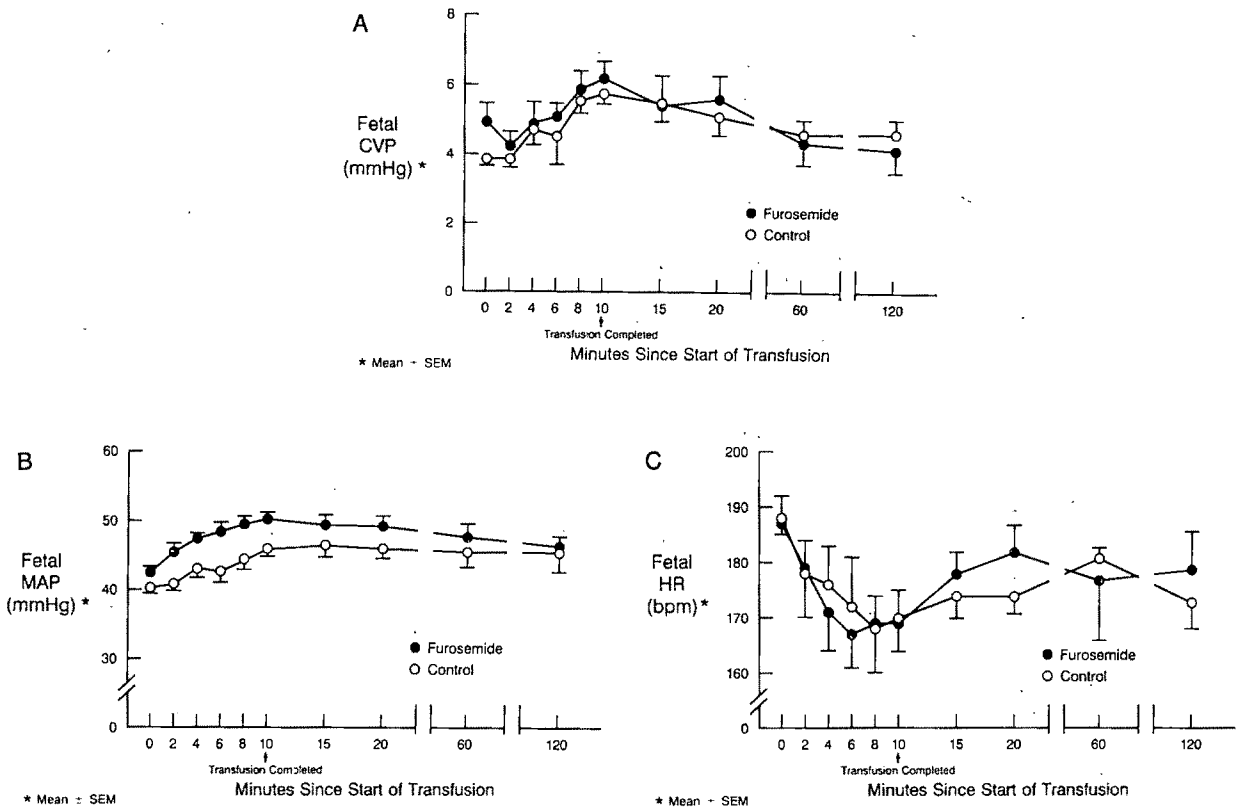


Fig. 1. A, Fetal central venous presure *(CVP)* responses over time. Transfusion significantly increased fetal central venous pressure at 8 and 10 minutes in the furosemide group and at each measurement between 6 and 20 minutes in the control group. **B,** Fetal mean arterial pressure *(MAP)* responses over time. Transfusion significantly increased fetal mean arterial pressure at each measurement between 4 and 120 minutes in the furosemide group and at each measurement between 6 and 120 minutes in the control group. **C,** Fetal heart rate *(HR)* responses over time. Transfusion significantly decreased fetal heart rate at each measurement between 4 and 10 minutes in the furosemide group and at each measurement between 4 and 20 minutes in the control group. Furosemide (2 mg/kg) or control saline solution was given at time zero and again at 5 minutes. Transfusion started at time zero and was completed at 10 minutes. All values are expressed as mean ± SEM.

control group. Hemorrhage resulted in a similar decrease in fetal hematocrit in the two groups (Table I). The two treatment groups were similar with regard to baseline maternal and fetal hemodynamic, blood gas, and acid-base measurements before transfusion on day 2 (Table II).

There was no significant difference between groups in the fetal hemodynamic response to transfusion (Fig. 1). There was a transient decrease in fetal central venous pressure between baseline and 2 minutes in the furosemide group, but the decrease was not statistically significant (Fig. 1, *A*). At 10 minutes, fetal central venous pressure had increased from 4.9 ± 0.5 to 6.2 ± 0.5 mm Hg in the furosemide group ($p = 0.01$) and from 3.9 ± 0.2 to 5.8 ± 0.3 mm Hg in the control group ($p = 0.0001$). Fetal mean arterial pressure increased from 42 ± 1 to 50 ± 1 mm Hg in the furosemide group ($p = 0.0001$) and from 40 ± 1 to 46 ± 1 mm Hg in the control group ($p = 0.0007$) (Fig. 1, *B*). At 10 minutes, fetal heart rate had decreased from 187 ± 2 to 169 ± 5 beats/min in the furosemide group ($p = 0.004$) and from 188 ± 4 to 170 ± 5 beats/min in the control group ($p = 0.0008$) (Fig. 1, *C*).

There was no significant difference between groups in the fetal arterial hematocrit, pH, or Po_2 responses to transfusion (Fig. 2). Specifically, transfusion significantly increased the fetal hematocrit at 15, 60, and 120 minutes in both groups ($p = 0.0001$) (Fig. 2, *A*). Transfusion did not significantly change fetal pH in either group (Fig. 2, *B*). At 120 minutes, fetal PO_2 had increased from 17 ± 1 to 19 ± 1 mm Hg in the furosemide group ($p = 0.03$) and from 19 ± 1 to 21 ± 2 mm Hg in the control group ($p = 0.05$) (Fig. 2, *C*). Finally, there was a small but significant decrease in PCO_2 at 15 minutes ($p = 0.01$) and at 60 minutes ($p = 0.005$) in the furosemide group, but not in the control group (Fig. 2, *D*).

Comment

Blood volume rapidly returns toward normal after hemorrhage in fetal lambs.[9, 10] Brace and Cheung[10] subjected 12 fetal lambs to hemorrhage (31 ± 2% of the

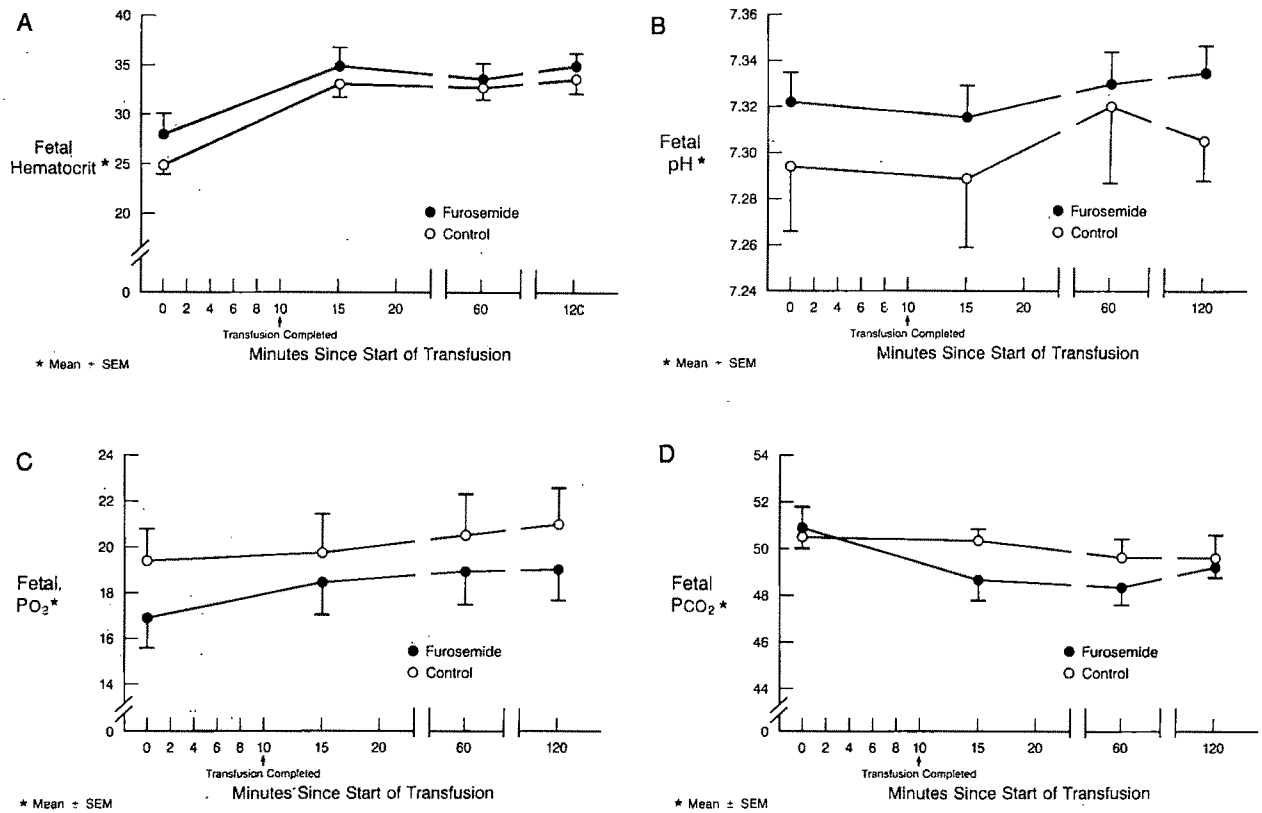


Fig. 2. A, Fetal hematocrit responses over time. Transfusion significantly increased fetal hematocrit at 15, 60, and 120 minutes in both groups ($p = 0.0001$). **B,** Fetal pH responses over time. Transfusion did not significantly change fetal pH in either group. **C,** Fetal P_{O_2} responses over time. Transfusion increased fetal P_{O_2} at 60 minutes ($p = 0.02$) and at 120 minutes ($p = 0.03$) in the furosemide group and at 120 minutes ($p = 0.05$) in the control group. **D,** Fetal P_{CO_2} responses over time. There was a small but significant decrease in P_{CO_2} at 15 minutes ($p = 0.01$) and at 60 minutes ($p = 0.005$) in the furosemide group, but not in the control group. Furosemide (2 mg/kg) or control saline solution was given at time zero and again at 5 minutes. Transfusion started at time zero and was completed at 10 minutes. All values are expressed as mean ± SEM.

initial blood volume over 2 hours). At the end of hemorrhage blood volume was reduced by only 14 ± 1%. Further, blood volume returned to normal by 3 hours after hemorrhage, and was increased 5 ± 2% above control at 24 hours after hemorrhage. The authors noted that "the restitution of blood volume occurred primarily because of a movement of fluid into the fetal circulation as indicated by a reduction in hematocrit."[10] In the present study, transfusion occurred approximately 18 hours after hemorrhage. Thus we presume that the fetal lambs were nearly normovolemic at the time of transfusion.

In the present study, rapid transfusion of approximately 20 ml/kg of packed red blood cells resulted in only modest increases in fetal central venous and arterial pressures. In addition, there was a decrease in fetal heart rate of approximately 18 beats/min, and an increase in fetal P_{O_2} of approximately 2 mm Hg. Brace[11] observed similar changes after intravascular transfusion of approximately 29 ml/kg of packed red blood cells over 30 minutes in anemic fetal lambs. Both Brace and we have speculated that the decreased fetal heart rate resulted from activation of the baroreceptors. The increased fetal P_{O_2} may have resulted from increased umbilical blood flow after transfusion. (This is consistent with the observation that transfusion of anemic human fetuses causes an acute decline of the umbilical artery systolic/diastolic ratio.[6] The increased diastolic flow velocity may reflect increased umbilical blood flow.)

Heretofore, one of us (C. P. W.) has consistently given furosemide (2 mg/kg estimated fetal weight) before and again during direct intravascular transfusion of anemic human fetuses. This rationale was on the basis of the observation that in adults, intravenous administration of furosemide rapidly increases venous capacitance and decreases cardiac filling pressures.[7] We speculated that furosemide might facilitate fetal adaptation to the abrupt increase in intravascular volume, first by dilating the fetal capacitance vessels, and later

by decreasing plasma volume by means of a diuresis. But in this study, furosemide did not significantly alter the hemodynamic response to rapid transfusion of the anemic fetal lamb. The hemodynamic changes were modest, with or without furosemide. We suggest three potential reasons for the absence of a difference between groups in the hemodynamic response to transfusion. First, it is possible that furosemide does not significantly dilate the capacitance vessels of fetal lambs. We did not study the hemodynamic effects of furosemide alone. But Lumbers and Stevens[12] noted that furosemide (2 mg/kg) significantly increased fetal diastolic arterial pressure when it was given to hypotensive fetal lambs. They did not evaluate the effect of furosemide on venous capacitance. Second, there are conflicting data with regard to the effect of furosemide on the urine output of fetal lambs.[12, 13] But third and most important, blood volume quickly returns toward normal after volume loading in fetal lambs.[14] Brace[11] noted that transfusion of anemic fetal lambs increased fetal blood volume by only 50% of the transfused volume because of a loss of plasma from the circulation.

One should be cautious before extrapolating results of any animal study to clinical practice. In addition, we note that the fetal lambs in this study were presumed to be healthy and nonhydropic. They had significant, but not severe, anemia.[2, 5, 15] Further, on day 1 of each experiment, fetal blood was replaced with normal saline solution. We did not determine fetal plasma protein concentrations, and we do not know whether hypoproteinemia occurred. (There is evidence that hypoproteinemia is an important factor in the pathophysiology of fetal hydrops.[16, 17]) Nonetheless, results of this study are consistent with observations made during transfusion of anemic human fetuses. Weiner et al.[18] observed that rapid intravascular transfusion of approximately 90 ml of packed red blood cells to nonhydropic anemic human fetuses increased umbilical venous pressure (corrected for amniotic fluid pressure) from 6.7 ± 1 to 10.9 ± 1 mm Hg. Nicolaides et al.[2] transfused 5 to 150 ml of packed red blood cells to anemic human fetuses at a rate as high as 15 ml/min without obvious evidence of fetal cardiovascular compromise. Nonetheless, Berkowitz et al.[5] argued that exchange, rather than direct, intravascular transfusion may be indicated in selected cases.

We conclude that rapid transfusion of the anemic fetal lamb resulted in modest increases in fetal central venous pressure and mean arterial pressure. Second, furosemide did not significantly alter the hemodynamic response to rapid transfusion.

REFERENCES

1. de Crespigny LC, Robinson HP, Quinn M, Doyle L, Ross A, Cauchi M. Ultrasound-guided fetal blood transfusion for severe rhesus isoimmunization. Obstet Gynecol 1985;66:529-32.
2. Nicolaides KH, Soothill PW, Rodeck CH, Clewell W. Rh disease: intravascular fetal blood transfusion by cordocentesis. Fetal Ther 1986;1:185-92.
3. Weiner CP. Cordocentesis for diagnostic indications: two years' experience. Obstet Gynecol 1987;70:664-8.
4. Copel JA, Grannum PA, Harrison D, Hobbins JC. The use of intravenous pancuronium bromide to produce fetal paralysis during intravascular transfusion. AM J OBSTET GYNECOL 1988;158:170-1.
5. Berkowitz RL, Chitkara U, Wilkins IA, Lynch L, Plosker H, Bernstein HH. Intravascular monitoring and management of erythroblastosis fetalis. AM J OBSTET GYNECOL 1988;158:783-95.
6. Weiner CP, Anderson TL. The acute effect of cordocentesis with or without fetal curarization and of intravascular transfusion upon umbilical artery waveform indices. Obstet Gynecol 1989;73:219-24.
7. Dikshit K, Vyden JK, Forrester JS, Chatterjee K, Prakash R, Swan HJC. Renal and extrarenal hemodynamic effects of furosemide in congestive heart failure after acute myocardial infarction. N Engl J Med 1973;288:1087-90.
8. Robillard JE, Matson JR, Sessions C, Smith FG. Developmental aspects of renal tubular reabsorption of water in the lamb fetus. Pediatr Res 1979;13:1172-6.
9. Brace RA. Fetal blood volume responses to acute fetal hemorrhage. Circ Res 1983;52:730-4.
10. Brace RA, Cheung CY. Fetal cardiovascular and endocrine responses to prolonged fetal hemorrhage. Am J Physiol 1986;251:R417-24.
11. Brace RJ. Ovine fetal cardiovascular responses to packed red blood cell transfusions. AM J OBSTET GYNECOL 1989;161:1367-74.
12. Lumbers ER, Stevens AD. The effects of frusemide, saralasin, and hypotension on fetal plasma renin activity and on fetal renal function. J Physiol 1987;393:479-90.
13. Chamberlain PF, Cumming M, Torchia MG, Biehl D, Manning FA. Ovine fetal urine production following maternal intravenous furosemide administration. AM J OBSTET GYNECOL 1985;151:815-9.
14. Brace RA. Fetal blood volume responses to intravenous saline solution and dextran. AM J OBSTET GYNECOL 1983;147:777-81.
15. Nicolaides KH, Rodeck CH, Millar DS, Mibashan RS. Fetal haematology in rhesus isoimmunisation. Br Med J 1985;290:661-3.
16. Phibbs RH, Johnson P, Tooley WH, Johnson BB, Sudman D, Schlueter M. Cardiorespiratory status of erythroblastotic newborn infants. II. Blood volume, hematocrit, and serum albumin concentration in relation to hydrops fetalis. Pediatrics 1974;53:13-23.
17. Nicolaides KH, Warenski JC, Rodeck CH. The relationship of fetal plasma protein concentration and hemoglobin level to the development of hydrops in rhesus isoimmunization. AM J OBSTET GYNECOL 1985;152:341-4.
18. Weiner CP, Pelzer GD, Heilskov J, Wenstrom KD, Williamson RA. The effect of intravascular transfusion on umbilical venous pressure in anemic fetuses with and without hydrops. AM J OBSTET GYNECOL 1989;161:1498-501.

Intrauterine growth retardation and the circulatory responses to acute hypoxemia in fetal sheep

Barry S. Block, MD,[a] Donald H. Schlafer, DVM, PhD,[b] Richard A. Wentworth, PhD,[c] Lois A. Kreitzer, MS,[b] and Peter W. Nathanielsz, MD, PhD[c]

Chicago, Illinois, and Ithaca, New York

Intrauterine growth retardation has been produced experimentally by umbilical placental embolization for 9 days (early intrauterine growth retardation) in pregnant sheep. Fetuses with early intrauterine growth retardation had a 20% decrease in mean body weight and 33% decrease in placental blood flow. However, the regional blood flow distribution was not significantly different at rest between the embolized and normally grown fetuses despite the 39% decrease in fetal arterial oxygen content. The purpose of this study was to determine the circulatory responses to acute hypoxemic stress in the early development of intrauterine growth retardation. We found that the regional blood flow distribution was not significantly different during imposed acute hypoxemia between the seven fetuses with early intrauterine growth retardation and seven nonembolized normally grown fetuses. We conclude that growth-retarded fetuses are able to meet basal metabolic oxygen requirements and to respond normally to imposed acute hypoxemia until the placental circulatory reserve capacity is depleted. (AM J OBSTET GYNECOL 1989;161:1576-9.)

Key words: Pregnancy, obstetrics, hypoxia, microspheres

Fetuses with intrauterine growth retardation (IUGR) tolerate the acute stress of labor poorly because of malnutrition and chronic hypoxemia.[1] Ethical constraints prevent studies of growth retardation or imposed hypoxemia in human fetuses; however, corresponding studies have been performed with experimental animals. Creasy et al.[2] embolized the maternal uteroplacental vascular bed for 21 to 28 days in sheep and reduced mean fetal body weight by 30% (late IUGR) and total placental blood flow by 53% in embolized fetuses, compared with nonembolized normally grown fetuses. They found that fetuses with late IUGR, at rest, had a redistribution of the circulation that provided preferential perfusion to the vital organs. This blood flow distribution was similar to the distribution seen in normally grown fetuses in response to acute hypoxemia,[3] with the exception that the proportion of combined ventricular output perfusing the placenta was decreased in fetuses with late IUGR at rest.[2] In response to imposed acute hypoxemia fetuses with late IUGR developed a more pronounced blood flow response to all vital organs except the placenta.[4]

Fetal umbilical placental embolization for a period of 9 days has previously been shown to reduce fetal weight by 20% (early IUGR) and to reduce total placental blood flow by 33%.[5] In contrast to fetuses with late IUGR, fetuses with early IUGR had a regional blood flow distribution at rest that was similar to that of nonembolized normally grown fetuses, despite the 39% decrease in fetal arterial oxygen content. Growth-retarded fetuses adapted to chronic oxygen deficiency by decreasing growth rate and thereby decreasing oxygen requirement. Thus a 33% reduction in total placental blood flow restricted normal fetal growth, but did not exceed the placental circulatory reserve capacity necessary to maintain basal metabolic oxygenation. The purpose of this study was to determine the circulatory responses to acute hypoxemic stress during the early development of fetal growth retardation.

Material and methods

Mixed-breed Rambouillet-Columbia pregnant ewes of known gestational ages were used in this study. Surgery was conducted with the ewes under halothane general anesthesia after a 24-hour fast. The maternal abdomen was prepared according to aseptic technique and the uterus was exposed through a midline abdominal incision. A pelvic limb of the fetus was exteriorized and polyvinyl catheters were advanced into the descending fetal aorta via the femoral artery, into the inferior vena cava via the femoral vein, and through a separate incision, the ascending aorta via the fetal ca-

From the Section of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, The University of Chicago,[a] and the Department of Pathology[b] and the Laboratory for Pregnancy and Newborn Research, Department of Physiology,[c] College of Veterinary Medicine, Cornell University.

Supported in part by grant HD 21350 from the National Institutes of Health and by The Harold Wetterberg Foundation.

Presented in part at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.

Reprint requests: Barry S. Block, MD, Department of Gynecology and Obstetrics, Room 3401, Loma Linda University, 11234 Anderson St., Loma Linda, CA 92350.

6/6/15672

rotid artery and the superior vena cava via the jugular vein. A polyvinyl amniotic fluid catheter was placed and the unterine incision was closed. Vinyl-insulated multistranded wire electrodes (Cooner Wire Co., Chatsworth, Calif.) were sewn into the uterus at two separate sites and the abdominal incision was closed. All catheters and the electrodes were exteriorized through the flank of the ewe. The maternal neck was prepared according to aseptic technique and polyvinyl catheters were placed in the carotid artery, jugular vein, and trachea.[6] Antibiotics were given to both ewe and fetus intravenously postoperatively for 4 days. The ewe received 250 mg ampicillin and 900 mg chloramphenicol intravenously twice daily. The fetus received 50 mg chloramphenicol intravenously and intraamniotically twice daily.

The fetal umbilical placental circulation was embolized by the general procedure previously described.[5] Briefly, on the sixth day after surgery, the fetuses were assigned to either the control or the embolized group. In the embolized group fetuses were injected over a 3-minute period into the descending aorta with carbonized latex 15 μm microspheres (8 to 16 million) in dextran that were suspended with sterile saline solution, q.s. to 5 ml, once or twice daily. The goal was to maintain an oxygen tension 5 to 7 mm Hg below control values in that fetus on the 2 days before embolization began. In the control group fetuses were injected over a 3-minute period into the descending aorta with a similar volume of dextran, q.s. to 5 ml in sterile saline solution twice daily.

Blood gases and pH were measured with an automated analyzer (ALB2, Radiometer, Copenhagen, Denmark) and corrected to a temperature of 39° C. Blood pressure and amniotic fluid pressure were determined with strain-gauge transducers (P23Db, Statham/Gould, Oxnard, Calif.) and recorded continuously on a polygraph (Dynograph R611, Beckman, Fullerton, Calif.). The percent of oxygen saturation of hemoglobin was measured with a hemoximeter (OSM2, Radiometer, Copenhagen). Arterial oxygen content was calculated as follows: O_2 content (ml/dl) = 1.34 (ml/gm Hgb) × [Hgb] (gm/dl) × Hgb O_2 saturation, where O_2 represents oxygen and Hgb represents hemoglobin.

Fetal arterial blood pressure was corrected by subtracting amniotic fluid pressure. Fetal heart rate was derived from the arterial pressure pulse by a cardiotachometer (9857B input coupler, Beckman, Fullerton, Calif.). Uterine electromyographic activity was displayed continuously. Fetal combined ventricular output and regional blood flow distribution were measured with 15 μm radionuclide-labeled microspheres (New England Nuclear, Boston) while the uterus was quiescent by methods previously described.[4]

The experimental protocol began after 5 days of recovery and 9 days of embolization. The protocol included 30 minutes of baseline recordings; then the first carotid arterial blood gas, pH, and regional blood flow determinations were performed. While the recordings continued 6 L/min of nitrogen was infused into the maternal trachea for 20 minutes and the second blood gas, pH, and regional blood flow determinations were performed. At the end of the experiment the ewe was killed and the uterus and its contents were removed, dissected, and weighed. The placental blood flow was calculated as the sum of the individual cotyledon and noncotyledonary membrane blood flows. The tissues were then carbonized and counted with an automated γ-counter and multiple-channel pulse height analyzer (Gamma Trac 2250, T.M. Analytic, Elk Grove Village, Ill.).

A two-factor analysis of variance for repeated measures and the Newman-Keuls test[7] were used to compare the mean values in the basal and hypoxemia periods for embolized and nonembolized normally grown (control) fetuses. The mean values for gestational age, birth weight, and total placental blood flow in the embolized and nonembolized normally grown fetuses were compared with the two-sample Student t test.[8] The level of significance chosen was $p < 0.05$. The values are expressed as the mean ± 1 SD.

Results

There were 12 singleton and two twin gestations (one in each group) included in this study. The gestational ages at the conclusion of the study were similar (128 ± 3 and 130 ± 4 days) in the seven embolized and seven control fetuses, respectively. Fetal umbilical placental embolization reduced the mean fetal body weight of the embolized fetuses 20% (2.99 ± 0.25 vs. 3.73 ± 0.58 kg, $p < 0.05$) and the total placental blood flow 31% (421 ± 82 vs. 606 ± 160 ml/min, $p < 0.05$) in the embolized fetuses compared with nonembolized normally grown fetuses. The brain-to-thymus weight ratio also was significantly increased in embolized fetuses (6.4 ± 2.2 vs. 3.3 ± 1.5, $p < 0.05$). Surprisingly, neither the fetal crown-rump length (46.2 ± 6.9 vs. 45.5 ± 3.7 cm) nor the brain-to-liver weight ratio (0.42 ± 0.09 vs. 0.43 ± 0.08) was significantly different in the embolized fetuses compared with the nonembolized normally grown fetuses.

The fetal arterial blood gas, pH, hemoglobin, heart rate, and arterial blood pressure values in the basal and hypoxemia study periods for the embolized and nonembolized normally grown fetuses are listed in Table I. Both the mean arterial oxygen tension and oxygen content were reduced significantly in the basal period in the embolized fetuses, and the oxygen content in both groups was reduced by approximately 50% during induced hypoxemia. The mean fetal arterial pH, hemoglobin, and carbon dioxide tension remained within

Table I. Fetal mean carotid arterial blood gases, pH, hemoglobin, blood pressure, and heart rate in control and embolized fetuses before basal and induced acute hypoxemia blood flow studies

	Control (n = 7)		*Embolized (n = 7)*	
	Basal	*Hypoxemia*	*Basal*	*Hypoxemia*
Oxygen tension (mm Hg)	25.8 ± 2.2	15.1 ± 1.6*	19.9 ± 1.8†	12.1 ± 1.5*
Oxygen content (mg/dl)	11.0 ± 2.6	5.1 ± 1.0*	6.8 ± 1.2†	3.6 ± 0.6*
Carbon dioxide tension (mm Hg)	50.9 ± 6.3	47.9 ± 3.6	53.7 ± 3.1	49.4 ± 2.9
pH	7.33 ± 0.05	7.34 ± 0.05	7.33 ± 0.06	7.33 ± 0.03
Hemoglobin (gm/dl)	10.7 ± 1.5	11.3 ± 1.2	10.6 ± 1.8	11.4 ± 2.1
Mean arterial blood pressure (mm Hg)	52 ± 9	50 ± 8	48 ± 6	48 ± 7
Heart rate (beats/min)	159 ± 13	144 ± 18*	163 ± 11	144 ± 20*

Values expressed as mean ± 1 SD.
*$p < 0.01$, hypoxemia versus basal.
†$p < 0.01$, embolized versus control.

Table II. Fetal combined ventricular output and its distribution in control and embolized fetuses during basal and induced acute hypoxemia periods

	Control (n = 7)		*Embolized (n = 7)*	
	Basal	*Hypoxemia*	*Basal*	*Hypoxemia*
Combined ventricular output (ml/min/kg)	564 ± 103	594 ± 151	482 ± 109	579 ± 130
Placental blood flow (ml/min/kg)	175 ± 37	212 ± 70	147 ± 16	192 ± 66
Distribution of cardiac output (%)				
Adrenal gland	0.05 ± 0.01	0.20 ± 0.13*	0.10 ± 0.04	0.26 ± 0.12*
Brain	2.7 ± 0.9	4.9 ± 2.1*	3.4 ± 1.1	4.9 ± 1.6*
Carcass	43.1 ± 5.9	40.7 ± 8.8	46.7 ± 5.8	45.0 ± 5.1
Heart	2.0 ± 1.0	6.6 ± 4.2*	2.6 ± 1.6	6.5 ± 3.4*
Kidney	1.6 ± 0.4	1.8 ± 0.4	2.1 ± 0.6	2.4 ± 0.9
Placenta	31.4 ± 6.1	35.5 ± 6.2*	31.5 ± 5.7	33.2 ± 9.5
Spleen	2.2 ± 0.7	1.5 ± 1.0*	.25 ± 2.4	1.4 ± 1.3*
Organ blood flow (mg/min/100 gm)				
Adrenal gland	257 ± 66	1026 ± 488*	385 ± 170	1116 ± 485*
Brain	161 ± 32	260 ± 65*	188 ± 65	259 ± 63*
Carcass	37 ± 8	34.1 ± 9.7	37.8 ± 9.1	36.6 ± 5.2
Heart	219 ± 72	649 ± 363*	248 ± 43	643 ± 215*
Kidney	126 ± 40	145 ± 40	143 ± 55	200 ± 55
Spleen	397 ± 125	267 ± 100*	520 ± 357	406 ± 33*

Values expressed as mean ± 1 SD. Comparisons of embolized versus control means were not significant ($p \geq 0.05$).
*$p < 0.05$, hypoxemia versus basal.

the normal range in both groups during induced hypoxemia. There was no significant difference in arterial blood pressure between the two groups in the basal or hypoxemia study periods. In addition, there was no significant difference in the bradycardiac fetal heart rate response between the embolized and nonembolized normally grown fetuses in response to acute hypoxemia.

The fetal combined ventricular output and regional blood flow distribution for the embolized and nonembolized normally grown fetuses are listed in Table II. In the basal study period the combined ventricular output and individual organ blood flow values were not significantly different, when calculated on either a weight-specific or percentage of combined ventricular output basis, between the embolized and nonembolized normally grown fetuses. In response to acute imposed hypoxemia, the combined ventricular output and blood flow to the placenta, on a weight-specific basis, were not significantly different between the embolized and nonembolized normally grown fetuses. Both the absolute blood flow and the proportion of combined ventricular output perfusing the adrenal gland, brain, and heart increased significantly in both groups in response to acute imposed hypoxemia.

Comment

Clearly, the ability to maintain normal growth and respond to adverse intrauterine conditions is related to the fetus' ability to adequately perfuse the placenta.

Fetal umbilical placental embolization decreased total placental blood flow and arterial oxygen content and produced a 20% reduction in fetal growth. At rest the regional blood flow distribution in fetuses with early IUGR was similar to the distribution in nonembolized normally grown fetuses. Thus the fetus with early IUGR with subnormal growth was able to meet basal metabolic oxygen requirements despite the 39% decrease in arterial oxygen content. In response to acute hypoxemia, the regional blood flow distribution in the fetus with early IUGR was not significantly different from the normally grown fetus. These findings suggest that the placental circulatory reserve capacity was adequate to maintain basal metabolic oxygenation in fetuses with early IUGR both at rest and in response to acute hypoxemia.

The regional blood flow response to acute hypoxemia in the nonembolized normally grown fetuses, characterized by preferential perfusion to the vital organs, is similar to the blood flow response previously reported.[3] In addition, the fetal arterial oxygen tension, oxygen content, blood pressure, and heart rate values for the nonembolized normally grown fetuses were similar to the values previously reported.[3, 4] The method of fetal umbilical-placental embolization was different from the method of maternal uteroplacental embolization. The explanation for choosing fetal embolization has been discussed in detail.[5] Briefly, we produced a significant degree of fetal hypoxemia by modification of a technique to increase placental vascular resistance and produce primary fetal placental vascular compromise described by Trudinger et al.[9]

To minimize concerns about natural growth retardation in twins, only the use of singleton gestations was planned. However, two ewes were noted at surgery to have twin gestations and they were assigned to different groups. Because the subsequent weight of the control and embolized twin fetuses at necropsy were similar (110% and 94%, respectively) to the mean weight of others in their group, they were not excluded from the study.

We have demonstrated that fetuses have a considerable capacity to adapt to chronic oxygen deficiency produced by decreased placental blood flow by decreasing growth rate and thereby decreasing oxygen requirement. The effects of chronic hypoxemia alone, produced without reduction in placental blood flow, have been previously reported. Kitanaka et al.[10] have shown that the fetus adapts to chronic hypoxemia produced by low oxygen infusion in a normobaric chamber for 21 to 28 days by maintaining fetal arterial oxygen content without reducing fetal weight. The effect of imposed acute hypoxemia on chronically hypoxemic normally grown fetuses has not previously been reported. However, the response to imposed acute hypoxemia in growth-retarded fetuses has previously been reported.[4] Maternal uteroplacental embolization for 21 to 28 days produced a 30% reduction in mean fetal body weight. These fetuses with late IUGR, in contrast to fetuses with early IUGR, have a distribution of combined ventricular output at rest similar to normally grown fetuses in response to acute hypoxyemia. In response to acute imposed hypoxemia, fetuses with late IUGR had a more pronounced blood flow response to all vital organs that normally grown fetuses, with the exception that the proportion of combined ventricular output perfusing the placenta did not change significantly in fetuses with late IUGR. These findings suggest that the 53% decrease in total placental blood flow exceeded the placental circulatory reserve capacity.

We conclude that the growth-retarded fetus adapts to chronic oxygen deficiency by decreasing growth rate and thereby decreasing oxygen requirement. The growth-retarded fetus is thus able to meet basal metabolic oxygen requirements and to respond normally to imposed acute hypoxemia until the placental circulatory reserve capacity is depleted.

We thank Tami Myers and Vivian Surman for their technical assistance.

REFERENCES

1. Gruenwald P. Growth and maturation of the foetus and its relationship to perinatal mortality. In: Butler NR, Alberman ED, eds. Perinatal problems. London: Livingstone, 1969:141-62.
2. Creasy RK, De Swiet M, Kahanpää KV, Young WP, Rudolph AM. Pathophysiological changes in the foetal lamb with growth retardation. In: Foetal and neonatal physiology. Proceedings of the Sir Joseph Barcroft Centenary Symposium, 1972. Cambridge, England: Cambridge University Press, 1973:398-402.
3. Cohn HE, Sacks EJ, Heymann MA, Rudolph AM. Cardiovascular responses to hypoxemia and acidemia in fetal lambs. AM J OBSTET GYNECOL 1974;120:817-24.
4. Block BS, Llanos AJ, Creasy RK. Responses of the growth-retarded fetus to acute hypoxemia. AM J OBSTET GYNECOL 1984;148:878-85.
5. Block BS, Schlafer DH, Wentworth RA, Kreitzer LA, Nathanielsz PW. The effects of decreased placental perfusion on regional blood flow distribution in intrauterine growth retarded fetal sheep. J Dev Physiol 1989 [In press].
6. Gleed RD, Poore ER, Figueroa JP, Nathanielsz PW. Modification of maternal and fetal oxygenation with the use of tracheal gas infusion. AM J OBSTET GYNECOL 1986; 155:429-35.
7. Winer BJ. Statistical principles and experimental design. New York: McGraw-Hill, 1971.
8. Zar JH. Biostatistical analysis. Englewood Cliffs, New Jersey: 1974.
9. Trudinger BJ, Stevens D, Connelly A, et al. Umbilical artery flow velocity waveforms and placental resistance: the effects of embolization of the umbilical circulation. AM J OBSTET GYNECOL 1987;157:1443-8.
10. Kitanaka T, Alonso JG, Gilbert RD, Siu BL, Clemons GK, Longo LD. Fetal responses to long-term hypoxemia in sheep. Am J Physiol 1989;256:R1348-54.

Prostaglandin E_2 metabolism in the human fetal membranes

Paul Y. C. Cheung, BSc, and John R. G. Challis, PhD, DSc
London, Ontario, Canada

Prostaglandins play a pivotal role in the initiation and maintenance of labor, but the sources that contribute to the production of primary prostaglandins (prostaglandin E_2 and prostaglandin $F_{2\alpha}$) remain controversial. In decidua, prostaglandin $F_{2\alpha}$ may be formed de novo or from prostaglandin E_2 through 9-ketoreductase activity, although it has been suggested that metabolism to 13,14-dihydro-15-ketoprostaglandin E_2 through 15-hydroxyprostaglandin dehydrogenase is dominant. To examine this possibility, and to determine changes with labor, we examined interconversions of prostaglandin E_2 to prostaglandin $F_{2\alpha}$, 13,14-dihydro-15-ketoprostaglandin E_2 and 13,14-dihydro-15-ketoprostaglandin $F_{2\alpha}$ by dispersed cells of decidua, chorion, and amnion obtained from patients at term elective cesarean section or after spontaneous labor. Incubations were performed with increasing concentrations (1 to 100 ng/ml) of prostaglandin E_2 for 2 and 4 hours, and products were measured by radioimmunoassay. The 13,14-dihydro-15-ketoprostaglandin E_2 was measured as 11-deoxy-13,14-dihydro-15-keto-11β,16ψ-cycloprostaglandin E_2. Basal output of prostaglandin E_2 increased between cesarean section and spontaneous labor from 190.6 ± 109 pg/10^5 cells (±SE) to 605.2 ± 346.8 in amnion, nondetectable to 24.5 ± 11.2 in chorion, and 18 ± 16 to 69 ± 25.8 in decidua. For both cesarean section and spontaneous labor patients there was very little conversion of prostaglandin E_2 by the amnion, substantial conversion to 13,14-dihydro-15-ketoprostaglandin E_2 by the chorion, and some conversion to 13,14-dihydro-15-ketoprostagalandin E_2 by the decidua. Percentage conversions did not change with labor. Prostaglandin $F_{2\alpha}$ was formed by all tissues but only in trace amounts. All tissues from one cesarean section patient showed substantial conversion of prostaglandin E_2 to prostaglandin $F_{2\alpha}$, but very little 13,14-dihydro-15-ketoprostaglandin E_2 or 13,14-dihydro-15-ketoprostaglandin $F_{2\alpha}$ formation, indicating either a decrease or deficiency in the 15-hydroxyprostaglandin dehydrogenase activity. We conclude that conversion of exogenous prostaglandin E_2 to prostaglandin $F_{2\alpha}$ can occur in intrauterine tissues, but this does not normally contribute significantly to the prostaglandin $F_{2\alpha}$ output by the human decidua and fetal membranes. (Am J Obstet Gynecol 1989;161:1580-5.)

Key words: Prostaglandin, fetal membranes, labor, metabolism

Prostaglandins (PGs) are involved in the initiation and maintenance of labor.[1,2] Any tissues capable of synthesizing or metabolizing primary PGs (PGE_2 and $PGF_{2\alpha}$) in utero would ultimately affect the concentrations of these PGs reaching the myometrium, the presumed site of PG action.[3,4] Because of their anatomical location, the human fetal membranes are highly suited for this purpose.

PGE_2 can be acted on by two enzymes, 15-hydroxyprostaglandin dehydrogenase (PGDH), leading to formation of 15-keto-PGE_2 and then to 13,14-dihydro-15-keto-PGE_2 (PGEM) or 9-ketoreductase to form $PGF_{2\alpha}$.[5] $PGF_{2\alpha}$ can then be metabolized by PGDH to 15-keto-$PGF_{2\alpha}$ and then to 13,14-dihydro-15-keto-$PGF_{2\alpha}$ (PGFM). PGDH activity has been demonstrated in all three tissues of the human fetal membranes with the order of activity as chorion > decidua > amnion.[2] Although 9-ketoreductase has been characterized extensively in the decidua,[6,7] it has not been investigated to the same extent in the amnion or chorion. Most of these studies have used homogenate or cytosolic preparations to study the characteristics of these enzymes. It has been suggested that inasmuch as the specific activity of PGDH is higher than the specific activity of 9-ketoreductase in decidual cytosolic fractions, PGE_2 would be preferentially metabolized to PGEM rather than to $PGF_{2\alpha}$.[7]

We have extended these studies to investigate the relative formation of PGEM, PGFM, and $PGF_{2\alpha}$ from exogenous PGE_2 by dispersed-cell preparations of separated human fetal membranes in vitro. We determined the potential for $PGF_{2\alpha}$ formation from PGE_2 by the amnion, chorion, and decidua, and examined whether this was altered with spontaneous labor.

Material and methods

Preparation of tissues. Human fetal membranes with attached decidua and placenta were obtained from

From the Departments of Obstetrics and Gynecology and Physiology, University of Western Ontario, The Lawson Research Institute, and St. Joseph's Health Centre.
Supported by the Medical Research Council of Canada (Group Grant in Reproductive Biology, J. R. G. C.).
Presented at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.
Reprint requests: Paul Cheung, The Lawson Research Institute, St. Joseph's Health Centre, University of Western Ontario, 268 Grosvenor St., London, Ontario, Canada N6A 4V2.
6/6/16241

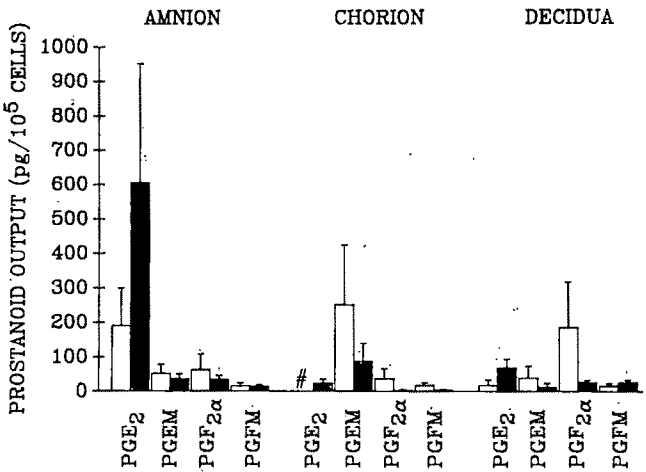


Fig. 1. Net basal output of PGE_2, $PGF_{2\alpha}$, PGEM, and PGFM by dispersed cells from amnion, chorion, and decidua obtained at term cesarean section (□) or spontaneous delivery (■). Values are mean ± SE for five patients at cesarean section and six patients at spontaneous delivery. (#, Nondetectable.)

Table I. Mean recovery of added PGE_2

Tissue	*Amount of PGE_2 added (ng/ml)*	*PGE_2 (% remaining)*	*% Converted*			*Total (mean ± SE)*
			$PGF_{2\alpha}$	*PGFM*	*PGEM*	
Cesarean section (n = 5)						
Amnion	1	37.0	2.2	0.7	0.5	40.4 ± 2.8
	10	31.6	0.8	ND	0.1	32.5 ± 4.5
Chorion	1	26.4	0.2	1.9	67.8	96.3 ± 21.6
	10	21.9	0.6	1.0	75.2	98.7 ± 27.6
Decidua	1	33.7	0.3	ND	8.4	42.4 ± 8.0
	10	22.4	0.6	0.1	5.9	29.1 ± 5.8
Spontaneous labor (n = 6)						
Amnion	1	39.1	0.6	0.4	2.6	42.7 ± 15.6
	10	47.2	0.3	0.1	0.9	48.5 ± 15.5
Chorion	1	32.3	0.3	2.8	100.3	135.7 ± 31.5
	10	34.1	0.2	1.3	78.5	114.1 ± 25.4
Decidua	1	43.7	0.4	1.0	9.3	54.4 ± 12.1
	10	44.5	0.3	0.2	2.6	47.6 ± 16.7

ND, Nondetectable.

women at term undergoing elective cesarean section (n = 6) or at spontaneous delivery (n = 6). Tissues were collected immediately after delivery in Dulbecco's phosphate buffered saline solution (pH 7.4). The amnion was separated manually from the choriodecidua. The tissues were then washed several times with Dulbecco's phosphate buffered saline solution to remove clotted blood. The chorion and decidua were then gently separated with fine forceps.

Cell dispersal. Cells were prepared with collagenase digestion as described by Skinner et al.[8] The rinsed tissues were cut into pieces (1 cm^2) and placed in separate 250 ml Erlenmeyer flasks. Freshly prepared collagenase (0.1%, Type I, Sigma Chemical Co., St. Louis, Mo.) and trypsin inhibitor (0.05%, Type I-S, Sigma Chemical Co.) in Dulbecco's phosphate buffered saline solution (30 ml) were added to each flask, and the flasks were tightly stoppered. The tissues were incubated for approximately 1 hour at 37° C in a shaking water bath with vigorous shaking every 15 to 25 minutes.

When the suspensions appeared uniform, cells were collected by centrifugation at 125 g for 10 minutes. The cells were then washed twice with Dulbecco's phosphate buffered saline solution with centrifugation at 125 g for 10 minutes and resuspended in 20 ml of Eagle's minimum essential medium (pH 7.4 with glutamine, without sodium bicarbonate) (Flow Laboratories, Mississauga, Ontario, Canada). Cell viability was assessed by exclusion of trypan blue dye. An aliquot (10 μl) of the cell suspension was added to Hematall isotonic diluent (10 ml), treated with 0.3% potassium cyanide (Hematall LA-Hgb reagent, Fisher Diagnostics, Unionville, Ontario, Canada), and the cells were counted with a Coulter counter. The cell suspension was then diluted so that the concentration of viable cells was 4×10^5 per milliliter of Eagle's minimum essential medium.

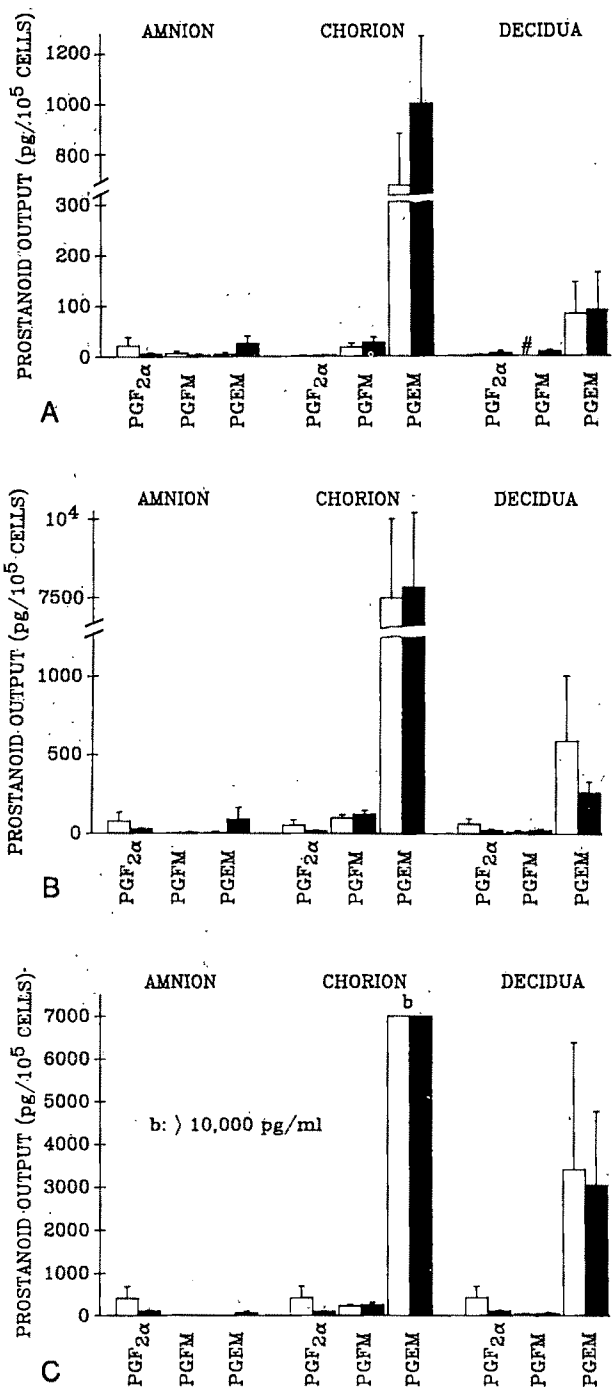


Fig. 2. Output of $PGF_{2\alpha}$, PGFM, and PGEM in presence of **A**, PGE_2, 1 ng/ml; **B**, PGE_2, 10 ng/ml; **C**, PGE_2, 100 ng/ml by amnion, chorion, and decidual cell preparations. Values are mean ± SE for five patients at term elective cesarean section (□) and six patients at spontaneous delivery (■). (#, Nondetectable.)

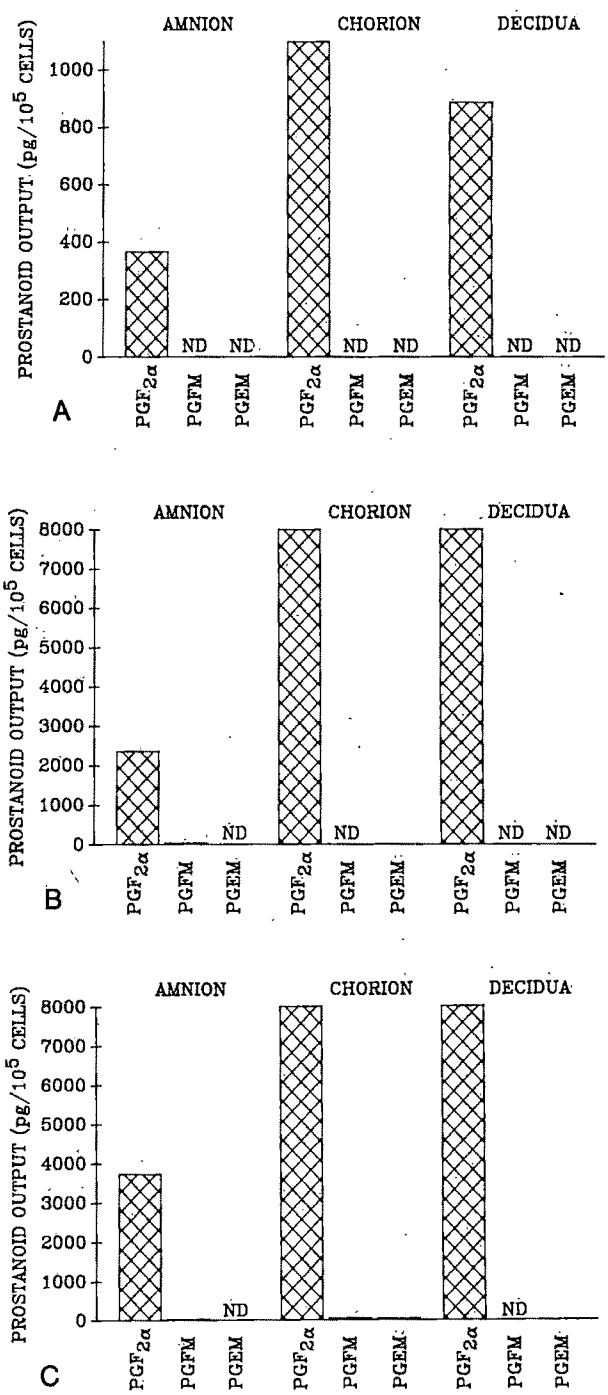


Fig. 3. Output of $PGF_{2\alpha}$, PGFM, and PGEM in presence of **A**, PGE_2, 1 ng/ml; **B**, PGE_2, 10 ng/ml; **C**, PGE_2, 100 ng/ml by amnion, chorion, and decidual cell preparations from one patient at term elective cesarean section. Output of $PGF_{2\alpha}$ by chorion and decidua in panels **B** and **C** were >8065 pg/10^5 cells. *ND*, Nondetectable.

Experimental protocol. Cell suspensions (0.5 ml) were added to polystyrene culture tubes (12 × 75 mm, Canlab, Toronto, Ontario, Canada). PGE_2 or vehicle control was added in 0.5 ml Eagle's minimum essential medium to the respective culture tubes so that the final cell concentration was 2 × 10^5 per milliliter and the final PGE_2 concentration was 0, 1, 10, 100 ng/ml. The cells were then agitated in a shaking water bath at 37° C for 2 and 4 hours under air. All incubations were performed in triplicate. Incubations were terminated

by freezing at −70° C. Controls included in this experiment were cells with media at time zero, test media incubated without cells for 2 and 4 hours, and cells with media but without added PGE_2 to obtain basal PG output at 2 and 4 hours. Frozen cell suspensions were thawed, mixed well, and centrifuged (1000 *g* for 10 minutes) before assay. The concentration of PGE_2 in media from cells at time zero was subtracted from that in media and cells at 2 and 4 hours (basal output) to determine the net basal output over that time. Net basal output was subtracted from total PG output in the presence of added PGE_2 to determine net stimulated output, and is expressed per 10^5 cells.

Overall mean cell viability after collagenase dispersal was 88.9% ± 5.4%. We have determined previously that there was no difference in viability between tissues, nor between cells obtained at elective cesarean section and spontaneous delivery.[9]

Prostaglandin assays. PGE_2, $PGF_{2\alpha}$, and PGEM were measured directly from the incubation media by specific radioimmunoassay. PGEM was measured as 11-deoxy-13,14-dihydro-15-keto-11β,16ψ-cycloprostaglandin E_2 by a commercial assay system from Amersham International (Amersham, United Kingdom). The same amount of medium was included in the assay standard curve. To measure PGFM, the media was first acidified and then extracted with diethyl ether. The extract was dried under a stream of nitrogen and assayed for PGFM.

The interassay and intra-assay coefficients of variation (%) for all four assays were 8.0 ± 2.3 and 3.3 ± 0.6 for PGE_2, 9.3 ± 3.3 and 5.7 ± 1.7 for PGEM, 7.7 ± 1.0 and 4.5 ± 0.3 for $PGF_{2\alpha}$, and 11.3 ± 1.6 and 2.8 ± 0.7 for PGFM.

Antibody specificity was calculated as 50% binding/binding in the absence of nonradioactive prostaglandin displacement. $PGF_{2\alpha}$ cross-reacted 0.18% with the PGE_2 antibody. There was <0.01% cross-reaction for the $PGF_{2\alpha}$ and PGFM antibody with PGs other than $PGF_{2\alpha}$ and PGFM, respectively. The PGEM antibody had <0.001% cross-reactivity with PGE_2, $PGF_{2\alpha}$, or PGFM.

Data analysis. Differences between cesarean section and spontaneous labor patients were sought with the Student *t* test.

Results

It became evident as the study progressed that one patient in the cesarean section group responded quite differently from the rest of the patients. Results for this individual are presented separately. For all other patients, the general response patterns at 2 hours and at 4 hours were similar, and results are presented for the 2-hour incubations only.

Basal PG output. Basal output of PGE_2 (Fig. 1) by amnion was greater than that for other prostanoids, and increased with cells from patients at spontaneous delivery. In chorion, the output of PGEM exceeded that of other PGs but did not change with labor. Net basal output of $PGF_{2\alpha}$ at cesarean section and of PGE_2 at spontaneous delivery from decidual cells was greater than that of PGFM or PGEM, but did not change with labor.

Effects of added PGE_2. The percent recovery of PGE_2 added to the medium at 1 ng/ml, incubated for 2 hours, and measured by radioimmunoassay was 90.8 ± 13.8% (n = 11). Table I shows the recovery of PGE_2, $PGF_{2\alpha}$, PGEM, and PGFM in the medium as a percentage of added PGE_2 after subtraction of net basal PG output. At both cesarean section and spontaneous delivery, recoveries from chorion were not significantly different from 100%, with PGEM being the major product. However, with amnion and decidual cell preparations, recoveries ranged between 30% to 55%, and most activity remained in PGE_2.

The profiles of PGs formed from added PGE_2 were similar at the three different concentrations of added potential substrate (Fig. 2). In amnion, conversion to $PGF_{2\alpha}$ was present, and on average higher at cesarean section than at spontaneous delivery. There was essentially no conversion to PGEM or PGFM by amnion cells at either cesarean section or spontaneous delivery. In chorion there was appreciable conversion to PGEM, but only trace amounts of $PGF_{2\alpha}$ and PGFM were detected. The formation of PGEM in chorion was not significantly different between cesarean section and spontaneous delivery patients. In the decidua there was negligible (<1.0%) formation of $PGF_{2\alpha}$ from added PGE_2. The major product was PGEM, although its formation was variable and did not differ significantly between cesarean section and spontaneous delivery patients.

PG output in one cesarean section patient. The pattern of basal and stimulated output of PGs was quite different in one of the cesarean section patients. In this patient, net basal output of PGE_2, $PGF_{2\alpha}$, and PGEM, and PGFM was undetectable in most tissues (Table II). After addition of PGE_2 (1 to 100 ng/ml) there was substantial formation of $PGF_{2\alpha}$ by amnion (up to 35%), chorion (up to 100%), and decidua (up to 80%), and minimal, if any, formation of PGEM or PGFM by any one of amnion, chorion, and decidual cell preparations (Fig. 3).

Comment

The possibility has been raised that conversion of PGE_2 to $PGF_{2\alpha}$ by intrauterine tissues, especially decidua, might contribute to the increase in $PGF_{2\alpha}$ and PGFM found in amniotic fluid and in maternal plasma during late pregnancy, and may contribute to the stimulus to myometrial contractility at parturition.[7] However, we found that although some $PGF_{2\alpha}$ is formed

Table II. Basal PG output at term cesarean section in one patient (pg/10^5 cells)

Tissue	*PGE_2*	*PGEM*	*$PGF_{2\alpha}$*	*PGFM*
Amnion	13	ND	ND	ND
Chorion	ND	ND	ND	ND
Decidua	ND	ND	ND	18

ND, Nondetectable.

from PGE_2 by all intrauterine tissues, quantitatively this was extremely small, particularly in comparison with the substantial metabolism of PGE_2 to PGEM. Therefore our results are in accord with the conclusions of Niesert et al.[7] and Kredentser et al.[10] that the activity of decidual 9-ketoreductase is unlikely to be a significant factor in the determination of the increase in the production of $PGF_{2\alpha}$ at parturition. Although previous studies have demonstrated PGE_2 9-ketoreductase activity in human decidual cytosol,[4, 7] the present results suggest that the major pathway of decidual $PGF_{2\alpha}$ production is through de novo synthesis. Our study provides new information on the output of PGEM by amnion, chorion, and decidua in the basal state and after added PGE_2 in vitro. We found that PGEM was the major prostanoid produced by chorion, and that in both chorion and decidua (but not in amnion) its output was considerably enhanced after addition of PGE_2. These observations are in accord with high PGDH activity in chorion, somewhat lower activity in decidua, and minimal activity in amnion.[2]

Although we recovered most of the added PGE_2 in PGE_2, PGEM, $PGF_{2\alpha}$, and PGFM for chorion, there was poor quantitative recovery from amnion and decidual cells. In the absence of cells, PGE_2 recovery was good. It seems likely that PGE_2 or its metabolites were taken up and retained to a greater extent by amnion and decidual cells than by chorion. However, because we were interested in relating PG output in vitro to secretion in vivo, we measured PG concentrations in the supernatant only. It is also possible that 15-keto-PGE_2 or 15-keto-$PGF_{2\alpha}$, which were not measured in this study, accumulate in the amnion and decidual cells, although previous studies of PG metabolism in these tissues[11] suggest that this is unlikely to account for the poor total recoveries. It is also possible that the rate of PG entry into cells varies between the tissues. Finally, we did not attempt to adjust cofactors in these experiments. PGDH is a nicotinamide adenine dinucleotide, oxidized form–dependent enzyme, whereas 9-ketoreductase uses nicotinamide adenine dinucleotide phosphate, reduced form.[6, 7] It remains possible that under our incubation conditions nicotinamide adenine dinucleotide phosphate, reduced form, availability is rate-limiting, resulting in an apparent predominance of PGDH activity, despite the demonstration of 9-ketoreductase in decidual cytosol preparations.

Previous studies suggested that 9-ketoreductase activity could be inhibited by progesterone, estradiol, and cortisol, and enhanced by calcium ion or oxytocin.[6] However, the concentrations of steroids used in those studies were high (1 mmol/L), and it is unlikely that endogenous steroid production by the cell types we used in vitro[12] would be sufficient to affect the activity of this enzyme. Further, if the influence of 9-ketoreductase is minimal in vivo, it seems unlikely that steroid modulation would have any physiological significance.

We found one patient in the cesarean section group in whom there was minimal PGDH activity in all tissues, and substantial conversion of PGE_2 to $PGF_{2\alpha}$. Thus, in the absence of PGDH, high levels of 9-ketoreductase were expressed. This patient was delivered as a repeat cesarean section in the current pregnancy. It is of interest that she had had three successive miscarriages at 16 to 20 weeks' gestation. Further detailed studies of this patient and her offspring are under way. The extremely low or undetectable PGDH activity in all tissues of this patient is intriguing. Erwich and Kierse,[13] from a study of placental PGDH activities in monozygotic and dizygotic pregnancies, have suggested that the level of PGDH is controlled by the fetus. A direct fetal effect could account for the results in chorion and amnion. However, the similar absence of PGDH in decidua raises the possibility that either an inhibitor or the lack of a stimulator to PGDH from the trophoblast-derived structures might also exert regulatory influences on the enzyme.

This patient clearly shows that 9-ketoreductase can be expressed in human amnion, chorion, and decidua. However, our results overall suggest that this enzyme does not normally contribute significantly to $PGF_{2\alpha}$ output by the fetal membranes or decidua at term.

REFERENCES

1. Novy MJ, Liggins GC. Role of prostaglandins, prostacyclins, and thromboxanes in the physiologic control of the uterus and in parturition. Semin Perinatol 1980;4:45-66.
2. Okazaki T, Casey ML, Okita JR, MacDonald PC, Johnston JM. Initiation of human parturition. XII. Biosynthesis and metabolism of prostaglandins in human fetal membranes and uterine decidua. Am J Obstet Gynecol 1981; 139:373-81.
3. Schillinger E, Prior G. Characteristics of prostaglandin receptor sites in human uterine tissue. Adv Prostaglandin Thromboxane Res 1976;1:259-63.

4. Wiqvist N, Lindblom B, Wickland M, Wilhemsson L. Prostaglandins and uterine contractility. Acta Obstet Gynecol Scand 1983;113(suppl):23-9.
5. Jarabak J. Early steps in prostaglandin metabolism in the human placenta. AM J OBSTET GYNECOL 1980;138:534-40.
6. Schlegel W, Kruger S, Korte K. Purification of prostaglandin E_2-9-oxoreductase from human decidua vera. FEBS Lett 1984;171:141-4.
7. Niesert S, Christopherson W, Korte K, Mitchell MD, MacDonald PC, Casey ML. Prostaglandin E_2 9-ketoreductase activity in human decidua vera tissue. AM J OBSTET GYNECOL 1986;155:1348-52.
8. Skinner KA, Challis JRG. Changes in the synthesis and metabolism of prostaglandins by human fetal membranes and decidua at labour. AM J OBSTET GYNECOL 1985; 151:519-23.
9. Olson DM, Skinner KA, Challis JRG. Prostaglandin output in relation to parturition by cells dispersed from human intrauterine tissues. J Clin Endocrinol Metab 1983; 57:694-9.
10. Kredentser JV, McCoshen JA, Odowichuk C. Fetal membrane prostaglndin F production has no significant contribution from conversion of amniotic prostaglandin E_2. In: Proceedings of the thirty-sixth annual meeting of the Society for Gynecologic Investigation. San Diego: Society for Gynecologic Investigation, 1989:297.
11. Keirse MJNC, Turnbull AC. The fetal membranes as a possible source of amniotic fluid prostaglandins. Br J Obstet Gynaecol 1976;83:146-51.
12. Challis JRG, Mitchell BF. Steroid production by the fetal membranes in relation to the onset of parturition. In: McNellis D, Challis J, MacDonald P, Nathanielsz P, Roberts J, eds. The onset of labor: cellular and integrative mechanisms. Ithaca, N.Y.: Perinatology Press, 1988:233.
13. Erwich JJHM, Keirse MJNC. Human placental 15-hydroxyprostaglandin dehydrogenase activity is under fetal genetic control. Prostaglandins 1988;35:123-31.

Make researching easier with the Ten-Year Cumulative Index (1978-1987) to the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY

The Ten-Year Cumulative Index is a complete guide to more than 30,000 pages of original articles, case reports, letters, and editorials published in the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY from 1978 through 1987 (volumes 130 to 157). Numbering more than 800 pages, the Cumulative Index includes both a subject and an author index. This hard-cover volume is $64.50 ($69.00 international). Prices include shipping. *Payment must accompany all orders and must be in U.S. funds, drawn on a U.S. bank.*

Contact The C.V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318 USA; phone (314) 872-8370, ext. 394. In the U.S., call toll-free: 1-800-325-4177, ext. 394. Please allow six weeks for delivery.

Increased placental progesterone may cause decreased placental prostacyclin production in preeclampsia

Scott W. Walsh, PhD, and Stephanie Coulter, BS
Houston, Texas

Placentas obtained from women with preeclampsia produce more thromboxane and less prostacyclin than normal. They also produce more progesterone than normal. This study tested whether a progesterone concentration equivalent to that in the medium after in vitro incubation of placentas from women with preeclampsia ($\geq 1.5 \times 10^{-5}$ mol/L) could cause an imbalance of increased thromboxane and decreased prostacyclin production by normal placentas. Fresh term placental tissues were incubated for 48 hours in the absence or presence of various concentrations of progesterone and/or estradiol. Prostacyclin and thromboxane were determined by radioimmunoassay of their stable metabolites, 6-keto-prostaglandin $F_{1\alpha}$ and thromboxane B_2. A progesterone concentration of 1.5×10^{-5} mol/L significantly ($p < 0.001$) inhibited prostacyclin production, compared with control, but lower concentrations did not. Estradiol at concentrations present in the medium after incubation of either normal or preeclamptic placental tissue did not significantly affect prostacyclin production, nor did it prevent progesterone at 1.5×10^{-5} mol/L from inhibiting prostacyclin. Thromboxane production was not affected by either progesterone or estradiol. *Conclusion:* In vitro addition of progesterone at a concentration equivalent to that in the medium after incubation of preeclamptic placentas inhibited prostacyclin production by normal placentas to a rate characteristic of preeclampsia. However, it did not increase thromboxane. *Speculation:* Increased trophoblast progesterone in preeclampsia may act by a paracrine mechanism to inhibit prostacyclin synthesis in placental vasculature. Progesterone's selective inhibition of prostacyclin without affecting thromboxane may be due to the compartmentalization of thromboxane production to trophoblast and stroma and prostacyclin production to placental vasculature. (AM J OBSTET GYNECOL 1989;161:1586-92.)

Key words: Progesterone, prostacyclin, thromboxane, preeclampsia, human placenta

Placentas obtained from women with preeclampsia produce more thromboxane and less prostacyclin than normal.[1] Prostacyclin, a potent vasodilator and inhibitor of platelet aggregation, opposes the vasoconstrictive and platelet aggregating effects of thromboxane. In vitro incubation of placental tissues obtained from women with mild preeclampsia results in progesterone concentrations that are 30% to 70% greater than normal.[2] The concentrations of estradiol in the incubation medium are the same as normal. This study was done to test the hypothesis that in vitro addition of progesterone at a concentration equivalent to that in the medium after incubation of placental tissue from women with preeclampsia can cause an imbalance of increased thromboxane and decreased prostacyclin production by normal placentas.

Material and methods

Fourteen term placentas were obtained within 5 minutes of vaginal ($n = 11$) or cesarean section ($n = 3$) delivery from healthy, normotensive women. The mode of delivery does not affect in vitro placental prostacyclin or thromboxane production rates.[1, 3] Institutional approval to use human placental tissues was obtained. Each placenta was placed in a sterile plastic bag, covered with ice, brought immediately to the laboratory, and processed under sterile conditions in a clean bench laminar flow type of hood. The umbilical arteries were gently flushed with Dulbecco's modified Eagle's medium (DMEM) (Gibco, Grand Island, N.Y.) until the chorionic plate arteries and veins were visibly clear of blood. Tissue samples were taken from various sites and always from the interior of the placenta to exclude contamination with chorion, decidua, large blood vessels, or bacteria after a vaginal delivery. Placental tissues were placed in DMEM that was oxygenated with 95% oxygen and 5% carbon dioxide. Tissues were gently cut and washed repeatedly until blanched of blood, then gently cut again into small pieces and blotted dry with sterile gauze with a consistent technique used for each

From the Perinatal Physiology Research Unit, Department of Obstetrics, Gynecology and Reproductive Sciences, and the Department of Physiology and Cell Biology, University of Texas Medical School at Houston.
Supported by grant HD20973 from the National Institute of Child Health and Human Development.
Presented at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989 (Abstract 215).
Reprint requests: Dr. Scott W. Walsh, Medical College of Virginia, Department of Obstetrics and Gynecology, P.O. Box 34, Richmond, VA 23298-0034.
6/6/15958

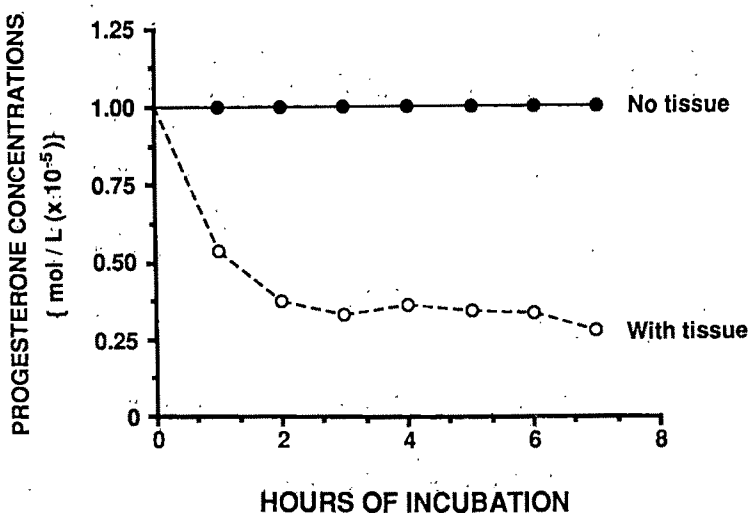


Fig. 1. Concentrations of progesterone in the incubation medium in the presence or absence of approximately 350 mg of human placental tissue. The molar concentration of progesterone added was 1×10^{-5} mol/L.

piece of tissue. Care was taken to assure as little trauma to the tissues as possible. Approximately 350 mg of tissue pieces was placed into 7 ml of DMEM in 2 × 3 six-well culture dishes (35 mm diameter for each well) (Costar, Cambridge, Mass.). Control wells were run in quadruplicate for each placenta and treatment wells were run in duplicate. Tissues were oxygenated with 95% oxygen and 5% carbon dioxide during 48 hours of incubation at 37° C in a Dubnoff metabolic shaker that was located inside a sterile hood.

The shaking incubator creates a shear stress on the tissues that is important for prostacyclin and thromboxane production. The use of 95% oxygen is necessary to assure viability of the inner cells of the tissue pieces. Cell viability of the tissue cultures is >95% as previously published.[3] Reduction of oxygen from 95% to 20% results in a significant decrease in prostacyclin production.[3]

The placenta both synthesizes and metabolizes progesterone and estradiol, but in vitro placental steroid synthesis is low in the absence of added precursor.[2] Therefore metabolism is greater than synthesis. For this reason progesterone and 17β-estradiol concentrations were determined in the incubation medium in the presence and absence of 350 mg of placental tissue. When the original incubation concentrations of progesterone were 1×10^{-4}, 1×10^{-5}, and 1×10^{-6} mol/L, the final incubation concentrations were 1.5×10^{-5} mol/L (85% reduction), 2.8×10^{-6} mol/L (72% reduction) and 4.5×10^{-7} mol/L (55% reduction), respectively. The rate of placental progesterone metabolism is shown in a representative figure for a starting concentration of 1×10^{-5} mol/L (Fig. 1). In the absence of placental tissue the concentrations of progesterone remain stable, but in the presence of placental tissue progesterone concentrations rapidly decline during the first 2 hours of incubation before reaching equilibrium and relatively stable concentrations. Placental metabolism of 17β-estradiol was similar to that of progesterone, but because physiologic concentrations of estradiol did not significantly affect prostacyclin or thromboxane production either alone or in combination with progesterone, its molar concentrations in the incubation medium are presented only as 10^{-x} for the sake of clarity, simplicity, and emphasis on progesterone. Progesterone and 17β-estradiol were initially diluted in 100% ethanol to a concentration of 10 mg/ml. Subsequent dilutions were made from these solutions with DMEM. The ethanol percentages in the experimental solutions were ≤0.586%.

Single 200 μl samples were collected at time 0 and at 8, 20, 32, and 48 hours of incubation from each incubation well. The time 0 sample was collected after all tissues were weighed and placed into their respective incubation wells and just before the shaking incubator was started. Sample volume was not replaced with fresh DMEM to avoid dilution of the incubation media. The production rates of thromboxane A_2 and prostacyclin were estimated by determining the concentrations in the incubation media of their stable metabolites, thromboxane B_2 (TXB_2) and 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$). The validation of each radioimmunoassay was published previously.[1, 3, 4] The concentrations of TXB_2 and 6-keto-$PGF_{1\alpha}$ in each sample were corrected for the volume of the incubation medium remaining at the time of sampling.

Data are expressed as picograms per milligram of wet tissue. The advantages of expressing the data as

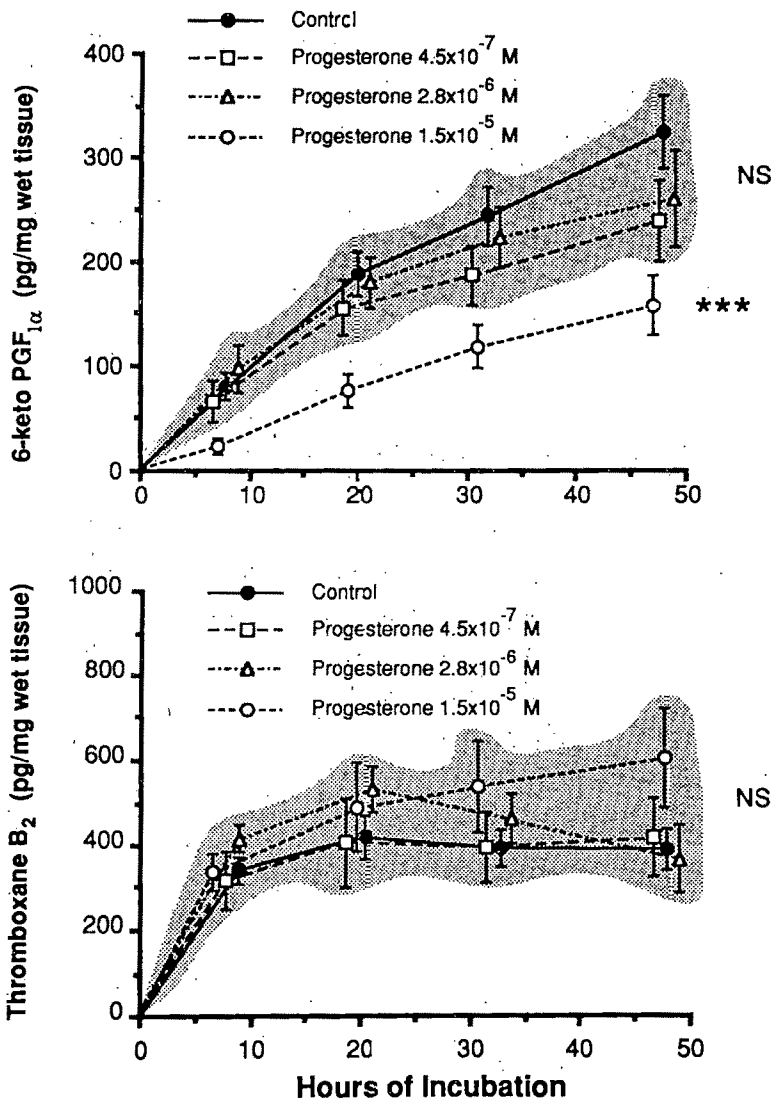


Fig. 2. Placental production of prostacyclin and thromboxane as estimated by the concentrations of their stable metabolites, 6-keto-$PGF_{1\alpha}$ and TXB_2, respectively, in the absence or presence of various concentrations of progesterone. The progesterone concentration of 1.5×10^{-5} mol/L is equivalent to that present in the medium after incubation of preeclamptic placental tissue.[2] Data represent mean ± SE (n = 14 for 6-keto-$PGF_{1\alpha}$, n = 10 for TXB_2). *Triple asterisk*, $p < 0.001$; *NS (shaded area)*, nonsignificant ($p > 0.05$).

per milligram of wet tissue, rather than as per milligram of dry tissue, protein, or deoxyribonucleic acid, are that it is simple and time efficient and it allows calculation of the total production rate for each placenta. We have validated the expression of the data as per milligram of wet tissue weight by comparing it with other methods. Comparison of picograms per milligram of wet tissue with picograms per microgram of protein results in significant and positive correlation coefficients for whole placental tissue of $r = 0.86$ for TXB_2 and $r = 0.95$ for 6-keto-$PGF_{1\alpha}$.[4] Correlation coefficients in five experiments for milligrams of wet tissue versus milligrams of dry tissue ranged from $r = 0.81$ to $r = 0.91$; for expression of the data as picograms per milligram of wet tissue versus picograms per milligram of dry tissue the correlation coefficients were $r = 0.94$ to $r = 0.98$ for TXB_2, and $r = 0.97$ to $r = 0.98$ for 6-keto-$PGF_{1\alpha}$.

Statistical differences between the concentration regression lines from 0 to 48 hours for 6-keto-$PGF_{1\alpha}$ or TXB_2 were assessed by two-way analysis of variance with orthogonal comparisons and the multivariate general linear hypothesis method. This is a revised version of a multivariate least-squares regression analysis allowing any linear contrasts on vectors of dependent and independent variables. A statistical computer software package was used.[5] Regression lines were considered significantly different at $p < 0.05$.

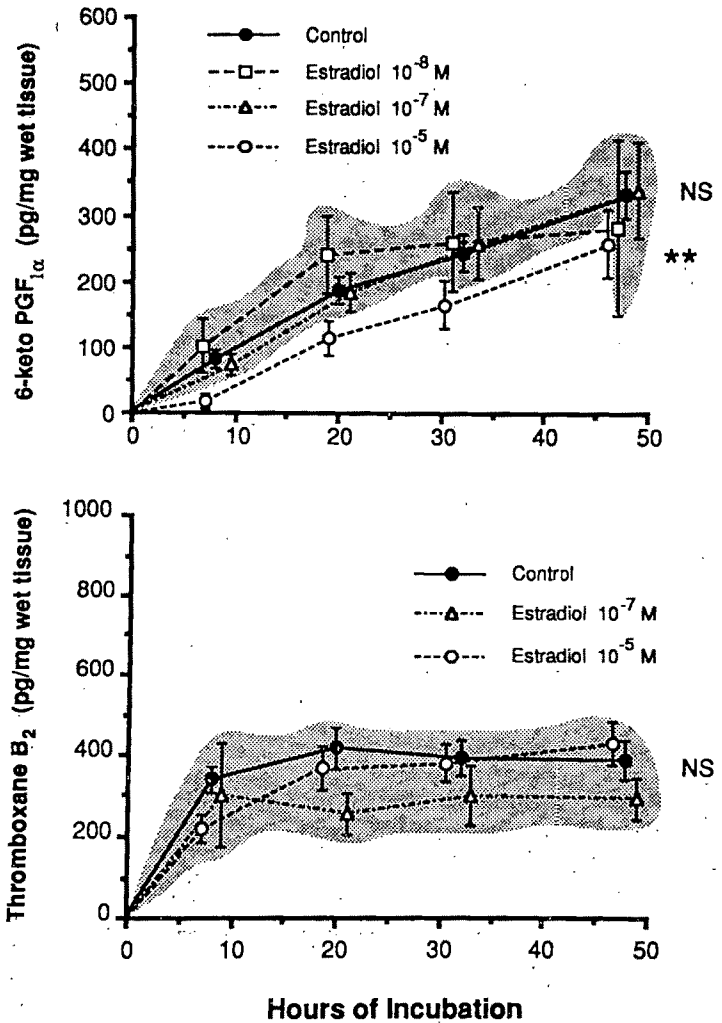


Fig. 3. Same as legend to Fig. 2 except placental tissues were incubated in the absence or presence of various concentrations of 17β-estradiol. The estradiol concentration of 10^{-5} mol/L is a supraphysiologic dose. *Double asterisk*, $p < 0.01$; *NS (shaded area)*, nonsignificant ($p > 0.70$).

Results

A progesterone concentration of 1.5×10^{-5} mol/L significantly inhibited placental prostacyclin production as indicated by significantly lower ($p < 0.001$) concentrations of 6-keto-$PGF_{1\alpha}$ in the incubation medium as compared with control (Fig. 2). Addition of lower concentrations of progesterone did not significantly affect 6-keto-$PGF_{1\alpha}$ concentrations compared with control ($p > 0.05$). An estradiol concentration of 10^{-5} mol/L significantly lowered ($p < 0.01$) 6-keto-$PGF_{1\alpha}$ concentrations, but addition of lower amounts of 10^{-7} and 10^{-8} mol/L did not significantly alter ($p > 0.70$) prostacyclin production (Fig. 3).

When progesterone was added to the incubation medium together with various amounts of estradiol, the progesterone concentration of 1.5×10^{-5} mol/L still significantly inhibited placental prostacyclin production because the concentrations of 6-keto-$PGF_{1\alpha}$ in the incubation medium were significantly lower ($p < 0.001$) than control (Fig. 4). Similar to the results obtained when progesterone was added alone, progesterone at lower concentrations in conjunction with estradiol did not significantly alter ($p > 0.10$) prostacyclin production.

Neither progesterone nor estradiol, either alone or in combination, significantly affected placental thromboxane production as indicated by the concentrations of TXB_2 (Figs. 2 to 4). Statistical probability levels were: 1.5×10^{-5} mol/L progesterone, $p > 0.07$; 2.8×10^{-6} mol/L progesterone, $p > 0.20$; 4.5×10^{-7} mol/L progesterone, $p > 0.98$; 10^{-5} and 10^{-7} mol/L estradiol, $p > 0.4$; progesterone plus estradiol, $p > 0.5$ for all doses. The TXB_2 data represent $n = 10$ rather than $n = 14$ because the TXB_2 radioimmunoassays for some of the experiments were not valid because of heat-damaged TXB_2 standards during shipment in August.

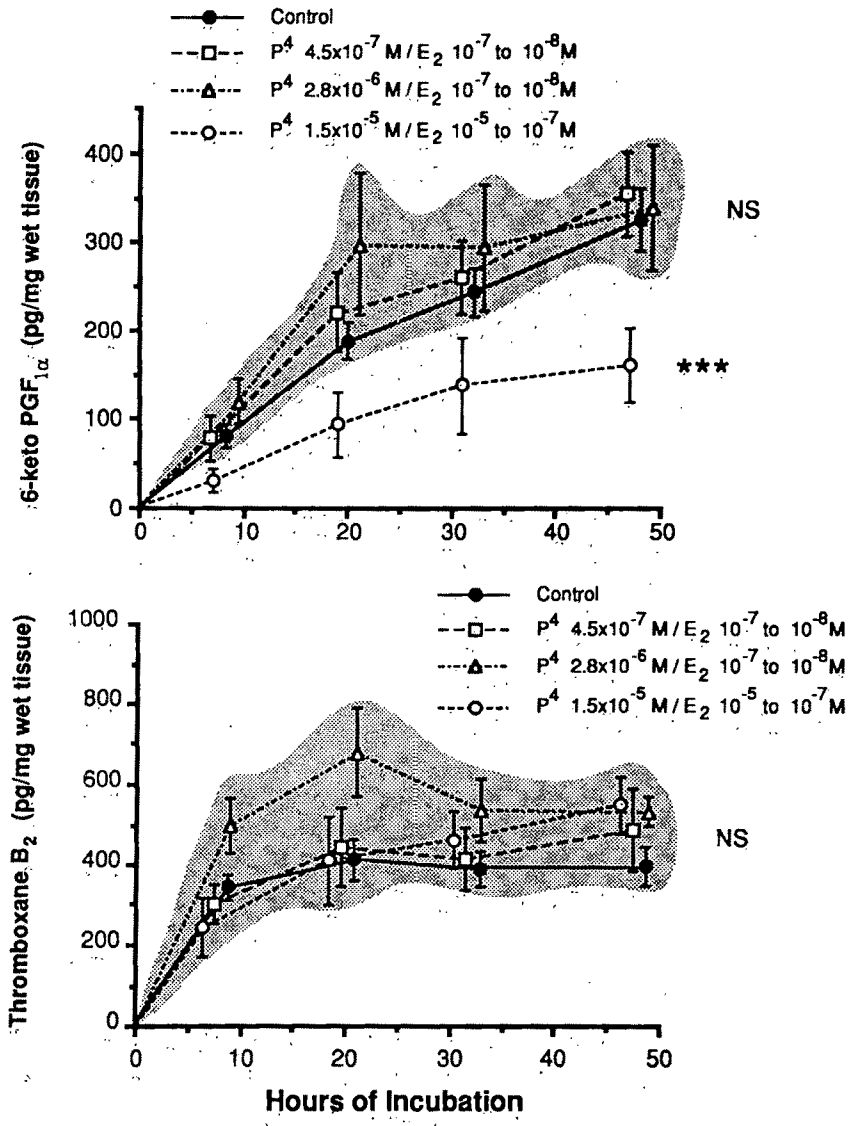


Fig. 4. Same as legend to Fig. 2 except placental tissues were incubated with various combinations of progesterone (P^4) plus estradiol (E_2). *Triple asterisk*, $p < 0.001$; *NS (shaded area)*, nonsignificant ($p > 0.10$).

In three experiments the concentrations of 6-keto-$PGF_{1\alpha}$ and TXB_2 were evaluated between 0 and 8 hours of incubation (i.e., at 1, 2, and 4 hours) in addition to the other sampling times. The concentrations of 6-keto-$PGF_{1\alpha}$ were linear and those of TXB_2 were curvilinear during this time interval. The inhibitory effect of progesterone at 1.5×10^{-5} mol/L on 6-keto-$PGF_{1\alpha}$ was present as early as 1 hour of incubation in two of three placentas.

Comment

Addition in vitro of progesterone at a concentration equivalent to that present in the incubation medium of preeclamptic placentas[2] significantly inhibited prostacyclin production by normal placentas to a rate characteristic of preeclampsia (Fig. 5).[1,3] Lower concentrations of progesterone did not significantly affect placental prostacyclin production (Figs. 2 and 4). Estradiol did not significantly alter prostacyclin production except at a concentration considerably higher than that present in the medium after incubation of either normal or preeclamptic placental tissue (Fig. 3).[2] The inhibitory effect of progesterone on placental prostacyclin production confirms the data of Myatt et al.[6] and Jogee et al.[7] These investigators found that progesterone, either alone or in combination with estradiol, suppressed prostacyclin production by dispersed term placental cells in culture. We speculate that increased trophoblastic production of progesterone in preeclampsia acts by a paracrine mechanism to locally inhibit prostacyclin production in the placental vasculature.

The concentration of 1.5×10^{-5} mol/L of proges-

terone used in this study, although higher than that in the maternal systemic circulation, could be physiologic for the placenta if one considers that progesterone exerts its effect in a paracrine, rather than an endocrine, manner. The in vitro production rates for progesterone previously reported,[2] on which the present study was based, are very similar to those obtained by in vivo studies in pregnant women when they are converted from nanograms per milligram of placental tissue per hour to milligrams per placenta per day. The in vitro progesterone production rates with pregnenolone or pregnenolone sulfate added as precursor ranged from 184 to 268 mg/day. This compares favorably with in vivo production rates reported for pregnant women of 100 to 250 mg/day.

The inhibitory effect of progesterone on placental eicosanoid production was selective because it inhibited prostacyclin but not thromboxane production. Since both prostacyclin and thromboxane are cyclooxygenase metabolites of arachidonic acid, this raises the question as to the mechanism whereby progesterone selectively inhibits prostacyclin without inhibiting thromboxane production in the human placenta. One possibility is that progesterone can selectively inhibit prostacyclin synthesis. The human placenta contains a protein that inhibits prostaglandin synthesis,[8] and progesterone, but not estradiol, inhibits the release of arachidonic acid from human endometrial and decidual cells within 15 minutes, presumably by inhibiting phospholipase activity.[9] We are not aware, though, of any evidence that progesterone can specifically inhibit prostacyclin synthase.

An alternate possibility for the selective inhibitory effect of progesterone on prostacyclin production is that prostacyclin and thromboxane are compartmentalized in the human placenta.[4, 10] Thromboxane is primarily produced in the trophoblastic and stromal tissues of term placentas, whereas prostacyclin is primarily produced in the placental vasculature. Therefore a testable hypothesis is that progesterone receptors are present only in the placental vasculature, resulting in an inhibitory effect on prostacyclin without affecting thromboxane production by the trophoblast and stromal tissue. This hypothesis is consistent with the low number of progesterone-specific receptors present in the human placenta.[11]

All of the doses of progesterone tested theoretically should have saturated the progesterone-specific receptors in the placenta. Despite this, progesterone significantly inhibited prostacyclin production only at the 1.5×10^{-5} mol/L dose. Myatt et al.[6] and Jogee et al.[7] also found significant inhibition of prostacyclin by a progesterone dose of 10^{-5} mol/L in dispersed term placental cells in culture. These observations, together with the finding of Wilson et al.[9] that progesterone

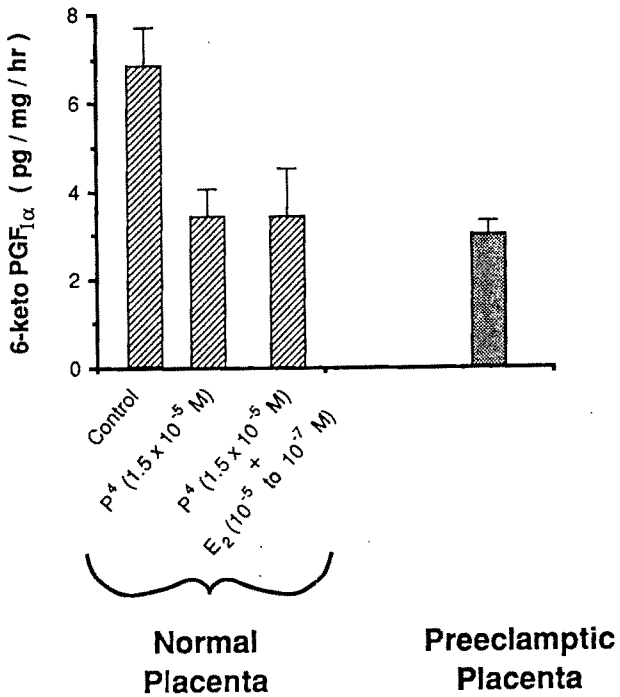


Fig. 5. Prostacyclin production rates (expressed as picograms of 6-keto-$PGF_{1\alpha}$ per milligram of wet tissue per hour) in placental tissues obtained from normal and preeclamptic pregnancies. At an in vitro concentration of progesterone equivalent to that in the medium after incubation of preeclamptic placentas,[2] prostcyclin production by normal placentas was inhibited to a rate characteristic of preeclampsia.[1, 3] Data represent mean ± SE.

rapidly inhibits phospholipase activity, suggest that progesterone can exert effects by a mechanism that may be separate from translocation of the progesterone-specific cytosol receptor to the nucleus with subsequent stimulation of transcription and translational processes.

This study was conducted to test the hypothesis that a progesterone concentration equivalent to that in the medium after in vitro incubation of preeclamptic placentas can cause an imbalance of increased thromboxane and decreased prostacyclin production by normal placentas. The data presented support one half of the hypothesis, that higher than normal placental progesterone concentrations can inhibit placental prostacyclin production, leading to an imbalance between thromboxane and prostacyclin. However, abnormally increased placental thromboxane production in preeclampsia appears to be independent of progesterone.

We acknowledge the expert technical assistance of Mr. Peter Brath.

REFERENCES

1. Walsh SW. Preeclampsia: an imbalance in placental prostacyclin and thromboxane production. AM J OBSTET GYNECOL 1985;152:335-40.
2. Walsh SW. Progesterone and estradiol production by nor-

mal and preeclamptic placentas. Obstet Gynecol 1988; 71:222-6.
3. Walsh SW, Behr MJ, Allen NH. Placental prostacyclin production in normal and toxemic pregnancies. AM J OBSTET GYNECOL 1985;151:110-5.
4. Nelson DM, Walsh SW. Thromboxane and prostacyclin production by different compartments of the human placental villus. J Clin Endocrinol Metab 1989;68:676-83.
5. Wilkinson L. SYSTAT: The system for statistics. Evanston, Illinois: SYSTAT, 1987.
6. Myatt L, Jogee M, Elder MG. Regulation of prostacyclin metabolism in human placental cells in culture by steroid hormones. In: Lewis PJ, Moncada S, O'Grady J, eds. Prostacyclin in pregnancy. New York: Raven Press, 1983:119-29.
7. Jogee M, Myatt L, Elder MG. Decreased prostacyclin production by placental cells in culture from pregnancies complicated by fetal growth retardation. Br J Obstet Gynaecol 1983;90;247-50.
8. Dembele-Duchesne MJ, Thaler-Dao H, Chavis C, Crastes de Paulet A. Some new prospects in the mechanism of control of arachidonate metabolism in human placenta and amnion. Prostaglandins 1981;22:979-1002.
9. Wilson T, Liggins GC, Aimer GP, Watkins EJ. The effect of progesterone on the release of arachidonic acid from human endometrial cells stimulated by histamine. Prostaglandins 1986;31:343-60.
10. Thorp JA, Walsh SW, Brath PC. Low-dose aspirin inhibits thromboxane, but not prostacyclin, production by human placental arteries. AM J OBSTET GYNECOL 1988;159: 1381-4.
11. Younes MA, Besch NF, Besch PK. Estradiol and progesterone binding in human term placental cytosol. AM J OBSTET GYNECOL 1981;141:170-4.

Aspirin differentially affects thromboxane and prostacyclin production by trophoblast and villous core compartments of human placental villi

D. Michael Nelson, MD, PhD, and Scott W. Walsh, PhD

St. Louis, Missouri, and Houston, Texas

Low-dose aspirin has been used as prophylactic treatment for preeclampsia and fetal growth retardation, but the physiologic mechanisms for the beneficial effect of this therapy are unknown. We studied the effects of aspirin on eicosanoid production by different compartments of normal term human placental villi. Duplicate parallel incubations ($n = 7$) of whole villi, villous core tissues, and trophoblast were established on Millicell platform dishes in minimum essential medium in the presence or absence of 1×10^{-4} or 1×10^{-5} mol/L aspirin. Production rates of thromboxane A_2 and prostacyclin were estimated by radioimmunoassay of their stable hydrolysis products, thromboxane B_2 and 6-keto-prostaglandin $F_{1\alpha}$, respectively. Our results indicate that 1×10^{-4} mol/L aspirin inhibits thromboxane production in whole villi and villous core tissues denuded of their trophoblast layer but not in isolated trophoblast cells. The same concentration of aspirin also inhibits prostacyclin production in the isolated villous core but not in whole villi and not in isolated trophoblast. We conclude that aspirin can selectively inhibit thromboxane production in whole placental villi and differentially affects thromboxane and prostacyclin production by the trophoblast and villous core compartments. (Am J Obstet Gynecol 1989;161:1593-8.)

Key words: Human placenta, thromboxane, prostacyclin, aspirin

The opposing physiologic effects of prostacyclin and thromboxane have led to speculation with regard to the role of these eicosanoids in normal and abnormal pregnancy.[1-3] Prostacyclin is a potent vasodilator and inhibitor of platelet aggregation, whereas thromboxane causes vasoconstriction and stimulates platelet aggregation. Local production in tissues is the key to the effects of these eicosanoids, since it is controversial whether thromboxane or prostacyclin are circulating hormones.[2] Many of the sequelae of preeclampsia can be explained by the action of unopposed thromboxane.[3] Low-dose aspirin has been used as a prophylactic treatment for preeclampsia,[4] other forms of hypertension in pregnancy,[5] and fetal growth retardation[6, 7] in women at increased risk to develop these conditions. The pathophysiologic mechanisms for the beneficial effect of this therapy are not known, but one proposed mechanism involves a preferential inhibition of thromboxane production by low-dose aspirin without a limitation of prostacyclin production.[8]

Human placental villi produce both thromboxane[3, 9] and prostacyclin.[3, 10] We have described a method to separate trophoblast from villous core stroma to compare in vitro eicosanoid production by these two villous compartments with eicosanoid production by intact villi.[11] In this article we report the effects of aspirin on eicosanoid production by the different compartments of human placental villi.

Material and methods

Human term placentas from seven uncomplicated pregnancies (38 to 41 weeks) were obtained immediately after delivery. All women were normotensive throughout pregnancy, were not taking medications, and denied smoking, alcohol use, or aspirin ingestion at the end of pregnancy. The method for isolation of trophoblast and villous core has been published in detail.[11] Briefly, villous tissue was rinsed repeatedly with a modified minimum essential medium to remove blood from the intervillous space and the tissue was treated 10 minutes at 37° C with the proteolytic enzyme dispase (Collaborative Research, Inc., Lexington, Mass.). After enzyme inactivation with ethylenediaminetetraacetic acid, trophoblast components released into the supernatant were harvested and dispase-treated villi were rinsed with three changes of minimum

From the Department of Obstetrics and Gynecology, Washington University School of Medicine, and the Department of Obstetrics, Gynecology, and Reproductive Sciences and the Department of Physiology and Cell Biology, University of Texas Medical School.

Supported in part by grants from the Jewish Hospital of St. Louis Faye Beth and Charles S. Baer Endowment Fund (to D. M. N.) and National Institute of Child Health and Human Development grants HD-22913 (to D. M. N.) and HD-20973 (to S. W. W.).

Presented at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.

Reprint requests: D. Michael Nelson, MD, PhD, Jewish Hospital Department of Obstetrics and Gynecology, 216 S. Kingshighway, St. Louis, MO 63110.

6/6/15654

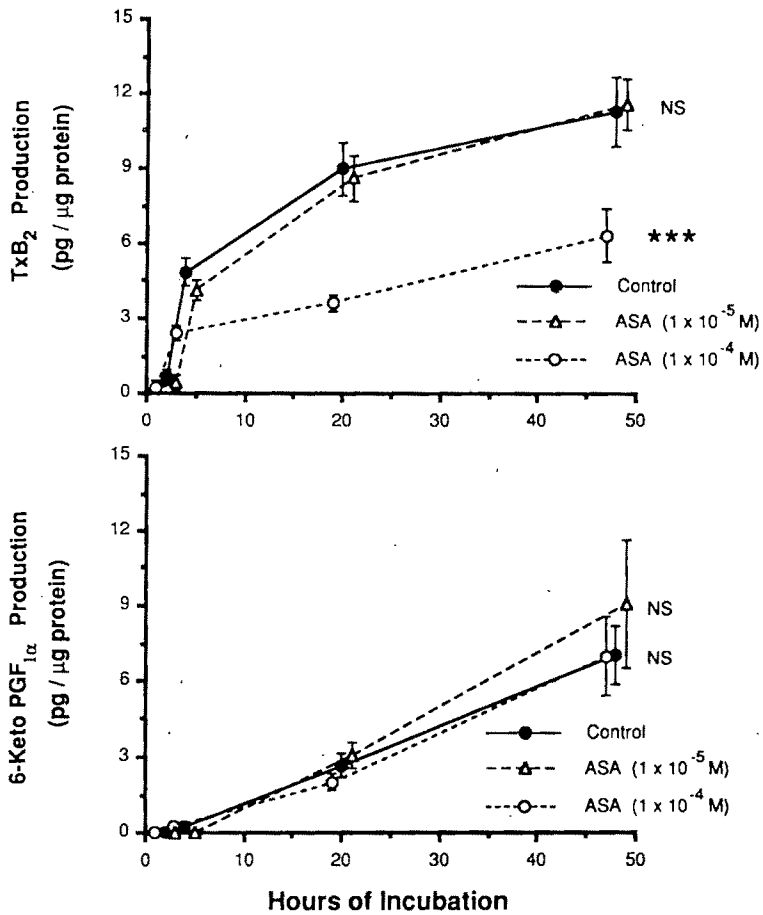


Fig. 1. Mean (±SE) concentrations of thromboxane B_2 *(TxB₂) (upper panel)* and 6-keto-$PGF_{1\alpha}$ *(lower panel)* in culture medium containing whole villi incubated in the absence *(control)* or presence of 1×10^{-4} or 1×10^{-5} mol/L aspirin *(ASA)*. Results are from four placentas with duplicates for each of the parallel incubations. (*NS*, Not significant; *triple asterisks*, $p < 0.001$.)

essential medium. Histologically the dispase-treated tissues consisted of villous core components denuded of the trophoblast layer; the trophoblast pellet contained both syncytial and cellular trophoblast.

Duplicate cultures of intact whole villi, villous core tissues, and trophoblast were established in the presence or absence of 1×10^{-3}, 1×10^{-4}, or 1×10^{-5} mol/L aspirin (Sigma Chemical, Co. St. Louis). All incubations were on Millicell platform dishes (Millipore Products Division, Bedford, Mass.) in serum-free minimum essential medium at 37° C in a 5% carbon dioxide and air atmosphere. Confirmation of viability under these conditions by tritiated leucine incorporation and trypan blue exclusion has been previously described.[11]

Aliquots of media obtained at 2, 4, 20, and 48 hours were analyzed for thromboxane A_2 and prostacyclin by specific radioimmunoassay of their stable hydrolysis products thromboxane B_2 and 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), as previously described.[11] Antibodies to thromboxane B_2 and 6-keto-$PGF_{1\alpha}$ were obtained from Advanced Magnetics, Inc. (Cambridge, Mass). Protein content of cells and tissues was determined by the method of Bradford[12] with bovine serum albumin as a standard.

The results are expressed as picograms of eicosanoid per microgram of protein with the mean ± SE calculated for each preparation at each time point. Statistical differences among regression lines were assessed by two-way analysis of variance with orthogonal comparisons and the multivariate general linear hypothesis method. A statistical computer software package was used.[13]

Results

Preliminary experiments (data not shown). Parallel incubations of whole villi, villous core tissues, and trophoblast were prepared from three placentas, and eicosanoid production was determined in the presence or absence of 1×10^{-3} to 1×10^{-5} mol/L aspirin. These studies showed that aspirin at 1×10^{-3} mol/L nonselectively inhibited ≥ 85% of the thromboxane B_2 and ≥70% of the 6-keto-$PGF_{1\alpha}$ produced by the tissues

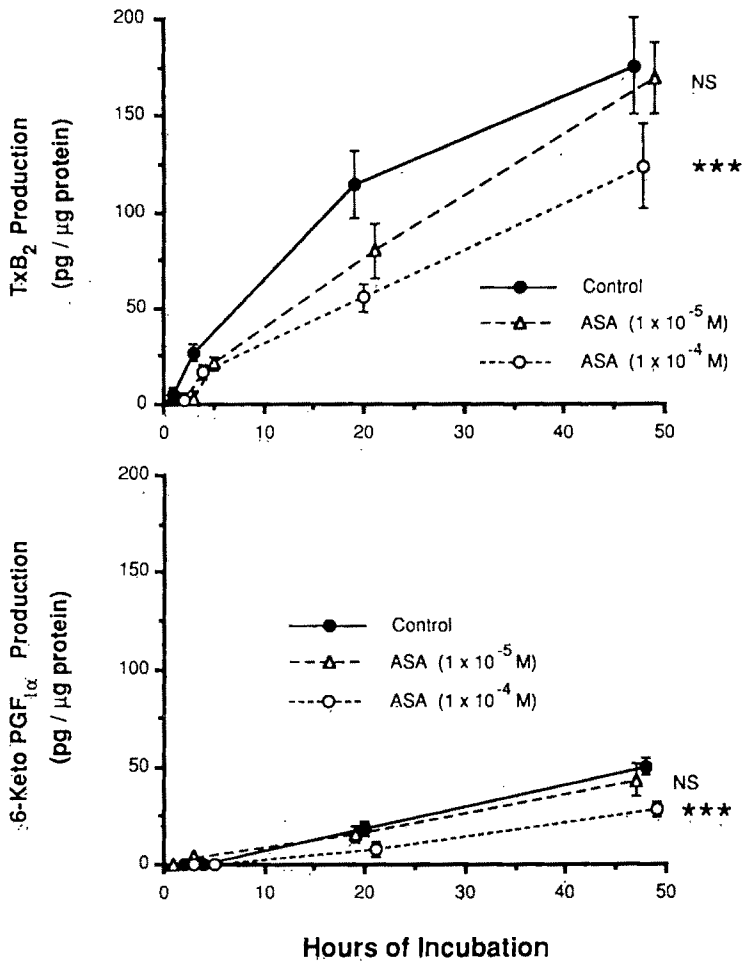


Fig. 2. Mean (±SE) concentrations of thromboxane B_2 *(TxB₂) (upper panel)* and 6-keto-$PGF_{1\alpha}$ *(lower panel)* in culture medium containing dispase-treated villous core tissues incubated in the absence *(control)* or presence of 1×10^{-4} or 1×10^{-5} mol/L aspirin *(ASA)*. Note the different scale for the ordinate compared with Fig. 1. Results are from four placentas with duplicates for each of the parallel incubations. (*NS*, Not significant; *triple asterisks*, $p < 0.001$.)

or cells during the 48-hour incubation. Aspirin at 1×10^{-5} mol/L did not appear to affect eicosanoid production in any of the incubations. We selected 1×10^{-5} and 1×10^{-4} mol/L aspirin for detailed study on the basis of our preliminary results and the results of a study that indicated these molar concentrations of salicylate were present in cord blood of women given a low dose of aspirin during labor.[14]

Eicosanoid production by whole villi. Thromboxane B_2 production by villi incubated with 1×10^{-5} mol/L aspirin was the same as control villi incubated without aspirin ($p > 0.5$; Fig. 1). Aspirin at 1×10^{-4} mol/L significantly inhibited thromboxane B_2 production ($p < 0.001$) by villi compared with villi incubated without aspirin (Fig. 1). Thromboxane B_2 inhibition by 1×10^{-4} mol/L aspirin was significant at 2 hours in culture; thromboxane B_2 levels in the media of the aspirin-treated tissues were only 31% of thromboxane B_2 levels in control incubations. This inhibition of thromboxane B_2 production by villi exposed to 1×10^{-4} mol/L aspirin continued over time: thromboxane B_2 levels were 50% of control villi at 4 hours, 41% of control at 20 hours, and 56% of control at 48 hours.

In contrast to the thromboxane B_2 results, neither 1×10^{-5} mol/L nor 1×10^{-4} mol/L aspirin significantly affected 6-keto-$PGF_{1\alpha}$ production by whole villi compared with villi incubated without aspirin ($p > 0.5$; Fig. 1).

Eicosanoid production by villous core tissues. Explants of villous core tissues produced increased concentrations of eicosanoids compared with whole villi, with a remarkable sixteenfold increase in the thromboxane B_2 concentration by 48 hours (Fig. 2). There was no difference in production of either eicosanoid by villous core tissues incubated with 1×10^{-5} mol/L aspirin compared with control tissues incubated without aspirin ($p > 0.2$; Fig. 2). However, 1×10^{-4} mol/L aspirin significantly inhibited both thromboxane

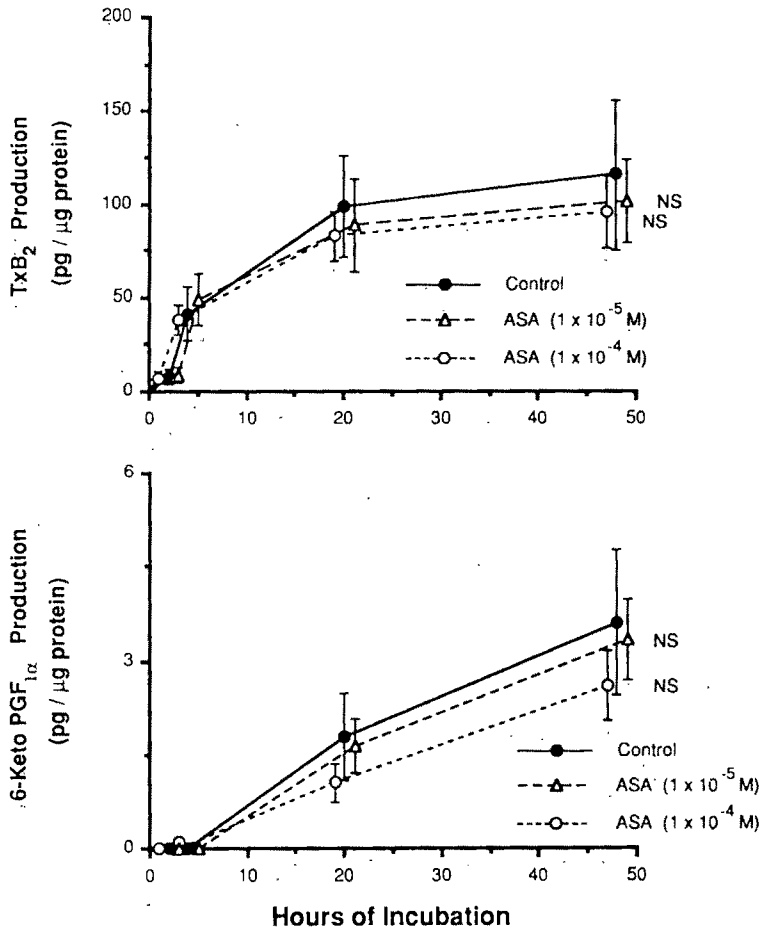


Fig. 3. Mean (±SE) concentrations of thromboxane B_2 *(TxB2) (upper panel)* and 6-keto-$PGF_{1\alpha}$ *(lower panel)* in culture medium containing trophoblast incubated in the absence *(control)* or presence of 1×10^{-4} or 1×10^{-5} mol/L aspirin *(ASA)*. Note the different scale for the ordinate compared with Fig. 1. Results are from four placentas with duplicates for each of the parallel incubations.

B_2 and 6-keto-$PGF_{1\alpha}$ production by villous core tissues compared with control tissues incubated without aspirin ($p < 0.001$; Fig. 2). Maximal inhibition of thromboxane B_2 production was present after 2 hours of incubation, with thromboxane B_2 levels 41% of control at this time. Inhibition continued throughout the study period, with the medium levels of thromboxane B_2 in the 1×10^{-4} mol/L aspirin-treated tissues 61% of control levels at 4 hours, 49% of control at 20 hours, and 71% of control at 48 hours. The 6-keto-$PGF_{1\alpha}$ production by control and aspirin-treated villous core tissues was not measurable in any of the incubations at 2 or 4 hours, but mean 6-keto-$PGF_{1\alpha}$ levels in media of villous core tissues exposed to 1×10^{-4} mol/L aspirin were 40% of control levels at 20 hours and 56% of control levels at 48 hours.

Eicosanoid production by trophoblast. Thromboxane B_2 was the predominant eicosanoid produced by trophoblast (Fig. 3). Neither concentration of aspirin significantly inhibited the thromboxane B_2 or 6-keto-$PGF_{1\alpha}$ production by isolated trophoblast cells ($p > 0.4$; Fig. 3).

Comment

The results indicate that 1×10^{-4} mol/L aspirin inhibits thromboxane B_2 production in whole villi and in villous core tissue denuded of its trophoblast layer but not in isolated trophoblast cells. The same concentration of aspirin also inhibits prostacyclin production in the isolated villous core but not in whole villi or isolated trophoblast. We conclude that aspirin can selectively inhibit thromboxane B_2 production in whole placental villi and differentially affects thromboxane B_2 and prostacyclin production by the trophoblast and villous core compartments. These results, in combination with our previous findings,[11] suggest that the trophoblast modulates villous core thromboxane B_2 production by a paracrine interaction. We speculate that aspirin mimics this trophoblast-inhibiting effect on thromboxane B_2 production by the placenta.

Whether the derangements in prostaglandin synthesis reported in preeclampsia represent primary or secondary phenomena is controversial.[1, 2] Recent studies have used low doses of aspirin to avoid the development of preeclampsia and fetal growth retardation in women at high risk for these complications of pregnancy.[4, 6-8] The beneficial effects of this treatment are attributed to a selective or preferential inhibition of thromboxane production without a significant alteration of prostacyclin production in different tissue compartments. Wallenburg et al.[4] administered placebo or 60 mg of aspirin daily to a group of women at high risk to develop preeclampsia, judged by their increased vascular sensitivity to infused angiotensin II. They found that aspirin alone was an effective prophylaxis for this complication of pregnancy. Beaufils et al.[5] administered daily 150 mg of aspirin and 300 mg of dipyridamole to a group of women at high risk to develop hypertension in pregnancy (not necessarily preeclampsia) and showed an improved outcome for the pregnancies in the treated group compared with untreated women. Treatment with 1 to 1.6 mg/kg/day aspirin in combination with 225 mg of dipyridamole reduced the risk of recurrence for intrauterine fetal growth retardation in a group of normotensive women with a history of intrauterine growth retardation.[6] Trudinger et al.[7] evaluated the fetal benefits of 150 mg aspirin daily in a group of women whose fetuses had elevated umbilical artery waveform systolic/diastolic ratios. Aspirin therapy was associated with an increase in birth weight, head circumference, and placental weight in the treated patients.

Maternal platelets are a source of thromboxane. Fitzgerald et al.[15] attributed the decrease in urinary metabolites of thromboxane after daily treatment of pregnant women with 40 mg of aspirin to a selective inhibition of the cyclooxygenase responsible for platelet thromboxane production. We have shown that normal placental villi also produce thromboxane, and an equal amount of prostacyclin,[3, 11] whereas villi from preeclamptic women produce a sevenfold excess of thromboxane compared with prostacyclin.[3] In addition, we found that trophoblast isolated from normal villi produced predominantly thromboxane, whereas the villous core compartment from normal villi denuded of the trophoblast layer produced a sixfold to ninefold excess of thromboxane compared with prostacyclin.[11] These data suggest that a trophoblast-derived inhibitor functioning in a paracrine interaction between the trophoblast and the villous core limits thromboxane production to maintain a physiologic balance of thromboxane and prostacyclin in the human placenta in normal pregnancy.

We questioned whether part of the beneficial effect of low-dose aspirin in complicated pregnancies might derive from a differential effect on thromboxane and prostacyclin production by different compartments of placental villi. Results of this study demonstrate that 1×10^{-4} mol/L aspirin selectively inhibits thromboxane production, without affecting prostacyclin production, by whole placental villi. This effect is the result of limiting villous core thromboxane production, because aspirin had no effect on eicosanoid production by isolated trophoblast. We speculate that aspirin may substitute for a trophoblast-derived inhibitor that normally limits villous core thromboxane production. We propose that this inhibitor may be absent in villi from preeclamptic women. Administration of low-dose aspirin would prevent the adverse physiologic effects of unopposed thromboxane in the placenta by differentially affecting eicosanoid production by villi.

The tissue levels of aspirin, or its active metabolite salicylate,[16] that result from the various low-dose aspirin regimens reported are not known. Plasma salicylate concentrations rapidly exceed those of aspirin after oral aspirin ingestion, and aspirin may be a "prodrug" for salicylate.[16, 17] Ylikorkala et al.[14] studied salicylate levels in umbilical cord blood after the administration of a single oral dose of 100 mg of aspirin to mothers in labor. The range of serum salicylate concentrations in the cord blood at delivery was between 1×10^{-5} mol/L to 0.9×10^{-4} mol/L. Tissue levels of salicylate may be as much as tenfold higher than serum levels.[17] We identified a differential effect on eicosanoid production by compartments of placental villi with 1×10^{-4} mol/L aspirin. This concentration should be within the ranges of aspirin and salicylate levels achieved in serum and tissues by clinical protocols that use aspirin as therapy for pregnancy complications.[4-8] Further studies will determine whether the effects of aspirin on eicosanoid production in placental compartments provide insight into the pathophysiology of preeclampsia.

We thank Peter Brath, Edward Curran, and Amy Marlo for their skillful technical assistance, and Dr. Ronald C. Strickler for his review of the manuscript.

REFERENCES

1. Friedman SA. Preeclampsia: a review of the role of prostaglandins. Obstet Gynecol 1988;71:122-37.
2. Walsh SW, Parisi VM. The role of arachidonic acid metabolites in preeclampsia. Semin Perinatol 1986;10:334-55.
3. Walsh SW. Preeclampsia: an imbalance in placental prostacyclin and thromboxane production. Am J Obstet Gynecol 1985;152:335-40.
4. Wallenburg HCS, Dekker GA, Makovitz JW, Rotmans P. Low-dose aspirin prevents pregnancy-induced hypertension and preeclampsia in angiotensin-sensitive primigravidae. Lancet 1986;1:1-3.
5. Beaufils M, Donsimoni R, Uzan S, Colau JC. Prevention of preeclampsia by early antiplatelet therapy. Lancet 1985;1:840-2.

6. Wallenburg HCS, Rotmans N. Prevention of recurrent idiopathic fetal growth retardation by low-dose aspirin and dipyridamole. Am J Obstet Gynecol 1987;157: 1230-5.
7. Trudinger BJ, Cook CM, Thompson RS, Giles WB, Connelly A. Low-dose aspirin therapy improves fetal weight in umbilical placental insufficiency. Am J Obstet Gynecol 1988;159:681-5.
8. Walsh SW. Low-dose aspirin: treatment for the imbalance of increased thromboxane and decreased prostacyclin in preeclampsia. Am J Perinatol 1989;6:124-32.
9. Mitchell MD, Bibby JG, Hicks BR, Redman CWG, Anderson ABM, Turnbull AC. Thromboxane B_2 and human parturition: concentrations in the plasma and production in vitro. J Endocrinol 1978;78:435-41.
10. Myatt L, Elder MG. Inhibition of platelet aggregation by a placental substance with prostacyclin-like activity. Nature 1977;268:159-60.
11. Nelson DM, Walsh SW. Thromboxane and prostacyclin production by different compartments of the human placental villus. J Clin Endocrinol Metab 1989;68:676-83.
12. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976; 72:248-54.
13. Wilkinson L. SYSTAT: The system for statistics. Evanston, Illinois: SYSTAT, Inc., 1987.
14. Ylikorkala O, Mäkilä UM, Kääpä P, Viinikka L. Maternal ingestion of acetylsalicylic acid inhibits fetal and neonatal prostacyclin and thromboxane in humans. Am J Obstet Gynecol 1986;155:345-9.
15. Fitzgerald DJ, Mayo G, Catella F, Entman SS, FitzGerald GA. Increased thromboxane biosynthesis in normal pregnancy is mainly derived from platelets. Am J Obstet Gynecol 1987;157:325-30.
16. Vane J, Botting R. Inflammation and the mechanism of action of anti-inflammatory drugs. FASEB J 1987;1:89-96.
17. Higgs GA, Salmon JA, Henderson B, Vane JR. Pharmacokinetics of aspirin and salicylate in relation to inhibition of arachidonate cyclooxygenase and antiinflammatory activity. Proc Natl Acad Sci USA 1987;84:1417-20.

In vitro release of endothelium-derived relaxing factor by acetylcholine is increased during the guinea pig pregnancy

Carl Weiner, MD, Ernest Martinez, BA, Liu Kang Zhu, MD, Abdi Ghodsi, BS, and David Chestnut, MD[a]

Iowa City, Iowa

The relaxation of isolated blood vessels by acetylcholine is dependent on the presence of intact endothelium and its release of a smooth muscle relaxing nitroso-like compound. Pregnancy is associated with altered vascular responsiveness to a variety of agents. Because many of these agents stimulate the release of endothelium-derived relaxing factor, we investigated in vitro the effect of pregnancy on acetylcholine-mediated relaxation of guinea pig uterine and carotid artery rings. The presence of intact and functional endothelium was confirmed both by examination under the scanning electron microscope and by vessel relaxation after the addition of acetylcholine. The addition of acetylcholine to the vessel bath produced dose-dependent relaxation of both carotid and uterine artery segments obtained from pregnant and nonpregnant animals after they had been submaximally preconstricted with phenylephrine. There was a significant increase in both response and efficacy to acetylcholine during pregnancy for both uterine and carotid arteries ($p < 0.0001$ for each). The concentrations of endothelial cells (cells per square micrometer) were similar in uterine arteries from pregnant and nonpregnant animals. We conclude that the most likely explanation for these findings is a pregnancy-mediated enhancement of endothelium-derived relaxing factor activity. (Am J Obstet Gynecol 1989;161:1599-605.)

Key words: Endothelium-derived relaxing factor, pregnancy, guinea pig, uterine artery, carotid artery

The response of vascular smooth muscle to a variety of vasoactive agents is altered during pregnancy.[1-3] Some of these alterations are mimicked in the castrated animal by estrogen replacement.[4-6] The relaxation of both large and resistance-size arteries caused by acetylcholine applied either in vitro or intravascularly in vivo depends on the functional integrity of the endothelium, which releases one or more vasodilating nitroso substances.[7-10] In the absence of a functional endothelium, acetylcholine causes vasoconstriction. Endothelium-derived relaxing factor release is stimulated by a variety of agents.[10] The effect of pregnancy acetylcholine-mediated relaxation has not been previously studied directly. However, the infusion of acetylcholine into the guinea pig uterus reduces resistance only during pregnancy or after estrogen treatment.[11-13] This loss of resistance was reported to be independent of vasodilator cholinergic nerves but reduced by protein synthesis inhibition.[13] If endothelium-derived relaxing factor activity were increased during pregnancy, it could account for at least part of the decline in vascular resistance and responsiveness to catecholamines during pregnancy. The present experiments were designed to determine whether the response of either systemic or reproductive organ arteries to acetylcholine is altered by pregnancy.

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, the Department of Anesthesia,[a] and the Perinatal Research Laboratories, University of Iowa School of Medicine.

Supported in part by United States Public Health Service Grant No. HD24494.

Presented in part at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.

Reprint requests: Carl Weiner, MD, Department of Obstetrics and Gynecology, The University of Iowa Hospitals and Clinics, Iowa City, IA 52242.

6/6/15671

Material and methods

The protocol was approved by the University of Iowa Animal Care Committee. Adult female, nonpregnant and pregnant (50 to 60 days' gestation; term, 65 to 68 days' gestation) guinea pigs (600 to 1000 gm) were anesthetized (ketamine hydrochloride, 80 mg/kg administered intraperitoneally; xylazine hydrochoride, 3 mg/kg administered intramuscularly). While 100% oxygen was applied by mask, the common carotid and main uterine arteries were removed en bloc with microsurgical instruments and an operating microscope. The carotid artery was selected to represent the systemic vasculature. The uterine artery was selected because it directly supplies the conceptus. The number of pups supplied by each uterine artery was recorded. These were small arteries. The inner diameter as measured under the operating microscope of the unstretched uterine artery from a nonpregnant guinea pig ranged from 90 to 150 μm. The inner diameter of the remaining vessels studied ranged from 150 to 200 μm.

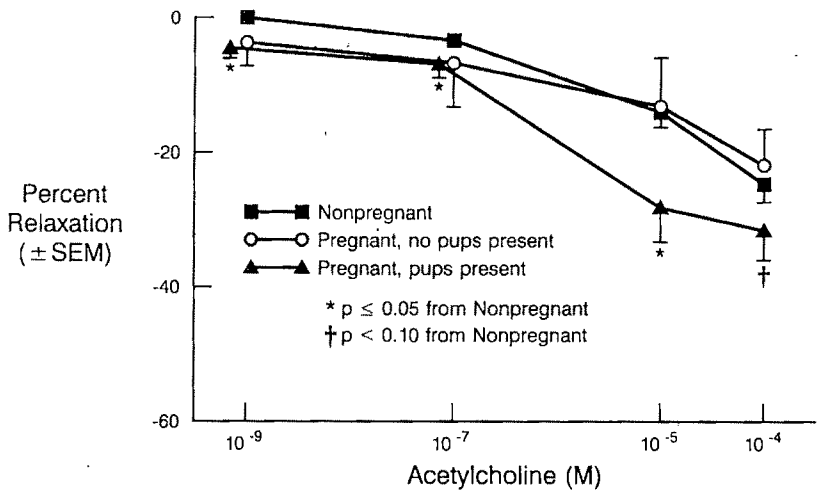


Fig. 1. Acetylcholine-stimulated relaxation of uterine artery preconstricted with phenylephrine was increased during pregnancy. Repetitive measures two-way ANOVA: dose, $p < 0.0001$; pregnancy status, $p < 0.0001$; interaction, $p < 0.0001$. Note that arterial segments from pregnant animals that supply a horn without a pup demonstrated an intermediate pregnancy effect (control, $n = 12$; no pups, $n = 3$; pups, $n = 8$). * and † Compared with nonpregnant animal.

The arteries were immediately placed in iced Krebs-Ringer bicarbonate solution (grams per liter: sodium chloride 6.914, potassium chloride 0.3505, calcium chloride dihydrate 0.328, magnesium sulfate heptahydrate 0.295, monopotassium phosphate 0.164, sodium bicarbonate 2.1, and glucose 2.0) previously aerated with 95% oxygen and 5% carbon dioxide. Under the microscope, the vessels were sharply cleaned of connective tissue, cut into 3 mm rings excluding areas with branches, and suspended on two 50 μm tungsten wire triangles. One triangle was hooked to a stationary rod and the other to a strain gauge transducer (Grass FT 0.03, Quincy, Mass.) for the measurement of isometric force. The rings were suspended in 25 ml dropaway baths filled with Krebs-Ringer solution maintained at 37° C and aerated with 95% oxygen and 5% carbon dioxide.[14, 15] All experiments were performed in the presence of desipramine (10^{-5} mol/L) to block neuronal, and hydrocortisone (10^{-5} mol/L) to block extraneuronal uptake of norepinephrine.[16, 17] Propranolol (10^{-5} mol/L) was added to inhibit β-adrenergic receptors.[18]

After at least 60 minutes of equilibration in the organ chamber, each ring was stretched to the optimal point of its length-tension curve as determined by the tension developed in response to potassium chloride (10^{-1} mol/L) added at each stretch level.[19] The optimal resting tension was defined as that point on the length-tension curve where the maximal constrictive response to potassium chloride was obtained. The arterial ring was then preconstricted with submaximal phenylephrine hydrochloride (10^{-7} mol/L) and endothelium-derived relaxing factor release stimulated by the addition of acetylcholine 10^{-9} to 10^{-4} mol/L) given in random order. The arterial ring was washed twice after each acetylcholine dose and rested for 15 minutes before the phenylephrine was again added to the bath. At the conclusion of the experiment, the rings were sharply opened longitudinally and the dimensions were measured. Then they were blotted dry and weighed. The vascular wall cross-sectional area was calculated so that the isometric force produced in response to phenylephrine could be normalized for muscle mass.[20] The response to acetylcholine was expressed in terms of the percent relaxation obtained from the phenylephrine-induced constriction and the efficacy of acetylcholine. Efficacy was defined as the percent relaxation obtained for that dose divided by the maximum relaxation obtained during the performance of the dose-response curve for the ring (i.e., the percent of the maximum).

To determine the effect of pregnancy on the number of endothelial cells and the effect of the experiment on the structural integrity of the endothelium, rings prepared identically and simultaneously with those used in experimentation and rings actually used in experimentation were placed in glutaraldehyde, mounted, and viewed under the scanning electron microscope. The area denuded of endothelium was estimated and the number of endothelial cells per micron^2 were counted. The pregnancy status of the animal from which the vessels were obtained was unknown to the investigator at the time these evaluations were made.

Drugs. The following drugs were used: acetylcholine hydrochloride, phenylephrine hydrochloride, desipramine, hydrocortisone, and propranolol (Sigma Chemical Co., St. Louis, Mo.); ketamine hydrochloride (Bristol Myers Co., Syracuse, N.Y.); and xylazine (Miles Laboratories, Shawnee, Kan.). All drugs and buffers were

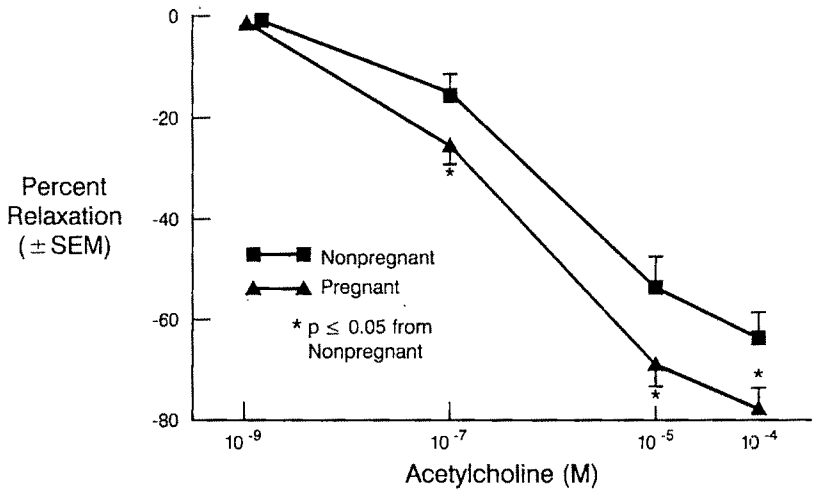


Fig. 2. Acetylcholine-stimulated relaxation of carotid artery preconstricted with 10^{-7} mol/L phenylephrine was increased during pregnancy (n = 13). Repetitive measures two-way ANOVA: dose, $p < 0.0001$; pregnancy status, $p < 0.0001$; interaction, $p = 0.0005$. * and † Compared with nonpregnant animal.

prepared fresh on the day of study with distilled water and were kept on ice. Concentrations are expressed as final molar concentration (mol/L) in the organ bath.

Statistics. Data are expressed as mean ± SEM; n indicates the number of animals. Uterine rings from pregnant animals were subcategorized by the presence or absence of at least one pup in the uterine horn they supplied. Quantitative differences in responses to acetylcholine between animals was a large source of variability. Therefore the effect of pregnancy and dose on the relaxation and efficacy of acetylcholine was determined by a two-way random block repeated measures analysis of variance (ANOVA) with either percent relaxation from the phenylephrine-induced constriction or efficacy as the dependent variable and the dose of acetylcholine and the pregnancy status as the independent variables (blocking by animal). If the mean values for the ANOVA were found to be significantly different, either a Scheffé test or an independent Student t test with appropriate correction for multiple comparisons was applied. Thus, despite the somewhat large SE because of interanimal variability, statistically significant differences between pregnancy and nonpregnant animals could be discerned. Comparisons of means from two groups used where appropriate were either a paired or unpaired Student t test (two-tailed). A p value < 0.05 was considered to indicate a significant difference.

Results

There was a significant dose-dependent acetylcholine-stimulated relaxation of the preconstricted carotid and uterine arterial rings from both pregnant and nonpregnant animals ($p < 0.0001$ for each) (Figs. 1 and 2). The percent of relaxation the preconstricted vessel achieved was greater in vessels from pregnant animals than that achieved in vessels from nonpregnant animals ($p < 0.0001$ for both uterine and carotid artery rings). Further, there was a significant interaction between pregnancy status and acetylcholine dose on the relaxation achieved for both the uterine ($p < 0.0001$) and carotid ($p = 0.0005$) arteries. The maximum relaxation observed for the carotid artery was approximately three times that observed for the uterine artery. The effect of pregnancy on the uterine artery was most pronounced at the lower concentrations of acetylcholine. Pregnancy was also associated with increased efficacy of acetylcholine for both carotid ($p < 0.0001$) and the uterine arteries ($p = 0.0002$) (Figs. 3 and 4). The interaction term between pregnancy status and dose was also significant for both the uterine ($p = 0.03$) and carotid ($p < 0.0001$) arteries.

Differences in such characteristics as vessel weight, area, and the tension developed in response to phenylephrine was examined. The carotid artery rings from the pregnant animal weighed less (1.61 ± 0.03 mg versus 1.83 ± 0.04 mg, $p < 0.0001$) and had a smaller surface area (1.5 ± 0.03 mm² versus 1.70 ± 0.04 mm², $p = 0.0001$) compared with the nonpregnant animal. However, there was no significant difference in the tension developed in response to 10^{-7} mol/L phenylephrine (53.7 versus 54.8 mg/mm², $p = 0.26$). The uterine artery rings from the pregnant animal weighed more (0.41 ± 0.02 mg versus 0.3 ± 0.01 mg, $p < 0.0001$) and had more surface area (0.85 ± 0.05 mm² versus 0.55 ± 0.02 mm², $p < 0.0001$). There was a significant increase in the tension developed in response to phenylephrine in the uterine artery during preg-

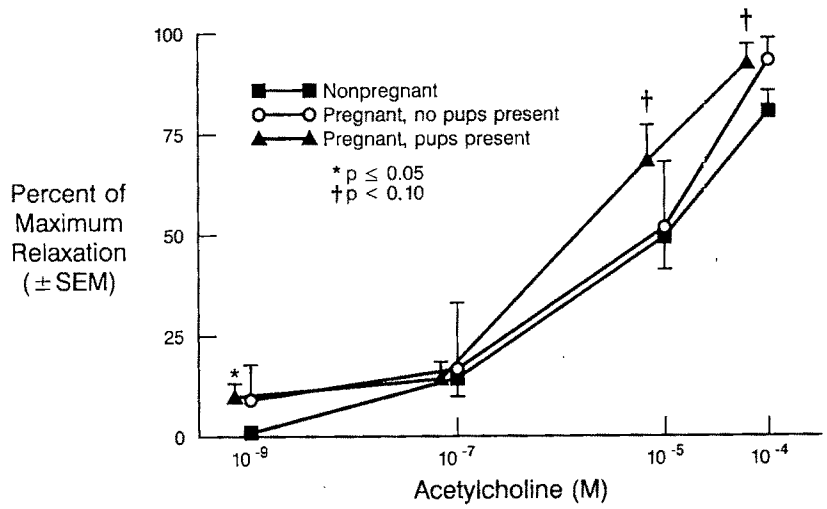


Fig. 3. Efficacy of acetylcholine for uterine artery (defined as percentage of relaxation per dose acetylcholine divided by maximum relaxation achieved in that arterial ring) preconstricted with phenylephrine (10^{-7} mmol/L) was increased during pregnancy (control, $n = 12$; no pups, $n = 3$; pups, $n = 8$). Repetitive measures two-way ANOVA: dose, $p < 0.0001$; pregnancy status, $p = 0.0002$; interaction, $p = 0.03$. * and † Compared with nonpregnant animal.

nancy (30 ± 2.7 mg/mm² versus 21 ± 2.3 mg/mm², $p = 0.01$).

Despite the small diameter of the uterine vessels, they were successfully suspended on the tungsten wire with minimal trauma (Fig. 5). There were no differences observed under the scanning electron microscope between uterine artery rings from pregnant (23 rings, 7 animals) and nonpregnant (16 rings, 5 animals) guinea pigs in terms of either the number of endothelial cells per square micrometer (pregnant, 13.2 ± 0.5; nonpregnant, 12.3 ± 0.6) or the percentage of surface area estimated visually to be denuded of endothelium both at the start and completion of the experiments (each <1%). However, because the surface area of the uterine rings from the pregnant animal was significantly larger than that of the nonpregnant animal, the calculated total number of endothelial cells per ring was also higher during pregnancy (pregnant, 12,205 ± 588; nonpregnant, 9904 ± 937; $p = 0.035$). Rings from both pregnant and nonpregnant animals revealed an apparent loss of endothelium (cells per square micrometer) during the course of the experiment (12 arteries from pregnant, before = 14.3 ± 0.5, after = 12.1 ± 0.7, $p = 0.02$; 8 arteries from nonpregnant, before = 14.1 ± 0.4, after = 10.4 ± 0.7, $p = 0.0003$). This may reflect a small degree of either tissue swelling or stretching during the course of the experiment inasmuch as the area denuded did not increase over the course of the experiment.

Comment

Numerous investigations during the past decade have documented the importance of the endothelium in a host of functions.[10, 21] In vitro studies of vascular smooth muscle function that did not specifically confirm endothelial integrity may have resulted in misleading conclusions concerning vascular responsiveness. For example, acetylcholine-mediated and serotonin-mediated relaxation is dependent on the presence of functional endothelium. In the absence of endothelium, the addition of a low concentration of either causes constriction rather than dilation.[10, 22]

The effect of pregnancy on endothelium-derived relaxing factor has not been studied directly. Bell and colleagues[11, 12] observed that acetylcholine produced hyperemia of the isolated guinea pig uterine horn only during pregnancy or after estrogen supplementation. Two recent reports have dealt with the effect of estrogen on endothelium-derived relaxing factor in the castrated rabbit. Gisclard et al.[23] reported that treatment with 17β-estradiol increased endothelium-dependent relaxation to acetylcholine (3×10^{-9} to 3×10^{-6} mol/L) in the femoral artery. In agreement with the present study, the enhancement was most obvious at the lower concentrations. Miller et al.[24] reported that treatment with 17β-estradiol enhanced acetylcholine-mediated relaxation of the uteroovarian artery obtained from castrated rabbits but did not alter the acetylcholine-mediated relaxation of either the carotid artery or the aorta from the same animal.

In studies by both Gisclard and Miller, the quantity of estrogen given increased the circulating estradiol concentration to estrus-like concentrations and did not alter norepinephrine sensitivity[24] as is typical of pregnancy.[25] Whereas their observations clearly demonstrate an estrogen affect on acetylcholine-mediated endothelium-dependent relaxation, their model does not necessarily mimic pregnancy. In the present study,

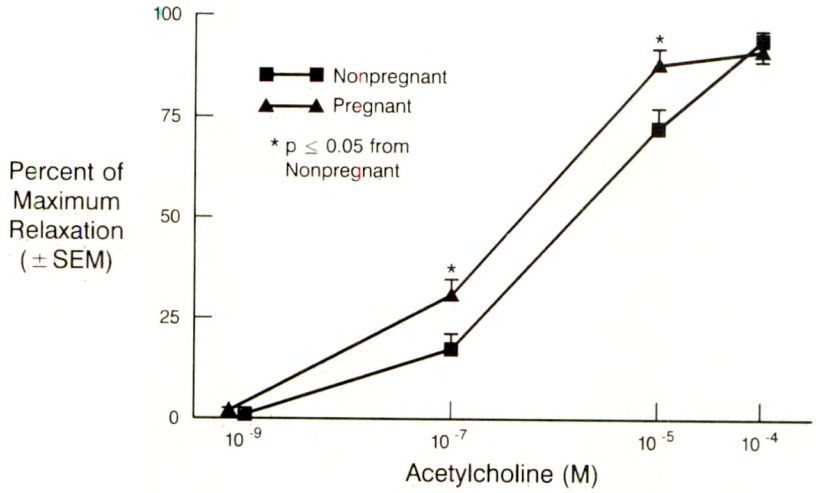


Fig. 4. Efficacy of acetylcholine for carotid artery preconstricted with phenylephrine (10^{-7} mmol/L) was enhanced during pregnancy ($n = 13$). Repetitive measures two-way ANOVA: dose, $p < 0.0001$; pregnancy status, $p < 0.0001$; interaction, $p < 0.0001$. * and † Compared with nonpregnant animal.

we documented a significant enhancement of acetylcholine-mediated relaxation and sensitivity for both the uterine and carotid arteries. That the response of these vessels was altered suggests that the stimulus that leads to the alteration is systemic rather than confined to the uterine vasculature at least by late pregnancy, as was studied here. We cannot explain the observation that the response of rings from uterine arteries that supplied a horn without a pup showed an effect intermediate to the nonpregnant and pregnant animals with a pup present.

Could our observations be a result of increased release of prostacyclin during pregnancy? Though the small diameter of the uterine artery precluded reproducible mechanical disruption of the endothelium without smooth muscle damage, and neither saponin nor indomethacin were used in the present investigation, the relaxation stimulated was very short-lived (<30 seconds total). This differs from prostacyclin. In addition, Gisclard et al.[23] documented that the enhancement of acetylcholine-mediated relaxation in the castrated rabbit after 17β-estradiol was unaltered by indomethacin. Thus the most likely explanation for our observation of increased relaxation and sensitivity to acetylcholine is an alteration of either endothelium-derived relaxing factor release or vessel responsiveness. The observed pregnancy-associated increase in the absolute number of endothelial cells (about 30%) is not likely the sole explanation because there was no significant change in the total number of endothelial cells per unit area. Further investigation is under way to examine this issue.

We cannot, at this time, determine whether the observed changes are a result of either increased release of endothelium-derived relaxing factor per activated muscarinic receptor, increased receptor number, or increased smooth muscle sensitivity to endothelium-derived relaxing factor. Each is plausible. In uterine artery from a castrated rabbit, 17β-estradiol replacement does not alter the endothelium-dependent re-

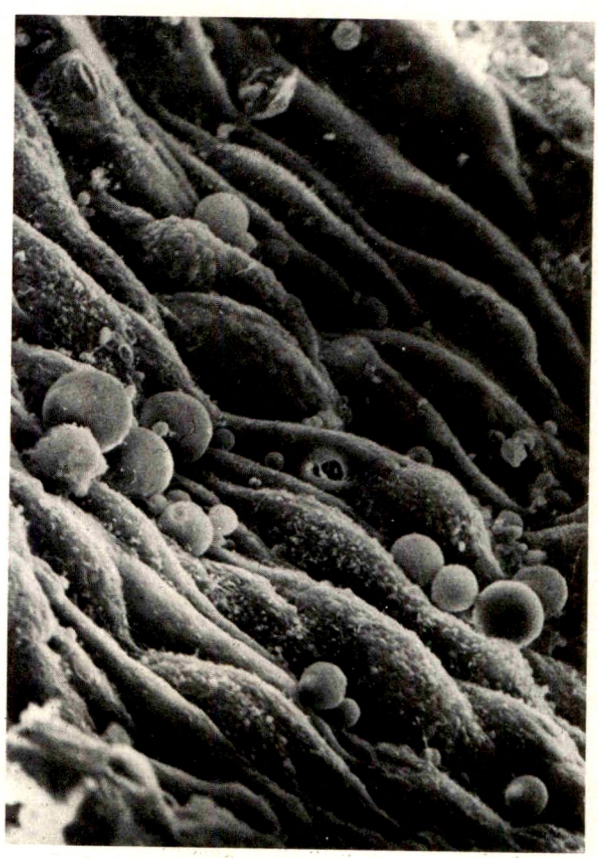


Fig. 5. Representative scanning electron microphotograph of uterine artery from nonpregnant animal after experiment. Note anatomically confluent endothelium.

sponses to the calcium ionophore A23187.[23] This would argue against increased sensitivity of the vascular smooth muscle to endothelium-derived relaxing factor, assuming A21387 and acetylcholine release the same factor (which is controversial[26]). And, as previously noted, estrogen replacement does not mimic pregnancy per se. Endothelial cells contain muscarinic receptors[27] and estrogens augment the number of muscarinic receptors in the rat hypothalamus.[28] Therefore one possible explanation of increased release of endothelium-derived relaxing factor in response to acetylcholine during pregnancy may be an estrogen-mediated increase in the number of endothelial cell muscarinic receptors. Blockade of these receptors prevents acetylcholine-stimulated release of endothelium-derived relaxing factor.

α-Adrenergic receptors appear to be involved in estrogen-induced rat uterine hyperemia.[29] The affinity and subtypes of β-adrenergic receptors is affected by estrogen treatment of rabbits and rats.[30, 31] Because the arterial rings in this study were preconstricted with phenylephrine, the observed effect of pregnancy may reflect an alteration of adrenergic responsiveness. The degree of vessel preconstriction might alter endothelium-derived relaxing factor release, and in this study there was a small but statistically significant difference in the force generated by the uterine arteries of pregnant and nonpregnant animals in response to phenylephrine (10^{-7} mol/L). Changes in disposition can be ruled out because neuronal and extraneuronal uptake of catecholamines were blocked. In conclusion, we believe the most likely explanation for the finding of increased acetylcholine-mediated arterial relaxation during pregnancy is a pregnancy-mediated enhancement of endothelium-derived relaxing factor activity.

REFERENCES

1. Griendling KK, Fuller ED, Cox RH. Pregnancy-induced changes in sheep uterine and carotid arteries. Am J Physiol 1985;17:H658-65.
2. Paller MS. Decreased pressor responsiveness in pregnancy: studies in experimental animals. Am J Kidney Dis 1987;9:308-11.
3. Massicotte G, St Louis J, Parent A, Schiffrin EL. Decreased in vitro responses to vasoconstrictors during gestation in normotensive and spontaneously hypertensive rats. Can J Physiol Pharmacol 1987;65:2466-71.
4. Resnik R, Killam AP, Battaglia FC, Makowski EL, Meschia G. The stimulation of uterine blood flow by various estrogens. Endocrinology 1974;94:1192-6.
5. Clark KE, Baker HA, Bhatnagar R, VanOrden DE, Brody MJ. Prevention of estrogen-induced uterine hyperemia by alpha-adrenergic receptor-blocking agents. Endocrinology 1978;102:903-9.
6. Naden RP, Rosenfeld CR. Systemic and uterine responsiveness to angiotensin II and norepinephrine in estrogen-treated nonpregnant sheep. Am J Obstet Gynecol 1985;153:17-25.
7. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 1980;288:373-6.
8. Griffith TM, Edwards DH, Davies RL, Harrison TJ, Evans KT. Endothelium-derived relaxing factor (EDRF) and resistance vessels in an intact vascular bed: a microangiographic study of the rabbit isolated ear. Br J Pharmacol 1988;93:654-62.
9. Ignarro LJ, Buga GM, Wood KS, Byrns RE, Chaudhuri G. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. Proc Natl Acad Sci USA 1987;84:9265-9.
10. Griffith TM, Lewis MJ, Newby AC, Henderson AH. Endothelium-derived relaxing factor. J Am Coll Cardiol 1988;12:797-806.
11. Bell C. Dual vasoconstrictor and vasodilator innervation of the uterine arterial supply in the guinea pig. Circ Res 1968;23:279-89.
12. Bell C. Oestrogen-induced sensitization of the uterine artery of the guinea pig to acetylcholine. Br J Pharmacol 1973;49:595-601.
13. Bell C, Coffey C. Factors influencing estrogen-induced sensitization to acetylcholine of the guinea pig uterine artery. J Reprod Fertil 1982;66:133-7.
14. Bevan JA, Osher JV. A direct method for recording tension changes in the wall of small blood vessels in vitro. Agents Actions 1972;2:257-60.
15. Hooker CS, Calkins PJ, Fleisch JH. On the measurement of vascular and respiratory smooth muscle responses in vitro. Blood Vessels 1977;14:1-11.
16. Kakner S. Sensitization of noradrenaline responses by inhibitors of extra neuronal uptake in coronary artery preparation. Br J Pharmacol 1974;51:453-5.
17. Green RS, Leffer CW. A comparison of the sensitivities of neonatal ovine pulmonary and femoral arteries to norepinephrine stimulation. Pediatr Res 1984;18:244-9.
18. Furchgott RF. The classification of adrenoreceptors. In: Blaschko H, Muscholl E, eds. Handbook of experimental pharmacology. New York: Springer-Verlag, 1972 vol 33:283.
19. Vanhoutte P, Leusen I. The reactivity of isolated venous preparations to electrical stimulation. Pflugers Arch 1969;306:341-53.
20. McMahon EG, Paul RJ. Effects of forskolin and cyclic nucleotides in isometric force in rate aorta. Am J Physiol 1986;19:C468-75.
21. Vanhoutte PM, Rubanyi GM, Miller VM, Houston DS. Modulation of vascular smooth muscle contraction by the endothelium. Ann Rev Physiol 1986;48:307-20.
22. Stewart DJ, Holtz J, Pohl U, Bassenge E. Balance between endothelium-mediated dilating and direct constricting actions of serotonin on resistance vessels in the isolated rabbit heart. Eur J Pharmacol 1987;143:131-4.
23. Gisclard V, Miller VM, Vanhoutte PM. Effect of 17 β-estradiol on endothelium-dependent responses in the rabbit. J Pharmacol Exp Ther 1988;244:19-22.
24. Miller VM, Aarkus LL, Vanhoutte PM. Effect of estrogens on adrenergic and endothelium-dependent responses in the ovarian artery of the rabbit. In: Halpern W, Pegram BL, Brayden JE, et al., eds. Resistance arteries. Syracuse, New York: Perinatology Press, 1988:136-45.
25. Weiner CP, Martinez E, Chestnut DH, Ghodsi A. Effect of pregnancy on uterine and carotid artery response to norepinephrine, epinephrine, and phenylephrine in vessels with documented functional endothelium. Am J Obstet Gynecol 1989;161:1605-10.
26. Rubanyi GM, Vanhoutte PM. Nature of endothelium-derived relaxing factor: are there two relaxing mediators? Circ Res 1987;61:1161-7.
27. Colburn P, Buonassisi V. Estrogen-binding sites in endothelial cell cultures. Science 1978;201:817-9.
28. Rainbow TC, Degroff V, Luine VH, McEwan BS. Estradiol-17β increases the number of muscarinic receptors in hypothalamic nuclei. Brain Res 1980;198:239-43.

29. Clark KE, Baker HA, Bhatnagar R, Van Orden DE, Brody JM. Prevention of estrogen-induced uterine hyperemia by alpha-adrenergic receptor blocking agents. Endocrinology 1978;102:903-9.
30. Colucci WS, Gimbrone MA, McLaughlin MK, Halpern W, Alexander RW. Increased vascular catecholamine sensitivity and alpha-adrenergic receptor affinity in female and estrogen treated male rats. Circ Res 1982;50:805-11.
31. Hoffman BB, Lavin TN, Lefkowitz RJ, Ruffalo RR. Alpha-adrenergic receptor subtype in rabbit uterus: mediation of myometrial contraction and regulation by estrogens. J Pharmacol Exp Ther 1981;219:290-5.

Effect of pregnancy on uterine and carotid artery response to norepinephrine, epinephrine, and phenylephrine in vessels with documented functional endothelium

Carl P. Weiner, MD,[a] Ernest Martinez, BA,[a] David H. Chestnut, MD,[b] and Abdi Ghodsi, BS[a]

Iowa City, Iowa

The effect of pregnancy on arterial sensitivity to vasoconstrictors is controversial. Some of the controversy may reflect methodologic differences. Vessel reactivity in vitro is altered by both the tension placed on the segment and the presence or absence of functional endothelium. We investigated the effect of pregnancy on guinea pig uterine and carotid arteries to norepinephrine, epinephrine, and phenylephrine. Each vessel segment was stretched to the optimal point along its length-tension curve, and functional endothelium was documented by acetylcholine-stimulated relaxation. A significant dose-response relationship was observed in each vessel for each agent (each $p < 0.0001$). Pregnancy was demonstrated to be associated with a significant reduction in both uterine artery response and sensitivity to norepinephrine, epinephrine, and phenylephrine. However, there was no consistent pregnancy-associated effect on carotid artery response and sensitivity. (Am J Obstet Gynecol 1989;161:1605-10.)

Key words: Pregnancy, catecholamines, endothelium, vascular smooth muscle, in vitro, guinea pig

Many pregnancy-mediated alterations of physiology are known, although the cause(s) are not always clear. Recently we reported enhanced endothelium-derived relaxing factor activity during pregnancy in the uterine and carotid arteries of the guinea pig.[1] Various agents including catecholamines, prostacyclin, histamine, serotonin, and bradykinin stimulate endothelium-dependent relaxation through endothelium-derived relaxing factor release.[2] It is also known that the degree of stretch placed on the vessel (shear or tangential) and the rate of flow through the vessel alters the release of endothelium-derived relaxing factor and consequently the response of the smooth muscle to various vasoconstrictors.[3-6] Prior in vivo study on the effects of catecholamines on uterine blood flow demonstrated decreased sensitivity to norepinephrine and epinephrine during pregnancy.[7-10] Results of the few studies on the effect of catecholamines on isolated vessels obtained from pregnant animals[11-13] seem at odds with the in vivo observations. To our knowledge in no instance has both functional endothelium been confirmed and the vessel placed at its individual optimal resting tension before in vitro experimentation. The purpose of this investigation was to determine the effect of pregnancy on arterial responsiveness in vitro to norepinephrine, epinephrine, and phenylephrine in a vessel stretched to its optimal resting tension and with its endothelium documented to be functionally intact.

From the Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology,[a] the Department of Anesthesia,[b] and the Perinatal Research Laboratories,[a, b] University of Iowa School of Medicine.

Supported in part by grant HD24494 from the United States Public Health Service.

Presented in part at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.

Reprint requests: Carl P. Weiner, MD, Department of Obstetrics and Gynecology, Division of Maternal-Fetal Medicine, The University of Iowa Hospitals and Clinics, Iowa City, IA 52242.

6/6/15650

Material and methods

This study was approved by the University of Iowa Animal Use Committee. Adult female nonpregnant

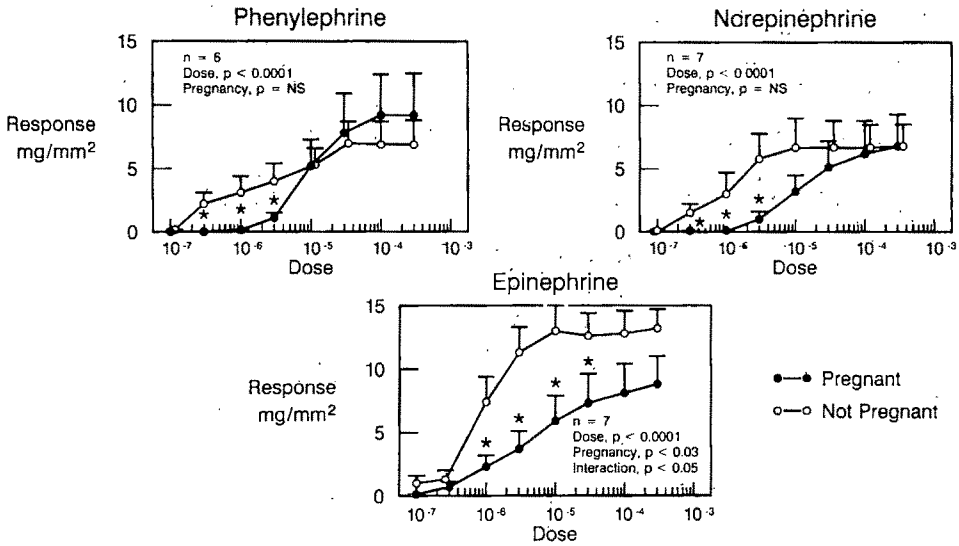


Fig. 1. Uterine artery force (milligrams per square millimeter) developed in response to phenylephrine, norepinephrine, and epinephrine. Dose reflects final molar concentration of drug in vessel bath. *p* Values for dose, interaction, and pregnancy result from two-way analysis of variance with dose and pregnancy status as independent variables. *Asterisk* reflects sensitivity (comparison of pregnant and nonpregnant animals' responses for individual doses).

and pregnant (50 to 60 days, term 65 to 68 days) guinea pigs (600 to 1000 gm) were anesthetized (ketamine 80 mg/kg intraperitoneally, xylazine 3 mg/kg intramuscularly). While 100% oxygen was applied by mask, the common carotid and main uterine arteries were removed en bloc with microsurgical instruments and an operating microscope. The common carotid artery was considered unlikely to be altered by pregnancy and served as a control vessel. The main uterine artery was selected because it directly supplies the conceptus and is thus most likely to be altered by pregnancy. The unstretched inner diameter of the uterine artery from the nonpregnant guinea pig ranges from 90 to 150 μm and would be considered resistance-sized, although in the guinea pig it is a conduit vessel.

The vessels were immediately placed in iced Krebs-Ringer bicarbonate solution (composed of sodium chloride, 6.914 gm/L; potassium chloride, 0.3505 gm/L; calcium chloride dihydrate, 0.328 gm/L; magnesium sulfate heptahydrate, 0.295 gm/L; potassium phosphate, monobasic, 0.164 gm/L; sodium bicarbonate, 2.1 gm/L; and glucose, 2.0 gm/L). Under the microscope, the arteries were cleaned sharply of connective tissue, cut into 3 mm rings, and suspended on two 50 μm tungsten triangles. One triangle was attached to a stationary rod and the other to a strain-gauge transducer (Grass FT 0.03) for the measurement of isometric force.[14, 15] The arterial segments were suspended in a 25 ml drop-away vessel chamber filled with Krebs-Ringer solution aerated with 95% oxygen and 5% carbon dioxide and maintained at 37° C. All experiments were performed in the presence of desipramine (10^{-5} mol/L) to block neuronal uptake and hydrocortisone (10^{-5} mol/L) to block extraneuronal uptake of catecholamines.[16, 17] Propranolol (10^{-5} mol/L) was added to inhibit β-adrenergic receptors.

After at least 60 minutes in the vessel bath, each ring was stretched to the optimal point of its length-tension curve as determined by the tension provoked by potassium chloride (10^{-4} mol/L) added at each stretch level. The optimal resting tension was defined as that point on the length-tension curve where the maximal response to potassium chloride was obtained. The presence of functional endothelium at the beginning (and end) of the experiment was documented by adding acetylcholine (10^{-4} mol/L) to the ring after preconstricting it with submaximal phenylephrine (10^{-7} mol/L). On the basis of prior study,[1] at least 20% relaxation was considered indicative of functional endothelium. In this same study, anatomic integrity of the endothelium was shown under the scanning electron microscope.

The following drugs were used in the experiments: acetylcholine chloride, norepinephrine hydrochloride, epinephrine hydrochloride, phenylephrine hydrochloride, desipramine, hydrocortisone, and propranolol hydrochloride (Sigma Chemical Co., St. Louis); ketamine hydrochloride (Bristol Myers Co., Syracuse, N.Y.); xylazine (Miles Laboratories, Shawnee, Kan.). All drugs and buffers were prepared fresh with distilled water on the day of study and kept on wet ice. All concentrations were expressed as the final molar concentration in the organ bath. After demonstrating functional endothelium, serial cumulative dose-response curves[18] were performed on each ring using norepinephrine, epinephrine, and phenylephrine applied in a random

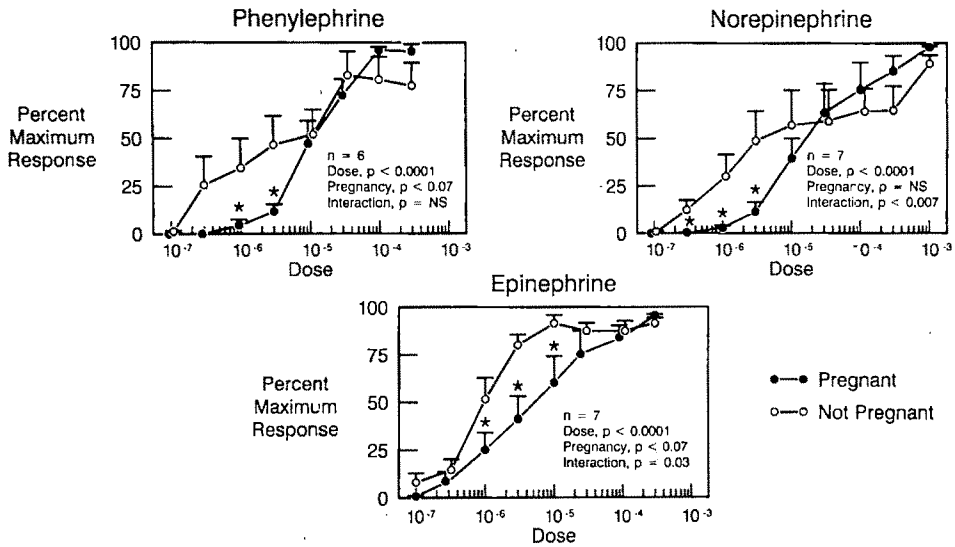


Fig. 2. Efficacy of phenylephrine, norepinephrine, and epinephrine on uterine artery. Dose reflects final molar concentration of drug in vessel bath. *p* Values for dose, interaction, and pregnancy result from two-way analysis of variance, with dose and pregnancy status as independent variables. *Asterisk* reflects sensitivity (comparison of pregnant and nonpregnant animals' responses for individual doses).

order (each 10^{-8} to 10^{-4} mol/L). At the conclusion of the experiment, the vessel segments were cut open longitudinally, the dimensions were measured, and the segments were blotted dry and weighed. The vessel wall cross-sectional area was calculated so that the isometric force generated could be normalized for muscle mass.[19] The response to an agent was expressed in terms of the force generated by each dose of the study agent (milligrams per square millimeter) and the force generated divided by the maximum force generated during the performance of that dose-response curve, i.e., the percent of the maximum.

Data are expressed as mean ± SEM; *n* indicates the number of animals. There were significant differences in the responses between animals. Therefore the effect of pregnancy and dose on vessel constriction was determined by a two-way random block analysis of variance with either response or percent response as the dependent variable and dose and pregnancy as independent variables (blocking by animal). If the mean values were found to be significantly different, a *t* test with Bonnferoni correction was used to reflect differences in sensitivity between pregnant and nonpregnant animals. Thus, despite relatively large standard errors as a result of interanimal variability, statistically significant differences between treatment groups and their dose response curves could be discerned. The comparisons were considered statistically different if $p \leq 0.05$.

Results

For each of the three agents studied a significant drug dose-response relationship was documented in uterine and carotid arteries obtained from pregnant and nonpregnant guinea pigs ($p < 0.0001$ for all). The response and sensitivity of the uterine artery were decreased during pregnancy for phenylephrine, norepinephrine, and epinephrine (Figs. 1 and 2). Each agent showed a higher ED_{30} (30% effective dose) during pregnancy ($p < 0.05$). There were no persistent differences observed for the response of carotid arteries obtained from pregnant and nonpregnant animals for any of the three agents (Fig. 3). However, there was a slight increase in the percent maximum response of norepinephrine for the carotid artery at the high end of the dose range ($p = 0.04$) during pregnancy (Fig. 4).

Comment

Although it is frequently stated that the sensitivity of the uterine artery to a variety of vasoconstrictors is reduced in vivo during pregnancy,[7-10] the finding is not universal in in vitro studies. Van Nimwegen and Dyer[11] compared strips of uterine artery obtained from nonpregnant women to those obtained from pregnant sheep. The tension on the strip was not standardized. They observed that norepinephrine and epinephrine in vessels from pregnant and nonpregnant donors were *equal* in vasoconstrictor activity. This particular technique has a high probability of damaging the endothelium. Assali et al.[12] compared uterine artery responsiveness to norepinephrine in vessels obtained from pregnant and nonpregnant sheep. They observed an *increase* in the response of the uterine artery during pregnancy to norepinephrine. In this study the investigators used vessels from different portions of the uterine arcade in an attempt to control for the changes in

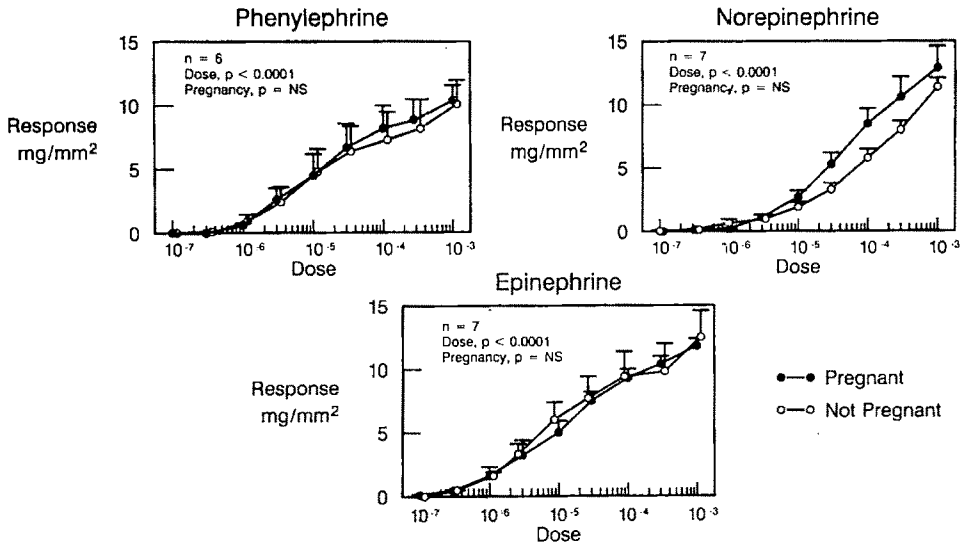


Fig. 3. Carotid artery force (milligrams per square millimeter) in response to phenylephrine, norepinephrine, and epinephrine. Dose reflects final molar concentration of drug in vessel bath. *p* Values for dose, interaction, and pregnancy result from two-way analysis of variance, with dose and pregnancy status as the independent variables. *Asterisk* reflects sensitivity (comparison of pregnant and nonpregnant animals' responses for individual doses).

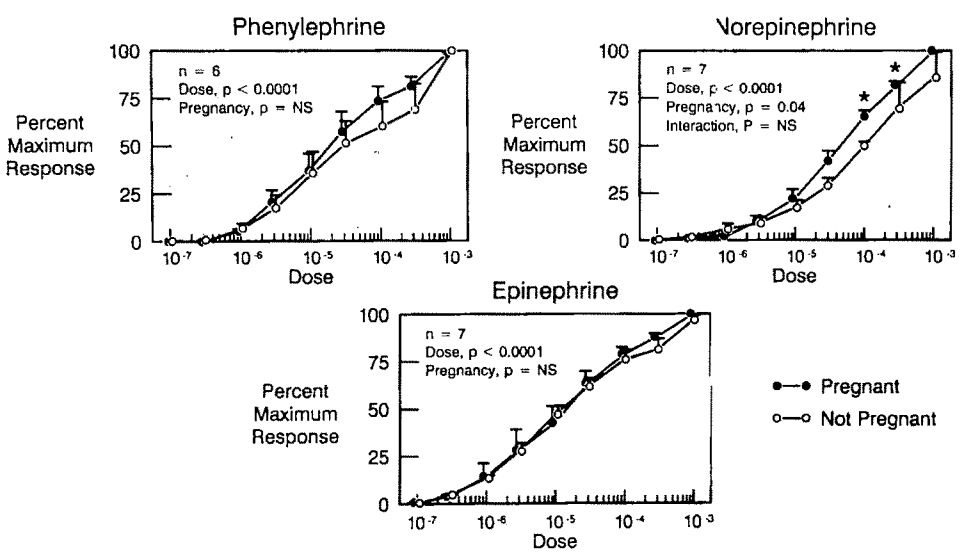


Fig. 4. Efficacy of phenylephrine, norepinephrine, and epinephrine in carotid artery. Dose reflects final molar concentration of drug in vessel bath. *p* Values for dose, interaction, and pregnancy result from two-way analysis of variance, with dose and pregnancy status as independent variables. *Asterisk* reflects sensitivity (comparison of pregnant and nonpregnant animals' responses for individual doses).

size of the uterine artery that occur with pregnancy, assuming that the segments would respond similarly if pregnancy were not a factor. Each of the aforementioned studies was performed before the general realization of the importance of the endothelium in vascular responsiveness. In 1985 Nunotani et al.[13] reported that the response of the uterine artery in the sow to norepinephrine was *enhanced* during pregnancy.[13] Functional endothelium was not documented. Our study of the guinea pig uterine artery with documented functional endothelium and stretched to its optimal resting tension supports the in vivo reports of decreased sensitivity to catecholamines during pregnancy.

The cause of this diminished sensitivity is unclear. Some investigators have suggested that it is mediated

by either estrogen or an estrogen-like substance.[20, 21] α-Adrenergic receptors are involved in estrogen-induced rat uterine hyperemia.[22] The affinity and subtypes of α-adrenergic receptors are affected by estrogen treatment of rabbits and rats.[23, 24] Thus the observed effect of pregnancy may reflect an alteration of adrenergic responsiveness, but this view is controversial.[21] In general, these studies have focused on receptor number, affinity, coupling, etc. However, there are other possibilities. There is an increase in uterine artery production of prostacyclin[25] and endothelium-derived relaxing factor[1] during pregnancy and catecholamines are capable of stimulating their release.[2] Thus altered α-receptor activity would not be necessary if α-stimulation was countered by the release of a vasodilatory substance. Further study is necessary to elucidate the roles of these agents in uterine artery responsiveness to catecholamines.

The effects of pregnancy on the response of systemic vessels to vasoconstrictors are also controversial. Both increases and no change in the sensitivity of the femoral artery to norepinephrine have been reported.[11, 26] Another study reported decreased responsiveness of the perfused tail artery of the rat to norepinephrine during pregnancy.[27] In our study, there was no consistent pregnancy-associated alteration in carotid artery response to the three agents studied.

The uterine artery undergoes rapid growth during pregnancy in association with a twentyfold to fortyfold increase in blood flow.[28] The increased diameter is not simply the result of passive dilation. Vascular smooth muscle hypertrophy and hyperplasia are fairly well documented in the uterine artery of a variety of species during pregnancy.[1, 28] We recently demonstrated an increase in uterine artery mass and endothelial number in the pregnant guinea pig.[1] This increase in smooth muscle content might theoretically be associated with an increase in the force-generating capacity of the vessel. However, this was not reproducible when the force generated by the arterial ring was corrected for cross-sectional area.

In summary, we observed that there is a pregnancy-related decrease in both the force generated and efficacy in the uterine artery to phenylephrine, norepinephrine, and epinephrine during pregnancy and that there is no consistent change in the force-generating capacity and efficacy of the guinea pig carotid artery during pregnancy when the endothelium is functional and the arterial segment is stretched to its optimal resting tension.

REFERENCES

1. Weiner CP, Martinez E, Zhu LK, Ghodsi A, Chestnut D. In vitro release of endothelium-derived relaxing factor by acetylcholine is increased during guinea pig pregnancy. Am J Obstet Gynecol 1989;161:1599-605.
2. Griffith TM, Lewis MJ, Newby AC, Henderson AH. Endothelium-derived relaxing factor. J Am Coll Cardiol 1988;12:797-806.
3. Rubanyi GM, Romero JC, Vanhoutte PM. Flow-induced release of endothelium-derived relaxing factor. Am J Physiol 1986;250(Heart Circ Physiol 19):H1145-9.
4. Holtz J, Forstermann U, Pohl U, Giesler M, Bassenge E. Flow-dependent, endothelium-mediated dilation of epicardial coronary arteries in conscious dogs: effects of cyclooxygenase inhibition. J Cardiovasc Pharmacol 1984; 6:1161-9.
5. Katusic ZS, Shepherd JT, Vanhoutte PM. Endothelium-dependent contraction to stretch in canine basilar arteries. Am J Physiol 1987;252(Heart Circ Physiol 21):H671-3.
6. Harder DR. Increased sensitivity of cat cerebral arteries to serotonin upon elevation of transmural pressure. Eur J Physiol 1988;411:698-700.
7. Anderson SG, Still JG, Greiss FC. Differential reactivity of the gravid uterine vasculatures: effects of norepinephrine. Am J Obstet Gynecol 1977;129:293-8.
8. Magness RR, Rosenfeld CR. Systemic and uterine responses to alpha-adrenergic stimulation in pregnant and nonpregnant ewes. Am J Obstet Gynecol 1986;155:897-904.
9. Greiss FC. Reactivities of the nongravid uterine vasculatures: effects of norepinephrine. Am J Obstet Gynecol 1978;131:778-9.
10. Barton MD, Killam AP, Meschia G. Response of ovine uterine blood flow to epinephrine and norepinephrine. Proc Soc Exp Biol Med 1974;145:996-1003.
11. Van Nimwegen D, Dyer DC. The action of vasopressors on isolated uterine arteries. Am J Obstet Gynecol 1974;118:1099-103.
12. Assali NS, Nuwayhid B, Brinkman CR, Tabsh K, Erkkola R, Ushioda E. Autonomic control of the pelvic circulation: in vivo and in vitro studies in pregnant and nonpregnant sheep. Am J Obstet Gynecol 1981;141:873-84.
13. Nunotani T, Matsuura S, Tamai T, Tatsumi N, Sagawa N, Mori T. The responses of isolated uterine arteries from pregnant sows to vasoconstrictive agents. Acta Obstet Gynaecol Jpn 1985;37:15-23.
14. Bevan JA, Osher JV. A direct method for recording tension changes in the wall of small blood vessels in vitro. Agents Actions 1972;2:257-60.
15. Hooker SC, Calkins PJ, Fleisch JH. On the measurement of vascular and respiratory smooth muscle responses *in vitro*. Blood Vessels 1977;114:1-11.
16. Furchgott RF. The classification of adrenoreceptors. An evaluation from the standpoint of receptors theory. In: Blaschko H, Muschool E, eds. Handbook of experimental pharmacology. New York: Springer-Verlag, 1972 vol 33:283.
17. Green RS, Leffer CW. A comparison of the sensitivities of neonatal ovine pulmonary and femoral arteries to norepinephrine stimulation. Pediatr Res 1984;18:244-9.
18. Van Rossum JM, Hurkmans JA, Wolters CJJ. Cumulative dose-response curves. II. Technique for the making of dose-response curves in isolated organs and the evaluation of drug parameters. Arch Int Pharmacodyn Ther 1963; 143:299-330.
19. McMahon EG, Paul RJ. Effects of forskolin and cyclic nucleotides in isometric force in rat aorta. Am J Physiol 1986;250:C468-75.
20. Resnik R, Killam AP, Battaglia FC, Makowski EL, Meschia G. The stimulation of uterine blood flow by various estrogens. Endocrinology 1974;94:1192-6.
21. Naden RP, Rosenfeld CR. Systemic and uterine responsiveness to angiotensin II and norepinephrine in estrogen-treated nonpregnant sheep. Am J Obstet Gynecol 1985;153:417-25.
22. Clark KE, Baker HA, Bhatnagar R, Van Orden DE, Brody JM. Prevention of estrogen-induced uterine hyperthemia by alpha-adrenergic receptor blocking agents. Endocrinology 1978;102:903-9.

23. Colucci WS, Gimbrone MA, McLaughlin MK, Halpern W, Alexander RW. Increased vascular catecholamine sensitivity and alpha adrenergic receptor affinity in female and estrogen treated male rats. Circ Res 1982;50:805-11.
24. Hoffman BB, Lavin TN, Lefkowitz RJ, Ruffalo RR. Alpha adrenergic receptor subtype in rabbit uterus: mediation of myometrial contraction and regulation by estrogens. J Pharmacol Exp Ther 1981;219:290-5.
25. Magness RR, Osei-Boaten K, Mitchell MD, Rosenfeld CR. In vitro prostacyclin production by ovine uterine and systemic arteries. J Clin Invest 1985;76:2206-12.
26. Moisey DM, Tulenko T. Increased sensitivity to angiotensin in uterine arteries from pregnant rabbits. Am J Physiol 1963;24≤(Heart Circ Physiol 13):H335-40.
27. Dogterm J, DeJong W. Diminished pressor response to noradrenaline of the perfused tail artery of pregnant rats. Eur J Pharmacol 1974;25:267-9.
28. Griendling KK, Fuller EO, Cox RH. Pregnancy-induced changes in sheep uterine and carotid arteries. Am J Physiol 1985;248(Heart Circ Physiol 17):H658-65.

Evaluation of pulsed Doppler echocardiography for measurement of aortic blood flow in the fetal lamb

Jean-Claude Veille, MD,[a] Michael Tavill, BA,[a] Mark Sivakoff, MD,[b] Ilan Cohen, MD,* Moshe Ben-Ami, MD,* Yuh-Cheng Yang, MD,* and Vasil Jovkovsky, MD*

Cleveland, Ohio

The purpose of this study was to assess the accuracy of a quantitative, range-gated, two-dimensional Doppler echocardiography method for measurement of blood flow in the fetal lamb descending aorta. Comparison was made between this method and measurements determined by an electromagnetic flowmeter placed directly on the aorta in the chest of the fetus. Stroke volume was manipulated acutely either by the removal of blood, the addition of fluid by injection, or by pharmacologic means. During each procedure, descending aortic blood flow was estimated by the Doppler ultrasonography method and compared to the flowmeter recording. The size of the lumen of the aorta determined by echocardiography was correlated with direct measurement during surgery and at autopsy. A total of 359 flow measurements were obtained in 15 fetuses. Doppler ultrasonographic flow studies and electromagnetic flowmeter measurements were found to be highly correlated ($r = 0.93$). The study demonstrates the accuracy and reliability of the two-dimensional pulsed Doppler echocardiography method for measurement of blood flow in the descending aorta in the fetal lamb. (Am J Obstet Gynecol 1989;161:1610-4.)

Key words: Pulsed Doppler echocardiography, fetal lamb

Doppler ultrasonography for measurement of cardiac output has been shown to be an accurate, reliable, noninvasive method in both adults and children. Several studies report a good correlation between cardiac output determined by Doppler ultrasonography and cardiac output measurements obtained with the Fick method[1] or thermodilution.[2, 3] Although quantitative ultrasonographic assessment of the human fetal cardiac function has been published,[4, 5] validation of this noninvasive technique is insufficient.

The purpose of this study was to evaluate the accuracy of blood flow rates measured in the fetal lamb descending aorta with range-gated, two-dimensional pulsed Doppler ultrasonography by comparing them to measurements obtained simultaneously with an electromagnetic flowmeter that had been previously placed on the descending aorta.

Material and methods

Experimental animals. A total of 15 fetal lambs were studied (mean body weight 3252 gm ± 846 gm) at a mean gestational age of 135.5 days ± 2.1 days (SD). Food was withheld from the ewe for 48 hours and water was withheld for 24 hours before surgery.

Lactated Ringer's solution (1000 ml) was given through a large bore catheter (No. 14) placed in the external jugular vein. General anesthesia was induced

From the Departments of Reproductive Biology[a] and Pediatrics (Cardiology),[b] MacDonald Hospital for Women, University Hospitals of Cleveland, Case Western Reserve University.
Supported by National Institutes of Health Research Grant No. HL-38296 from the National Institute of Child Health and Human Development (J. C. V.).
Presented in part at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.
Reprints not available.
**Visiting Research Fellows.*
6/6/15658

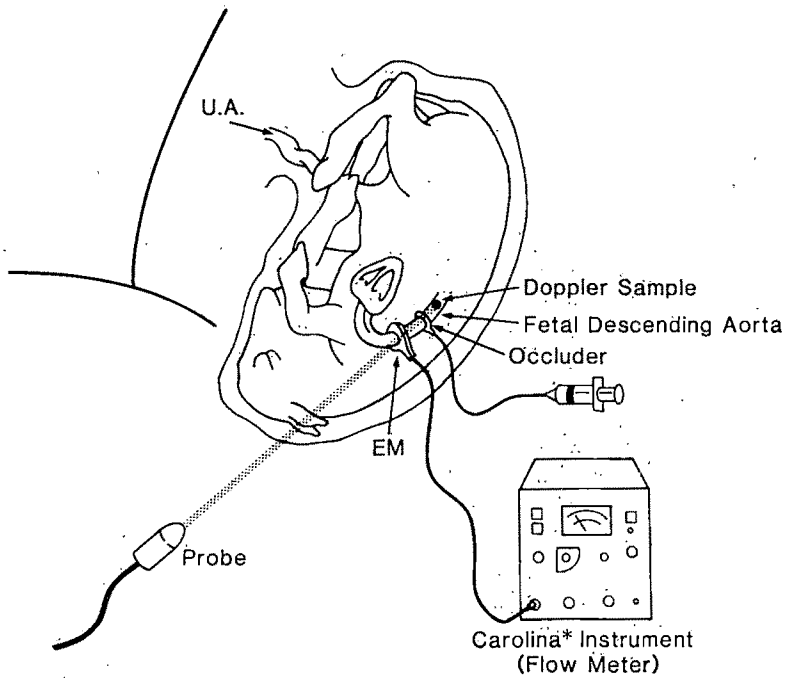


Fig. 1. Diagrammatic illustration of placement of flow probe and occluder on fetal descending aorta, adjacent to ductus.

with ketamine 4 mg/kg and was maintained with ketamine 3 mg/kg per hour administered intravenously. Ventilation was continued through a tracheostomy with inspired gas of ≥50% oxygen in nitrous oxide. Muscle relaxation was achieved with 0.15 mg/kg of pancuronium bromide.

Electromagnetic probe placement. The sheep was shaved and a midline incision was made to expose the uterus. An area distal to the cotyledons (usually close to the uterine septum) was chosen to avoid bleeding and abruptio placentae. A purse-string suture was placed around the myometrium with 1-0 chromic catgut. A second purse-string suture was placed around the amnion to decrease the loss of amniotic fluid. The fetal lamb was then partially delivered to the level of the sternum. A temporary suture was placed to secure the chest and the back of the fetus to the myometrium. Care was taken to avoid compression of the umbilical cord during the surgery. The fetal external jugular vein was identified and a large caliber (inner diameter 3.0 mm) plastic tube was introduced into the lumen of the vessel and advanced to the level of the atrium. The tube was fixed by sutures to the skin of the neck. The chest was opened at the level of the fifth rib and the pleura was carefully incised to expose the descending aorta. The adventitia was opened longitudinally, the size of the vessel was measured, and a precalibrated cuff-type electromagnetic flow probe (In Vivo Metric System, Healdsburg, Calif.) with a lumen diameter of either 8 mm or 10 mm was placed distally and adjacent to the ductus arteriosus and secured with ligatures. A water-inflatable occluder of a corresponding diameter was secured distally to the electromagnetic flowmeter. Occlusion of the vessel was accomplished transiently by the injection of water into the tubing with a 3 ml syringe. All occluders were checked at the time of the surgery. Both the tubing and cable were externally fixed to the chest wall. The chest was closed and the fetus was returned completely to the uterine cavity. The purse-string sutures were tied after introduction of normal saline solution (0.9% wt/vol) into the amniotic cavity to restore the amniotic fluid loss. The maternal skin incision was closed.

Electromagnetic study. Returning signals were displayed on a Carolina Medical Electronics flowmeter (model FM 501, Burlington, N.C.), properly grounded to the sheep, and mean flow was transcribed on a two-channel recorder (Gould, Cleveland, Ohio). The flow probes used were previously calibrated for their linearity and sensitivity with an in vitro preparation. The flow probe was attached to a segment of external jugular vein from a sheep that had been put to death, and 500 ml of heparinized blood was introduced at a constant rate into a closed circuit circulation system. The flow rate was checked with a beaker and stopwatch technique. A gradual restriction to flow was applied distal to the segment of vessel to vary flow rate. The vessel was maintained distended and wet to ensure a good electrical contact between the vessel and the flow probe. An excellent linear correlation

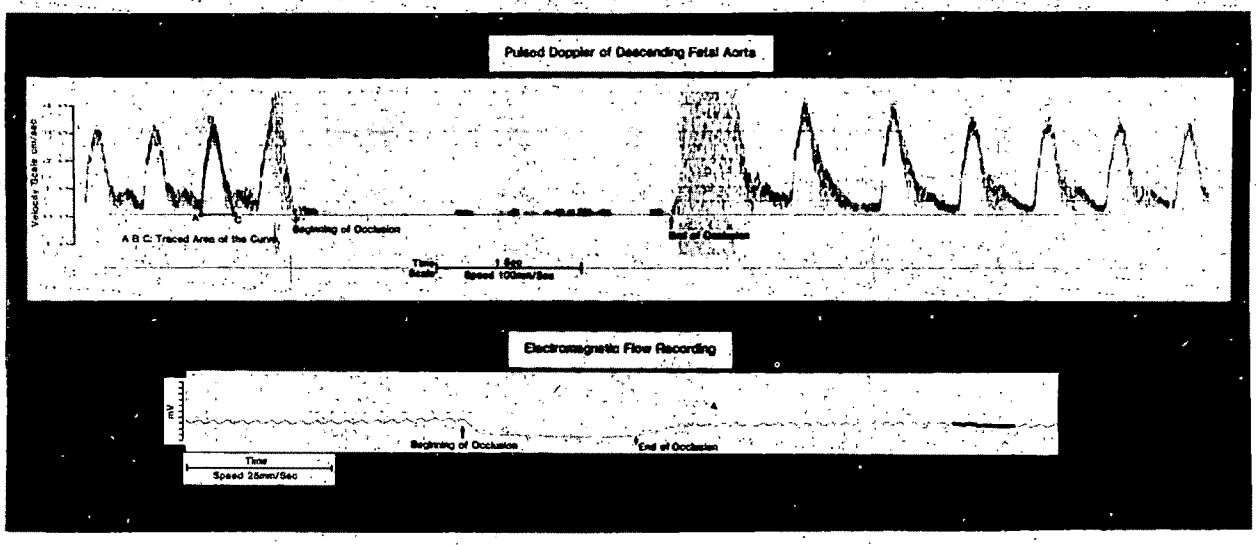


Fig. 2. Typical Doppler tracing of descending aorta of fetal sheep. Waveform was analyzed by placement of digital pen on point *A* of curve and tracing curve out (i.e., joining point *A* to *B* to *C*). This was done to obtain time velocity integral. Quantity of blood flowing through that vessel was calculated with use of area of that vessel. Results are expressed in cubic centimeters per beat.

was shown between voltage and flow for both flow probes ($r = 0.98$, $p < 0.01$, $n = 35$) for probe No. 10, and $r = 0.97$, $p < 0.01$, $n = 27$ for probe No. 8).

Doppler ultrasonography and blood flow measurement. The probe (Ultra Mark 8 mechanical sector, Advanced Technology Laboratories, Bothell, Wash.) was placed (scan heads with either 3 or 5 MHz frequencies, Fig. 1) on the maternal abdominal wall and the descending fetal aorta was identified. The size of the vessel was evaluated on a still frame during systole (inner to inner walls). Comparison between aortic measurements obtained by ultrasonography and those at autopsy was 7.4 mm ± 2 mm and 7.5 mm ± 2 mm, respectively. By placing the probe parallel to the vessel with the two-dimensional echocardiographic image, thereby keeping the angle close to zero, it was possible to keep the sample volume as small as possible. The Doppler ultrasonographic frequency shift was automatically corrected for the angle. The velocity scale was increased to provide maximum magnification of the Doppler ultrasonographic display. A hard copy was obtained with a strip chart recorder set at a speed of 100 mm/sec.

Experimental protocol. Twenty milliliters of blood or fluid was rapidly removed or added at a time through the large bore catheter placed in the external jugular vein of the fetal lamb. After completion of each withdrawal, simultaneous recordings of the two-dimensional Doppler ultrasonographic and electromagnetic flow measurements were obtained. A 30-second vessel occlusion was obtained at each recording to establish a dynamic calibration baseline zero. An average of 20 to 25 data points were obtained per animal. Pharmacologic agents atropine, epinephrine, or ritodrine (0.01 mg increments) were injected directly into the fetus to affect heart rate and thus the quantity of blood ejected per beat through that particular vessel.

Digitizing protocol. An average of the three cardiac cycles that immediately preceded the vessel occlusion was analyzed. The Doppler ultrasonography curve area was traced with a tablet digitizer and a stylus tip (Digisonics, Houston, Tex.) to include points A, B, and C (Fig. 2). The ascending part of the curve was usually a fine line that was easily traced. The middle of the most dense spectral display was traced. All signals below the baseline were not included in the tracing analysis (Fig. 2). This area under the curve is defined as the time velocity integral. The size of the vessel was obtained by two-dimensional echocardiography. The area of the vessel was computed by multiplying half of the diameter by π (½ of diameter^2 × π). The actual volume of blood that flowed through that vessel per heart beat was computed automatically by multiplying time velocity integral and the area of the vessel. Intraobserver variability for the tracing of the curves was limited because measurements were obtained by two investigators (J. C. V., M. S.).

Statistical analysis. A commercially available statistical package (Northwest Statpak, Portland, Ore.) was used to calculate the correlation coefficient between the actual flow measurement obtained with the electromagnetic flow probe and the pulsed Doppler ultrasonography measurement.

Results

A total of 359 measurements of the quantity of blood flow through the descending fetal aorta were ob-

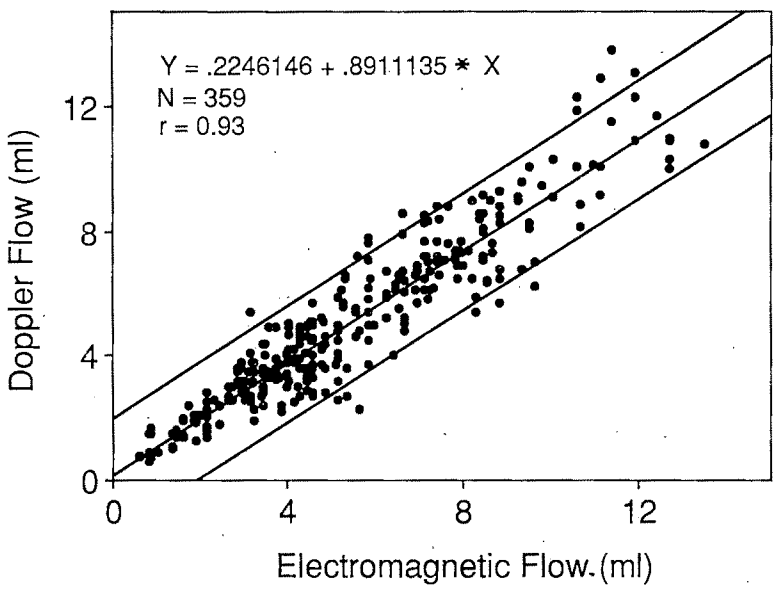


Fig. 3. Linear relationship between quality of blood flowing through fetal descending aorta per beat. Note that values are in ml (or ml/beat) because heart rate is not taken into consideration.

tained simultaneously over a range of 2 to 15 ml/beat with two-dimensional pulsed Doppler echocardiography and electromagnetic flow probe on 15 fetal lambs. There was an excellent direct linear correlation between electromagnetic flow and Doppler ultrasonography readings ($r = 0.93$, $p < 0.001$; Fig. 3) Interobserver variability (i.e., between two observers) and intraobserver variability (i.e., same curve traced by the same observer at different times) were found to be <5%.

Comment

This study suggests that two-dimensional pulsed Doppler ultrasonography can be used to reliably measure the quantity of blood flow in the descending aorta of the fetal lamb. Estimation of flow with this noninvasive technique gave an accurate calculation of flow, as compared to an invasive method such as an electromagnetic flowmeter. In this study, the electromagnetic flow probe was used as our reference standard. Although probes were "factory" precalibrated and had an electronic zero, we recalibrated all the flowmeters with a beaker and stopwatch technique with both a mechanical and electronic zeroing to correct for any possible signal drift. Furthermore, the phasic nature of the studies of blood flow pattern rendered a dynamic calibration necessary, especially when studying low flow rates.[6] Each period of analysis was coupled with a mechanical zero for reference. Care was taken not to constrict or kink the segment of the blood vessel that was being analyzed. The transducers used were chosen to maintain contact around the vessel to thereby minimize movement of the transducer. In our earlier experiments poor electromagnetic signals and gross artifacts occurred because of poor vessel contact. Careful scrutiny of placement and fixation of the electromagnetic flowmeter yielded more consistent results. The acute nature of these experiments did not allow for fibrosis to occur as a means of securing the flow probe. Nonetheless, the consistency of the signals attested to the constant contact between the flow probe and the vessel. As mentioned earlier, all the occlusions were placed downstream to the transducer to prevent additional breaks in the contact between flow probe and vessel. During the calibration of the flow probe, we checked its accuracy over the whole range of its scale to determine its sensitivity. Its reliability was found to be excellent at both low and high flow velocity. As we removed blood from the fetus and reinjected saline solution, the fetal hematocrit was altered and thus could have affected the sensitivity of the flow probe.[7, 8] Notwithstanding the above comments, we showed that the flow measurements obtained with electromagnetic flowmeters were precise and reproducible, and that we were justified in their use as our reference standard.

Estimation of the quantity of blood flow with the use of Doppler ultrasonography greatly depends on the ability to accurately measure the size of the vessel (i.e., its cross-sectional area) and to keep the angle between the ultrasound beam and the direction of the blood flow as close to zero as possible. With the use of various focal depths (maximum display depth, 25 cm) we were able to measure the descending aortic diameter with reliable accuracy. A small error in measurement of the vessel size could result in a large flow error because the

diameter is squared to calculate the area and the flow through the vessel under study.[9] Maintenance of the angle as low as possible gave the best and most reliable Doppler ultrasonographic tracings as judged by best correlation with electromagnetic measurements. In all analyses, the angle was kept close to 20°. Such deviation in the angle should not alter estimation of the quantity of blood flow, as the cosine of the angle is close to 1,[10] and error of measurements will be <6%.[11]

With pulsed Doppler ultrasonography we were able to place the sample volume in close proximity to the electromagnetic flow probe. This is a major advantage in the use of such a technique, as the precise location and depth of sample are known. Even with such positioning, there was some spectral dispersion of the Doppler ultrasonography velocity curve. Although the middle of the most dense portion of the velocity shift was traced, there was an element of subjectivity in locating this zone. By standardization of these tracings we improved the reproducibility and correlation with electromagnetic flow probe measurements.

Despite certain theoretical and practical difficulties in the use of two-dimensional Doppler echocardiography to quantify flow through deep vessels such as those of the intrauterine fetus, we have shown that these can be overcome. Our results indicate that this method can reliably determine, in a noninvasive manner, the quantity of blood flow through the descending aorta in the ovine fetus.

We thank Dr. Mark Morton of the Department of Cardiology at Oregon Health Sciences University for his indispensable help and Dr. Merrill Adams for his technical assistance.

REFERENCES

1. Loeppky JA, Hoekenga DA, Greene ER, Luft UC. Comparison of noninvasive pulsed Doppler and Fick measurements of stroke volume in cardiac patients. Am Heart J 1984;107:339-46.
2. Rose JS, Nanna M, Rahimmtoola SH, et al. Accuracy of determination of changes in cardiac output by transcutaneous continuous-wave Doppler computer. Am J Cardiol 1984;54:1099-101.
3. Alverson DC, Eldridge M, Dillon T, et al. Noninvasive pulsed Doppler determination of cardiac output in neonates and children. J Pediatr 1982;101:46-50.
4. St. John Sutton MG, Gewitz MH, Shah B, et al. Quantitative assessment of cardiac chamber growth and function in the normal human fetus: a prospective longitudinal echocardiographic study. Circulation 1984;69:645-54.
5. Veille JC, Sivakoff M, Nemeth M. Evaluation of the human fetal cardiac size and function. Am J Perinat [In press].
6. Hainsworth R. The measurement of blood flow. Cardiovasc Physiol 1983;P303:1-24.
7. Cuthbertson EM, Gilfillan RS. Evaluation and calibration of square-wave electromagnetic flow probes for chronic implantation. Med Res Eng 1971;10:10-24.
8. Hickman R, Crosier JH, Smith P, et al. Problems with an electromagnetic blood flowmeter. S Afr Med J 1975; 49:261-4.
9. Valdes-Cruz LM, Horowitz S, Mesel E, Sahn DJ, et al. A pulsed Doppler echocardiographic method for calculation of pulmonary and systemic flow: accuracy in a canine model with ventricular septal defect. Circulation 1983;3: 597-602.
10. Reid MH, Mackay RS, Lentz BMT. Noninvasive blood flow measurements by Doppler ultrasound with applications to renal artery flow determinations. Invest Radiol 1980;15:323.
11. Shuster AH, Nanda NC. Doppler examination of the heart, great vessels and coronary arteries. In: Nanda NC, ed. Doppler echocardiography. New York: Igaku-Shoin, 1985:77-129.

Lymph flow rate response to angiotensin II is decreased in pregnant sheep

Guillermo J. Valenzuela, MD, and Lawrence D. Longo, MD
Loma Linda, California

Pregnancy in humans is associated with a number of physiologic changes including interstitial fluid retention (edema) and a decrease in the systemic vascular response to infused angiotensin II. In nonpregnant sheep angiotensin II increases the lymph flow rate by what appears to be a direct effect on the lymphatic vessels. The purpose of this study was to test the hypothesis that during pregnancy the lymph flow rate response to angiotensin II infusion is decreased in relation to that of the nonpregnant state. We speculate that a decrease in lymph flow may explain the interstitial fluid retention observed during human pregnancy. In nine nonpregnant and five pregnant chronically catheterized ewes, we infused angiotensin II at rates of 0.1, 10, and 1000 ng/kg/min during a 5-minute period, with intervals of at least 15 minutes between doses. At the highest angiotensin II dose, peak lymph flow rate increased 286% in pregnant ewes compared with an increase of 344% in the nonpregnant sheep ($p < 0.05$). No changes occurred in the intravascular volume, plasma or lymph protein concentration, or venous pressure. The arterial pressure responses to angiotensin II were decreased in pregnant sheep ($p < 0.05$). These results are compatible with a model for fluid retention in pregnancy in which a decreased lymph flow rate plays a significant role in interstitial fluid retention. (Am J Obstet Gynecol 1989;161:1615-9.)

Key words: Angiotensin II, fluid balance, pregnancy, lymph flow

Human pregnancy is associated with a number of cardiovascular changes, including increases of approximately 40% in blood volume, about 50% in cardiac output, and the retention of several liters of fluid in the interstitium.[1] The fluid distribution across the capillaries follows the balance between the hydrostatic and oncotic pressures on both sides of the capillaries as described by Starling's law.[2] The resulting forces are modified by the area of the surface of interchange and the protein permeability of the capillary walls.[3] Fluid and protein, representing ~30% of the total protein mass in the intravascular system, are transferred into the interstitium and returned to the systemic circulation by the lymphatic system. Thus one may speculate that relatively small changes in the lymph flow rate could produce significant retention of protein and fluid, thus altering the forces that affect the interstitial space oncotic and interstitial fluid pressure. We have previously postulated that angiotensin II may have a direct effect on lymphatic flow in nonpregnant ewes,[4] a concept supported by the findings of Dabney et al.[5] of the effects of angiotensin II in the dog's hind-paw lymphatics. During pregnancy, several animal species, including sheep[6] and man,[7] experience a decrease in systemic arterial pressure response to angiotensin II infusion, despite the fact that circulating levels of angiotensin II in the pregnant sheep during angiotensin II infusion are not statistically different from those in nonpregnant animals.[8] Therefore we tested the hypothesis that lymph flow response to angiotensin II infusion is decreased in pregnancy as compared with nonpregnant controls. Such a finding would suggest that during pregnancy the lymphatic system behaves in a manner similar to that of the systemic vascular system with a decreased responsiveness to vasoactive hormones. We hypothesize that if lympahtic contractility is decreased and if at the same time interstitial compliance is increased, more protein and fluid would remain within the interstitium and appear clinically as edema.

From the Division of Perinatal Biology and the Department of Obstetrics and Gynecology, Loma Linda University.
Presented at the Thirty-fifth Annual Meeting of the Society for Gynecologic Investigation, Baltimore, Maryland, March 17-20, 1988.
Reprint requests: Guillermo J. Valenzuela, MD, Department of Obstetrics and Gynecology, San Bernardino County Medical Center, 780 E. Gilbert St., San Bernardino, CA 92404.
6/6/16510

Material and methods

We used nine nonpregnant and five pregnant ewes, with body weights of 30 to 80 kg. The animals were obtained from a local breeder and housed in individual pens with artificial light (14 hours of light and 12 hours of dark) and ambient temperature of 22° C. The data on the nonpregnant animals have been described in detail elsewhere.[4]

Surgery was performed with the animals under general anesthesia as described in detail elsewhere.[4] Briefly, anesthesia was induced with intravenous Biotal (500 mg) and maintained by inspiration of 1.5% halothane

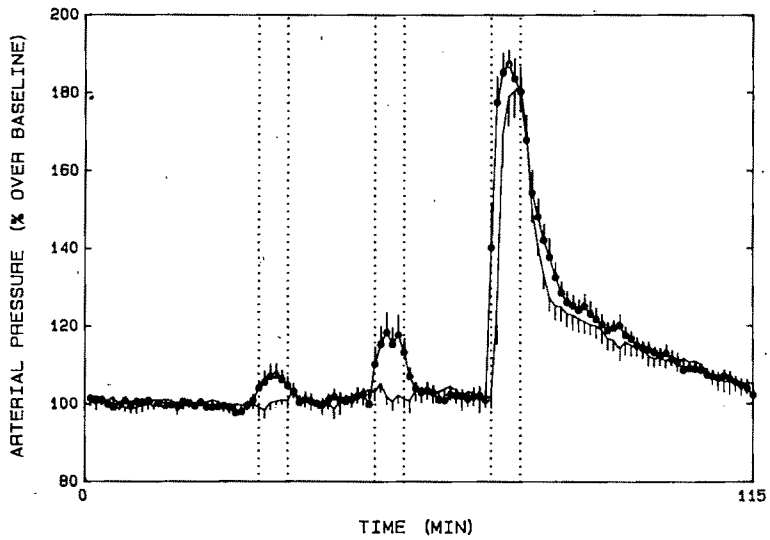


Fig. 1. Mean ± SEM arterial pressure (expressed as percent over baseline) is presented for pregnant (●——●) and nonpregnant (——) animals. Angiotensin II infusion is represented by interval between vertical *dotted lines*; the doses used were 0.1, 10, and 1000 ng/min/kg, respectively. The nonpregnant animals responded with higher arterial pressures ($p < 0.05$).

balanced with oxygen. Tygon catheters (inside diameter 1.27 mm) were placed through a neck incision in the thoracic duct, and one catheter was also placed in one of the internal jugular vein tributaries. The connection between these two vessels was made externally so as to allow the lymph to circulate undisturbed while allowing us easy access to the lymphatic vessels at the time of the experiments. We also catheterized a femoral artery and vein. Splenectomy was performed through a left flank incision. An incision was made 2 cm parallel to the left rib cage, centered about the midaxillary line. Once the incision was made into the peritoneal cavity, the loose fascial planes attached to the splenic capsule were released by blunt dissection. The spleen was then delivered through the incision, stretching the pedicle containing the splenic artery and vein; this pedical was then doubly clamped and tied with 1-0 nylon sutures. Occasionally other small vascular connections at the inferior pole were also ligated. Average blood loss from splenectomy was <50 ml, and the procedure added <10 minutes to the surgery. Postoperatively the animals received heparin, 30,000 IU intravenously daily and 15,000 IU subcutaneously twice a day.

Experimental protocol. The experiments were conducted 4 to 14 days after surgery. We measured lymph flow rate by draining the lymph into a continuously weighed vial ~30 cm below the level of the thoracic duct. The rate was calculated every 10 seconds from the differential of the vial's weight by the use of an online program. We returned the lymph to the animal every 10 minutes, by infusing the vial's content into the jugular vein. Samples for hematocrit and plasma and lymph protein concentration were obtained during the control period and immediately before and after each of the angiotensin II infusions. Protein concentrations were determined by a hand-held refractometer (American Optical, Buffalo). Arterial and venous pressures were measured continuously and averaged every 30 seconds; the values were stored for later analysis.

After a control period of 30 minutes, we infused angiotensin II at a dose of 0.1 ng/kg animal weight per minute for 5 minutes into the jugular vein. Then after a 15-minute observation period, we infused a dose of 10 ng/kg/min for 5 minutes followed by an observation period of 30 minutes. We then administered a third angiotensin II infusion at a rate of 1000 ng/kg/min for 5 minutes. Finally, all functions were measured for 40 minutes.

Data analysis. The data are presented as average ± SEM. To eliminate interanimal variability, the arterial pressure and the lymph flow rate are presented as percent over initial control. Group comparisons were performed by unpaired t test, and the changes with time for each variable were tested with a two-way analysis of variance (separated for pregnant and nonpregnant animals). The lymph flow rate responses were also analyzed by measuring the total area under the curve for pregnant and nonpregnant animals. We plotted the responses to angiotensin II infusion and cut out and weighed the area under the curve for both pregnant and nonpregnant ewes. Also we measured the amount of lymph collected during the first 10 minutes after the angiotensin II infusion for pregnant and nonpregnant animals.

Results

The values for pregnant and nonpregnant animals during the control period are presented in Table

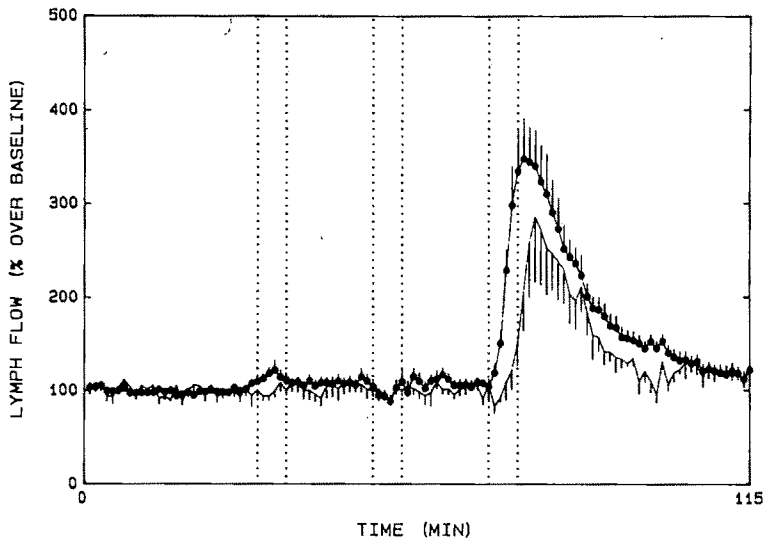


Fig. 2. Average ± SEM lymph flow rates (expressed as milliliters per minute over baseline) responses to angiotensin II are presented in nonpregnant (●——●) and pregnant (——) animals. Vertical *dotted lines* represent angiotensin II infusion doses of 0.1, 10, and 1000 ng/min/kg. There was no effect at lower doses, but at higher doses the response was significantly lower for pregnant ewes ($p < 0.05$).

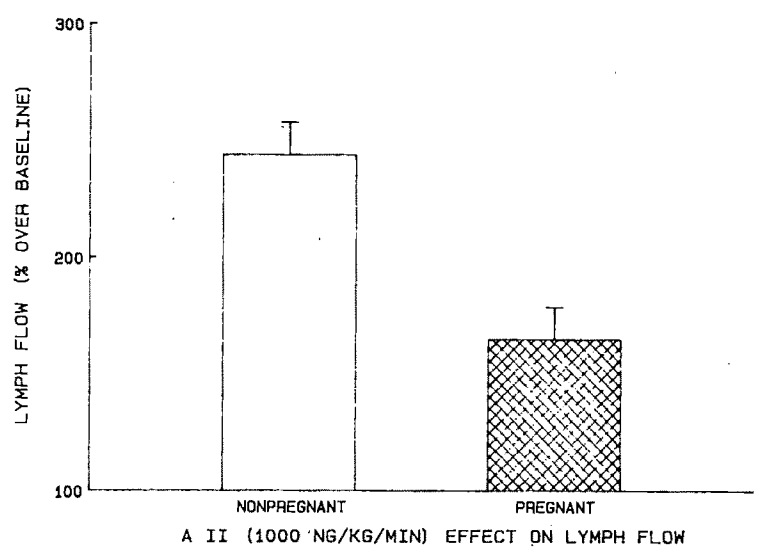


Fig. 3. Excess of lymph flow in response to the higher angiotensin II infusion dose (expressed as % over baseline) for pregnant and nonpregnant animals. The difference is significantly lower for pregnant animal ($p < 0.05$).

I. During the control period the arterial pressure was significantly higher for the nonpregnant ewes ($p < 0.05$). There were no statistical differences between the groups for lymph flow rate, venous pressure, hematocrit, plasma, or lymph protein concentration.

Fig. 1 presents the percent change of arterial pressure over baseline in association with angiotensin II infusion for both pregnant and nonpregnant ewes; there was a statistically higher pressor response at all angiotensin II doses in the nonpregnant animals ($p < 0.05$). Fig. 2 presents the lymph flow response for both groups expressed as the percent change over baseline. There was no change in the lymph flow rate at the two lower doses of angiotensin II even though arterial pressure was elevated in the nonpregnant animals. In absolute terms, after the maximum angiotensin II dose, lymph flow rate increased from a baseline of 2.46 ± 0.69 to 2.98 ± 0.7 ml/min in pregnant animals, as compared with the change from baseline of 2.93 ± 0.15 to 5.07 ± 0.57 ml/min for the nonpregnant group ($p < 0.05$). The ratio of the total area under the curve for nonpregnant/pregnant response was 1.65 ($p < 0.05$). The excess lymph flow during the first 10 minutes after angiotensin II infusion at the highest dose was also greater for the nonpregnant animals (Fig. 3) ($p < 0.05$).

Table I. Values during 30-minute control period

	Nonpregnant (mean ± SEM)	*Pregnant (mean ± SEM)*	*p Value*
Arterial pressure (mm Hg)	100.3 ± 3.7	84 ± 4.2	<0.05
Venous pressure (mm Hg)	7.9 ± 1.1	9.1 ± 0.7	NS
Lymph flow (ml/min)	2.93 ± 0.15	2.46 ± 0.69	NS
Hematocrit (%)	28.84 ± 1.17	26.24 ± 3.66	NS
Plasma protein (gm/dl)	6.3 ± 0.8	6.8 ± 0.2	NS
Lymph protein (gm/dl)	4.4 ± 0.5	5.1 ± 0.6	NS

There were no changes with time for venous pressure, hematocrit, plasma protein, or lymph protein in association with angiotensin II infusion for the pregnant or nonpregnant ewes ($p > 0.1$).

Comment

An understanding of fluid dynamics during pregnancy is important because appropriate fluid retention is associated with improved perinatal outcome.[9, 10] Furthermore, many clinical conditions such as preterm labor, heart disease, kidney disease, and preeclampsia are characterized by alterations of the total amount of fluid retained or its distribution. For instance, preterm labor is characterized by an expansion of blood volume less than that of the normal pregnant women who is delivered at term. In contrast, preeclampsia is characterized by a total fluid retention within normal limits, but it is distributed chiefly to the interstitium when the disease is more pronounced.[10]

The present finding of a relatively decreased lymph flow in response to angiotensin II infusion in pregnant animals as compared with nonpregnant animals is relevant to the problem of fluid dynamics during pregnancy. This is because it is compatible with pregnancy-associated hyporesponsiveness of the lymphatics to pressor hormones as a possible mechanism for interstitial fluid retention. For instance, a 5% lower fluid transport by the lymphatics may result in an interstitial fluid retention of ~100 ml/day; thus in a few days it could produce the retention of a significant amount of fluid and protein. One might argue that the increased lymph flow resulted from the increase in arterial pressure observed with the angiotensin II infusion. However, angiotensin II acts on the smaller arterioles and does not affect veins,[11] so we would not expect an effect on the capillary hydrostatic pressure. The lack of venous pressure change can be used as indirect evidence that the hydrostatic capillary pressure was not significantly changed. If the origin of the increase in lymph flow were the intravascular fluid, we would have expected a change in the microhematocrit in splenectomized animals. Furthermore, in experiments in which a greater transfer of fluid is obtained by increasing the local blood flow, there is a delay before the increase in lymph flow is apparent.[12] In addition, there were no changes in interstitial fluid pressure (as reflected by a lack of changes in venous pressure) or in the plasma or lymph protein concentrations (therefore the oncotic pressures remained stable). Thus with these direct and indirect measurements all parameters suggest that the increased lymph flow did not originate from the systemic circulation. Another possibility is that the excess lymph originated by mobilization of interstitial fluid; however, the lack of change in venous pressure strongly suggests that interstitial fluid pressure remained stable during the angiotensin II infusion.

We speculate that the decreased response to angiotensin II seen in pregnancy results in a decrease in lymph flow. Although the absolute values for lymph flow (expressed as milliliters per minute per kilogram) are not different for pregnant and nonpregnant animals in these results or in our previous study,[13] if a correction factor for the placenta, fetus, and amniotic fluid weights is used, the pregnant animals display a 20% lower lymph flow rate. The mechanism may involve a down-regulation of angiotensin II receptors similar to that thought to occur during pregnancy in rabbits.[14] Another possible mechanism could be the release and/or synthesis of compounds with vasodilator capability produced by angiotensin II infusion during pregnancy; it is well known that blocking prostaglandin production produces a disappearance of the angiotensin II resistance of pregnancy.[15]

Mechanisms other than alterations in the lymph flow rate can be postulated to explain edema formation during pregnancy. For instance, estrogen increases capillary permeability, thus augmenting the fluid and protein transferred across the capillary wall. The administration of fluid also produces an increase in the capillary passage of fluid; however, the steady state is maintained by a severalfold increase in the lymph flow rate, resulting in no net fluid retention in the interstitium.[2] For fluid and protein to be retained in the interstitium, it is also necessary that there be changes in the interstitial fluid space compliance. In this manner the interstitial space may contain more fluid without increasing the pressure. Otherwise, the elevation in interstitial fluid pressure would produce an increase in the lymph flow rate. Estrogen infusion produces fluid retention, mainly in the interstitium,[16] and the decrease of physiologic concentration of estrogen by castration in sheep decreases interstitial fluid space compliance.[17] The fluid and protein retention occur until a critical interstitial fluid volume is reached beyond which the interstitial pressure increases at the same rate as that

of a nonpregnant animal. At this time the lymph flow rate and the response to fluid infusion return to normal, as demonstrated by us in the pregnant sheep.[13]

In conclusion, pregnancy in sheep is associated with a decreased lymph flow rate response to angiotensin II infusion, a phenomenon similar to the response observed in the systemic circulation. This hyporesponsiveness may play a role in the fluid retention of pregnancy.

REFERENCES

1. Metcalfe J, Stock MK, Barron DH. Maternal physiology during gestation. In: Knobil E, Neill J, eds. Physiology of reproduction. New York: Raven Press, 1988:2145-76.
2. Guyton AC, ed. Textbook of medical physiology. 6th ed. Philadelphia: WB Saunders, 1981:370-82.
3. Renkin EM. Some consequences of capillary permeability to macromolecules: Starling's hypothesis reconsidered. Am J Physiol 1986;250:H706-10.
4. Valenzuela GJ, Hewitt CW, Graham AD. A II infusion increases thoracic duct lymph flow in chronically catheterized sheep. Am J Physiol 1987;252:R853-8.
5. Dabney JM, Buehn MJ, Dobbins DE. Constriction of lymphatics by catecholamines, carotid occlusion, or hemorrhage. Am J Physiol 1988;255(Heart Circ Physiol 24): H514-24.
6. Rosenfeld CR, Gant NF. The chronically instrumented ewe: a model for studying vascular reactivity to A II in pregnancy. J Clin Invest 1981;67:486-92.
7. Chesley LC, Talledo E, Bohler CS, Zuspan FP. Vascular reactivity to A II and norepinephrine in pregnant and nonpregnant women. Am J Obstet Gynecol 1965;91: 837-42.
8. Naden RP, Coultrup S, Arant BS, Rosenfeld CR. Metabolic clearance of angiotensin II in pregnant and nonpregnant sheep. Am J Physiol 1985;249(Endocrinol Metab 12):E49-55.
9. Longo LD, Hardesty JS. Maternal blood volume: measurements, hypothesis of control, and clinical considerations. Rev Perinat Med 1984;5:35-59.
10. Goodlin RC, Quaife MA. The significance, diagnosis and treatment of maternal hypovolemia as associated with fetal/maternal illness. Semin Perinatol 1981;5:163-74.
11. Flemming JT, Harris PD, Joshua IG. Endogenous prostaglandins selectively mask large arteriole constriction to angiotensin II. Am J Physiol 1987;253:H1573-80.
12. Lewis GP, Winsey NJP. The action of pharmacologically active substances on the flow and composition of cat hind limb lymph. Br J Pharmacol 1970;40:446-60.
13. Valenzuela GJ, Wood LL, Brace RA. Thoracic duct lymph flow in the pregnant sheep and responses to blood volume expansion. Am J Physiol 1986;250:R1095-8.
14. Brown GP, Venuto RC. Angiotensin II receptor alterations during pregnancy in rabbits. Am J Physiol 1986; 251:E58-65.
15. Everett RB, Worley RJ, MacDonald PC, Gant NF. Effect of prostaglandin synthetase inhibitors on pressor response to angiotensin II in human pregnancy. J Clin Endocrinol Metab 1978;46:1007-10.
16. Ueda S, Fortune V, Bull BS, Valenzuela GJ, Longo LD. Estrogen effects on plasma volume, arterial blood pressure, interstitial space, plasma proteins, and blood viscosity in sheep. Am J Obstet Gynecol 1986;155:195-201.
17. Valenzuela GJ, Brace RA, Longo LD. Lymphatic and vascular responses to fluid infusion in castrated and noncastrated sheep. Am J Physiol 1987;252:R1114-8.

Atrial natriuretic factor: The effects of pregnancy and a high-salt diet on atrial levels

Lony C. Castro, MD, Chander P. Arora, PhD, Jane L. Davis, MD, Calvin J. Hobel, MD, and Hassan Parvez, PhD

Los Angeles, California, and Paris, France

The chronic and relatively acute changes in blood volume that occur during pregnancy and post partum may be associated with alterations in the content of atrial natriuretic factor in the atria. We measured the concentration of atrial natriuretic factor in the right and left atria of virgin, pregnant, and postpartum Sprague-Dawley rats by radioimmunoassay as well as the concentration of atrial natriuretic factor in the atria and plasma of term pregnant rats on a high-salt diet. Neither right nor left atrial concentrations of atrial natriuretic factor were elevated during pregnancy in animals on a normal diet, but both were increased during the first 2 days after delivery. Term pregnant rats on a high-salt diet showed a small increase in atrial natriuretic factor levels in the left atria only, without any change in plasma atrial natriuretic factor levels. We conclude that relatively acute changes in fluid and electrolyte balance are more likely than chronic ones to be associated with alterations in atrial natriuretic factor concentrations in the atria.
(AM J OBSTET GYNECOL 1989;161:1620-3.)

Key words: Atrial natriuretic factor, pregnancy, rats, blood volume

Atrial natriuretic factor (ANF) is a family of peptides secreted by the heart that is involved in fluid and electrolyte homeostasis.[1] The cardiac atria of nonpregnant mammals respond to acute and chronic blood volume expansion with an increase in the synthesis and release of ANF.[2, 3] ANF in turn decreases the blood volume sensed by the atria through its effects on the vascular, renal, and endocrine systems.[4, 5]

Normal pregnancy in both humans and rats is a state of chronic volume expansion,[6, 7] and rats have been widely used as a model to study the interactions between ANF and blood volume. We have previously reported that plasma ANF levels are not elevated in the pregnant rat at term, when blood volume expansion is maximal, but increase during the first 48 hours post partum.[8] This has led us to suggest that factors other than blood volume may play a role in the regulation of the release of ANF during pregnancy and after delivery.

However, atrial ANF content does not always change in parallel with plasma levels as it represents the difference between ANF synthesis and secretion.[9, 10] Therefore, information about the concentration of ANF in the atria in addition to knowledge with regard to plasma levels is necessary to determine whether either pregnancy or the puerperium is associated with an alteration in atrial ANF synthesis.

In this study, we measured the concentration of ANF in the right and left atria of virgin, pregnant, and postpartum Sprague-Dawley rats to determine whether the chronic and relatively acute changes in blood volume associated with pregnancy and the early puerperium alters the concentration of ANF in the atria. Atrial and plasma ANF levels were also measured in a subgroup of pregnant rats placed on a high-salt diet to evaluate whether the atria of the term pregnant rat are able to alter ANF synthesis or secretion in response to a sodium load.

Material and methods

Animals studied. A total of 55 virgin, timed pregnant, and postpartum Sprague-Dawley rats (Simonsen, Gilroy, Calif.) from 10 to 16 weeks old were used in this study. The experimental protocol was approved by the animal research committee. All animals were housed ≥6 days before study in an environment in which light and temperature were controlled. The animals were studied during proestrus (determined by vaginal smear) and on days 11 to 15, 18, and 21 of pregnancy (term was day 21). Post partum they were studied between 0 and 24 hours, 25 to 48 hours, and 30 days after delivery. A total of 48 rats received a "normal" diet that consisted of standard rat chow with free access to tap water. A total of 7 rats received standard rat chow and tap water on days 1 to 16 of preg-

From the Division of Maternal-Fetal Medicine, Cedars-Sinai Medical Center, and the Neuropharmacology Unit, University of Paris.
Supported in part by Biomedical Research Support Grant No. SO7RR05486.
Presented at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.
Reprint requests: Lony C. Castro, MD, Department of Obstetrics and Gynecology, North Tower, Room 3210, Cedars-Sinai Medical Center, 8700 Beverly Blvd., Los Angeles, CA 90048.
6/6/15964

nancy. The drinking water was then replaced with 1% sodium chloride from days 17 to 21 (high-salt diet).

Blood and tissue preparation. After the rats were killed by cervical dislocation, 2 to 2.5 ml of blood was obtained from the abdominal aorta and placed in chilled tubes that contained ethylenediaminetetraacetic acid (1 mg/ml) and aprotinin (500 kallikrein-inhibitor U/ml, Sigma Chemical Co., St. Louis, Mo.). The plasma was separated by centrifugation, extracted on C-18 Sep-Pak cartridges (Waters Associates, Milford, Mass.) and assayed for ANF. The plasma ANF levels measured in the animals on a normal diet have been previously reported.[8]

Immediately after blood sampling the right and left atria were dissected in situ, snap frozen in liquid nitrogen, and stored at −50° C until they were processed. The atria were extracted under acidic conditions to minimize proteolytic degradation. Two milliliters of boiling 1.0 mol/L acetic acid was added to each frozen tissue sample. The tissue was then homogenized, placed in a boiling water bath for 10 minutes, cooled on ice, and centrifuged at 30,000 *g* for 30 minutes at 4° C. Half of the resultant supernatant was used for protein determinations and half was dried in a Speed Vac concentrator (Savant Instruments, Hicksville, N.Y.) and stored at −50° C until determination of ANF content by radioimmunoassay (RIA). To evaluate ANF recoveries in tissue extracts, atrial samples were homogenized in acetic acid and divided. ANF 99-126 (Peninsula Laboratories, Belmont, Calif.) was then added to half of each sample at a concentration that was severalfold greater than that present in the atria. The tissue samples were then processed as described above and the concentration of ANF was determined by RIA.

RIA for ANF. Tissue and plasma concentrations of ANF were measured with a double antibody RIA, the details of which have been previously published.[8] This assay uses a rabbit anti-ANF antiserum that cross-reacts 100% with ANF 99-126. The intra- and interassay variabilities were 4% and 8%, respectively. Extraction recoveries in plasma were 93% ± 3% (n = 10). Extraction recoveries in tissue were 78% ± 1% (n = 6). Results are not corrected for recoveries. Protein concentrations were measured with the Bradford method.[11]

Statistics. Results are expressed as the mean ± SEM. Differences between groups were analyzed with either the one-way or two-way analysis of variance with the Newman-Keuls test or the paired or unpaired Student t test. A p value <0.05 was considered significant.

Results

The ANF concentrations in the right and left atria of virgin, pregnant, and postpartum Sprague-Dawley rats are shown in Figs. 1 and 2, respectively. Neither right nor left atrial ANF concentrations changed significantly during pregnancy and they were not significantly elevated, compared with those in virgin rats. During the first 2 days post partum, atrial ANF levels increased twofold to threefold and returned toward levels present in virgin rats by 30 days post partum. At no time was there any significant difference between right and left atrial ANF concentrations; ANF levels in both atria showed similar patterns of change.

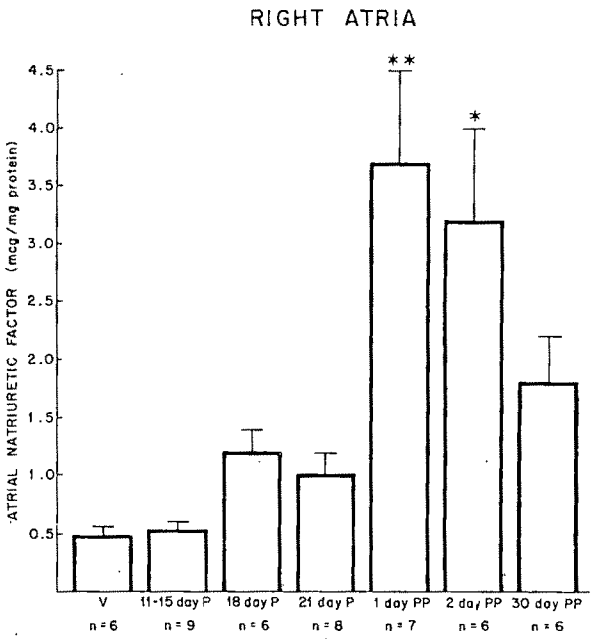


Fig. 1. Concentration of ANF in right atria of virgin *(V)*, pregnant *(P)*, and postpartum *(PP)* Sprague-Dawley rats. *Double star*, $p < 0.01$ versus virgin rats, 11- to 15-, 18-, and 21-day pregnant rats; $p < 0.05$ versus 30-day postpartum rats. *Star*, $p < 0.01$ versus virgin rats, 11- to 15- and 21-day pregnant rats; $p < 0.05$ versus 18-day pregnant rats and 30-day postpartum rats.

Table I shows the effects a high-salt intake over a 4-day period at the end of pregnancy had on atrial and plasma ANF concentrations. The eight pregnant rats on a normal diet represent a subgroup of animals for which plasma ANF values have been previously reported.[8] The high-salt intake produced a modest but significant elevation in left atrial ANF concentration. There was no significant alteration in right atrial or plasma ANF levels.

Comment

Atrial stretch appears to be the primary determinant of ANF release,[12] but other factors such as blood pressure and myocardial work[13] may be important in the regulation of ANF synthesis and secretion in vivo. We previously showed that plasma ANF levels are elevated on days 16 to 18 of pregnancy and during the first 2 days post partum, but are comparable to those in virgin

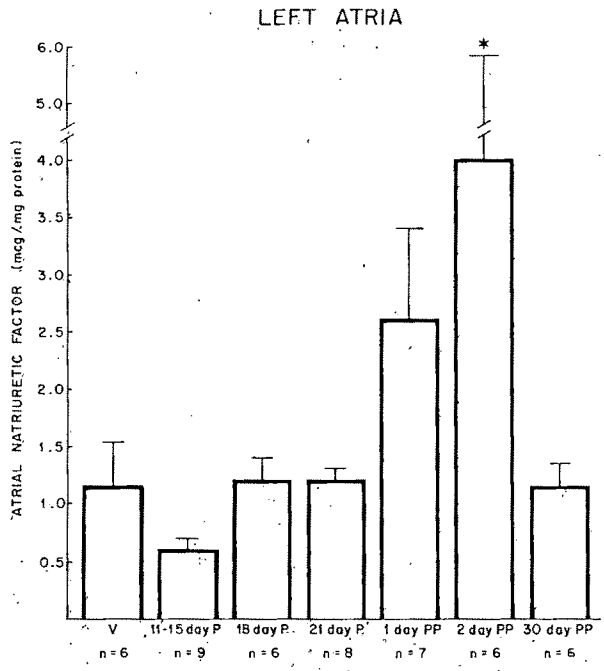


Fig. 2. Concentration of ANF in left atria of virgin *(V)*, pregnant *(P)*, and postpartum *(PP)* Sprague-Dawley rats. *Star*, $p < 0.05$ versus 11- to 15-, 18-, and 21-day pregnant rats.

Table I. 21-day pregnant rats

	Normal diet $n = 8$	*High-salt diet* $n = 7$
ANF, right atria (μg/mg protein)	1.0 ± 0.2	1.2 ± 0.5
ANF, left atria (μg/mg protein)	1.2 ± 0.1	1.8 ± 0.2*
ANF, plasma (pg/ml)	63 ± 6	53 ± 8

*$p < 0.05$ versus left atria, normal diet.

rats on days 11 to 15 and 21 of pregnancy.[8] These findings, coupled with the lack of significant change in the concentration of ANF in the right and left atria during pregnancy reported here, suggest that during the second half of pregnancy any changes in the rate of secretion of ANF are accompanied by similar changes in the rate of ANF synthesis by the atria. These data also suggest that the chronic blood volume expansion present in the term pregnant rat (day 2[7]) is not associated with any significant change in atrial ANF synthesis or secretion when compared to the virgin rat. We previously proposed that this may be a result of the decrease in blood pressure[14] and cardiac index,[15] which have been reported to occur after day 19 of pregnancy. These changes could conceivably result in alterations in myocardial work and atrial stretch opposite to those expected by volume expansion alone. However, our data do not rule out the possibility that in the term pregnant rat an increase in the synthesis and secretion of ANF could be accompanied by an equal increase in ANF clearance or volume of distribution, resulting in tissue and plasma ANF levels that are similar to those in the virgin rat. The data reported here confirms a previously published study[16] that did not show changes in rat atrial ANF content during pregnancy as measured with a bioassay. However, our data are in disagreement with a study[17] in which cardiac ANF levels were found to be elevated at term, whereas plasma levels were decreased. A possible explanation for this difference is that the term pregnant rats in our study were in labor and the accompanying hemodynamic changes may have increased ANF secretion when compared with nonlaboring animals at a similar gestational age.

ANF levels in the atria were elevated during the first 48 hours post partum, a time when plasma ANF levels are known to be high. There are at least two possible reasons for this. After delivery there is closure of the uteroplacental shunt with a probable elevation in blood pressure and increase in myocardial work. The resultant increase in ANF secretion may then be coupled with an even greater increase in ANF synthesis and a concomitant rise in the amount of ANF stored in the atria. Similar changes in the synthesis and secretion of ANF have been reported to occur during the development of spontaneously hypertensive rats.[10] Alternatively, closure of the uteroplacental shunt may be associated with a significant decrease in the clearance of ANF from the circulation. The increase in ANF levels seen in the atria may then reflect a decrease in ANF secretion. Reports that confirm the presence of ANF receptors in the human placenta[18] and of an umbilical arteriovenous difference in plasma ANF levels[19] lend support for the concept of the placental circulation as a possible site of ANF clearance.

The observation that left atrial ANF levels increased after 4 days of sodium loading, whereas plasma levels did not change, suggests that the term pregnant rat retains the ability to respond to relatively short-term changes in sodium intake with alterations in the synthesis of ANF even though ANF secretion did not apparently change. Sodium loading in spontaneously hypertensive rats has been reported to be associated with increased plasma ANF levels only during the first 24 hours after the initiation of the high-salt diet. ANF levels were not elevated thereafter.[20] In a more recent study, pregnant and nonpregnant uninephrectomized rats treated for 3 weeks with deoxycorticosterone and a high-sodium diet showed no alterations in plasma ANF levels; however, the concentration of ANF in the atria was not measured.[21] The dissociation we observed between left and right atrial ANF levels in response to the high-sodium diet is of interest in light of a report

that levels of ANF messenger ribonucleic acid in male Wistar rats are elevated in the right atria but not in the left atria after 1 week of a high-sodium diet.[22] Whether our findings represent a true difference in the response of pregnant versus nonpregnant rats to a sodium load or merely reflect differences in experimental protocols remains to be determined.

Whereas a definitive conclusion about any alteration in ANF synthesis that may occur during pregnancy or post partum will require a knowledge of the levels of ANF messenger ribonucleic acid, it appears that relatively acute changes in fluid and electrolyte balance are more likely than chronic ones to be associated with alterations in atrial ANF concentrations.

REFERENCES

1. Laragh JH. Atrial natriuretic hormone, the renin aldosterone axis and blood pressure—electrolyte homeostasis. N Engl J Med 1985;313:1330-40.
2. Lang RE, Tholken H, Ganten D, Luft FC, Ruskoaho H, Unger Th. Atrial natriuretic factor—a circulating hormone stimulated by volume loading. Nature 1985;314:-264-6.
3. Espiner EA, Nicholls MG. Human atrial natriuretic peptide. Clin Endocrinol 1987;26:637-50.
4. Winquist RJ. Modulation of vascular tone by atrial natriuretic factor. Blood Vessels 1987;24:128-31.
5. Atlas SA, Maack T. Effects of atrial natriuretic factor on the kidney and the renin-angiotensin-aldosterone system. Endocrinol and Metab Clin North Am 1987;16:107-43.
6. Chesley LC. Plasma and red cell volumes during pregnancy. Am J Obstet Gynecol 1972;112:440-50.
7. Barron WM. Volume homeostasis during pregnancy in the rat. Am J Kidney Dis 1987;9:296-302.
8. Castro LC, Arora C, Parvez S, Parvez H, Valenzuela G, Hobel CJ. Plasma atrial natriuretic peptide levels during the rat estrous cycle, pregnancy and puerperium. Am J Obstet Gynecol 1989;160:15-9.
9. Takayanagi R, Tanaka I, Maki M, Inagami T. Effects of changes in water-sodium balance on levels of atrial natriuretic factor messenger RNA and peptide in rats. Life Sci 1985;36:1843-8.
10. Kato J, Kida O, Nakamura S, Sasaki A, Kodama K, Tanaka K. Atrial natriuretic polypeptide (ANP) in the development of spontaneously hypertensive rats (SHR) and stroke prone SHR (SHRSP). Biochem Biophys Res Commun 1987;143:316-22.
11. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976; 72:248-54.
12. Edwards BS, Zimmerman RS, Schwab TR, Heublein DM, Burnett JC. Atrial stretch, not pressure, is the principal determinant controlling the acute release of atrial natriuretic factor. Circ Res 1988;62:191-5.
13. Rankin AJ. Mechanisms for the release of atrial natriuretic peptide. Can J Physiol Pharmacol 1987;65:1673-9.
14. Pike R. Sodium requirement of the rat during pregnancy. In: Lindheimer MD, Katz AI, Zuspan FP, eds. Hypertension in pregnancy. New York: John Wiley, 1976:207-15.
15. Chemtob S, Potvin W, Varma D. Selective increase in placental blood flow by atrial natriuretic peptide in hypertensive rats. Am J Obstet Gynecol 1989;160:477-9.
16. Kristensen CG, Nakagawa Y, Coe FL, Lindheimer MD. Effect of atrial natriuretic factor in rat pregnancy. Am J Physiol 1986;250:R589-94.
17. St. Louis J, Parent A, Gutkowska J, Genest J, Schiffrin E. Vasorelaxation and vascular binding sites for atrial natriuretic peptide in pregnant rats. Am J Physiol 1988; 254:H1027-33.
18. Hatjis CG, Grogan DM. Atrial natriuretic peptide receptors in normal human placentas. Am J Obstet Gynecol 1988;159:587-91.
19. Castro LC, Arora CP, Roll KE, Sassoon D, Hobel CJ. Perinatal factors influencing atrial natriuretic peptide levels in umbilical arterial plasma at the time of delivery. Am J Obstet Gynecol 1989;161:623-7.
20. Gradin K, Hedner J, Hedner T, Towle AC, Petterson A, Persson B. Effects of chronic salt loading on plasma atrial natriuretic peptide in the spontaneously hypertensive rat. Acta Physiol Scand 1987;129:67-72.
21. Nadel AS, Ballerman BJ, Anderson S, Brenner BM. Interrelationships among atrial peptides, renin and blood volume in pregnant rats. Am J Physiol 1988;254:R793-800.
22. Lattion AL, Aubert JF, Fluckiger JP, Nussberger J, Waeber B, Brunner HR. Effect of sodium intake on gene expression and plasma levels of ANF in rats. Am J Physiol 1988;255:H245-9.

Is a decrease in plasma oncotic pressure enough to explain the edema of pregnancy?

Guillermo J. Valenzuela, MD
Loma Linda, California

The balance of fluid across capillaries is given by the Starling equation. Because the plasma protein concentration (one of its components) is decreased in pregnancy, we decided to explore the question as to whether hypoproteinemia with intact protein mass (produced by blood volume expansion) or hypoproteinemia with decreased total protein mass (produced by removal of circulating proteins) alters the oncotic pressure differences across capillaries. We calculated the oncotic pressure difference obtained in seven nonpregnant ewes during periods of normoproteinemia and hypoproteinemia; the influence of fluid infusion under both conditions was also observed. There was an increase in the oncotic pressure difference across the capillary wall during hypoproteinemia produced by a decrease in the total protein mass ($p < 0.01$); however, the response to hypoproteinemia produced by fluid infusion was similar ($p > 0.1$). The venous pressure (used as an index of interstitial fluid pressure changes) did not differ in either hypoproteinemia or fluid infusion. Capillary permeability was decreased during hypoproteinemia, as evidenced by a higher lymph/protein ratio of labeled albumin during the control period ($p < 0.05$). In conclusion, during a state of decreased plasma protein concentration similar to that of pregnancy, the difference in the oncotic pressure and hydrostatic pressure forces acting to prevent transfer of fluid to the interstitium is increased. Therefore other factors that influence fluid transfer across the capillaries must be investigated to explain the edema of pregnancy. (Am J Obstet Gynecol 1989;161:1624-7.)

Key words: Oncotic pressure, fluid balance, edema

Human pregnancy is characterized by many changes in fluid homeostasis including the retention of several liters of fluid in the interstitium.[1] Under normal conditions the fluid balance across the capillaries follows the Starling equation, with hydrostatic capillary pressure and the interstitial fluid oncotic pressure forcing fluid out of the capillary, and the plasma oncotic pressure and interstitial fluid pressure driving the fluid into the capillary. The resulting net movement of fluid and protein is toward the interstitium, from where it is then returned to the systemic circulation via the lymphatics.[2] In the nonpregnant state normal regulatory mechanisms maintain fluid balance with a variability of <1%, despite wide variations in daily water intake[3]; therefore, the interstitial fluid retention seen during pregnancy must be a result of alterations in the components of the Starling equation. Arterial pressure is decreased, whereas central venous pressure is unchanged during pregnancy[4]; therefore, capillary hydrostatic pressure is probably not increased. Thus the edema cannot be explained by hydrostatic forces. For these reasons, we decided to test the hypothesis that a decrease in the plasma oncotic pressure in a nonpregnant animal would be accompanied by a decrease in the oncotic pressure difference across the capillary wall and would favor the retention of fluid in the interstitial space—a finding that would help to explain the edema of pregnancy. The data used in this experiment have been reported in detail elsewhere,[5] with the exception of the calculations that were made for the oncotic pressure measurements, and the data on labeled albumin appearance in the lymph.

From the Department of Obstetrics and Gynecology and Division of Perinatal Biology, Loma Linda University.
Presented at the Thirty-fifth Annual Meeting of the Society for Gynecologic Investigation, Baltimore, Maryland, March 17-20, 1988.
Reprint requests: Guillermo J. Valenzuela, MD, Department of Obstetrics and Gynecology, 780 E. Gilbert St., San Bernardino, CA 92404.
6/6/15957

Material and methods

The experiments were performed on seven nonpregnant ewes that were obtained from a local breeder and housed in standard laboratory conditions. After general anesthesia was induced the surgery was performed as described in detail elsewhere.[6] The vessels catheterized included the femoral artery and vein, the left thoracic duct lymphatic, a tributary of the internal jugular vein, and the left jugular vein.

Experimental protocol. The experiments were conducted on postoperative day 4 and postoperative day 9. Labeled albumin (10 μCi) (Mallinckrodt, St. Louis, Mo.) was injected and samples from both the plasma

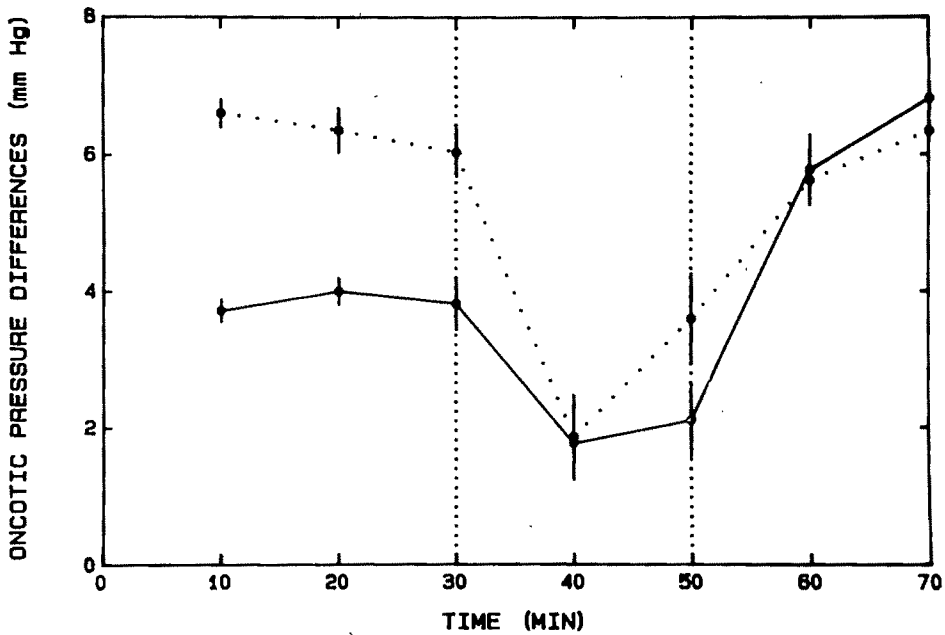


Fig. 1. Differences in oncotic pressure across capillaries are represented during period of normoproteinemia *(continuous line)* and hypoproteinemia *(dotted lines)*. *Vertical lines* represent infusion of fluid. Oncotic pressure differences are significantly higher during hypoproteinemia.

and lymph was obtained at 60 minutes for counts. A normoproteinemic control period of 30 minutes preceded an infusion of 6% of body weight of isosmotic intravenous fluid (Isolyte, Abbott Laboratories, North Chicago, Ill.) administered over 20 minutes. Lymphatic flow measurements and plasma protein samples were obtained every 10 minutes throughout the experiment. There was a 20-minute recovery period. Plasma and lymphatic protein concentration levels were determined by a standard manual refractometer (American Optical, Keene, N.H.). The venous pressure was measured on a continuous basis. Subsequently, the lymphatic fluid was allowed to drain to the exterior for 5 days. A similar protocol of fluid infusion was repeated on postoperative day 9. In four animals, the protein concentrations that were obtained by refractometry were confirmed by measurement of the protein concentrations with the Lowry method in some of the samples.

The different protein fraction concentrations were measured and found unaltered.[5]

Oncotic pressure calculations. In the samples that were obtained during the control period and at the time of peak lymphatic flow, we found that there was no alteration in the protein fraction that was transferred to the interstitium during the hypoproteinemic control period or during the peak fluid infusion period. We calculated the oncotic pressure with the formula developed by Demling et al.[7] in which:

$$\pi_o = 2.47^* (LPC) + 0.51_{,p} = 2.22^* (PPC) + 1.87$$

where π_o = lymphatic oncotic pressure, LPC = lymph protein concentration, π_p = plasma oncotic pressure, and PPC = plasma protein concentration. The albumin counts were expressed as counts per minute per milliliter and the results were expressed as lymph/protein ratio.

Results

The oncotic pressures for plasma during the normoproteinemic and hypoproteinemic period were 21.9 ± 1.4 mm Hg (day 1) and 12.5 ± 1.7 mm Hg (day 5) ($p < 0.01$), and 15.1 ± 1.2 and 7.5 ± 1.4 mm Hg ($p < 0.01$) for the lymph on days 1 and 5, respectively. The average ± SE values for the differences in the oncotic pressure results during the baseline period and during the time of fluid infusions are presented in Fig. 1 (values obtained at 10-minute intervals). The responses to fluid infusion were identical when the normoproteinemic and hypoproteinemic periods were compared ($p > 0.1$). The venous pressures for the normoproteinemic control period and fluid infusion were 9.8 ± 0.1 mm Hg (SE) and 14.6 ± 0.9 mm Hg, whereas the corresponding values for the hypoproteinemic period were 7.4 ± 0.1 mm Hg and 11.6 ± 0.6 mm Hg (a nonsignificant difference when normoproteinemic and hypoproteinemic groups are compared). The labeled albumin lymph/plasma ratio was 0.4 ±

0.01 for the control period and 0.30 ± 0.15 for the hypoproteinemic period ($p < 0.05$).

Comment

Human pregnancy is characterized by many cardiovascular changes including a 40% increase in blood volume and the retention of several liters of fluid in the interstitium.[1] Although the causes for this fluid retention are largely unknown, Longo[8] has proposed that an increase in the circulating concentration of estradiol and other hormones or the placenta acting as an arteriovenous shunt may be the mechanism(s) responsible for these changes. The understanding of fluid balance during human pregnancy is of vital importance because abnormalities in fluid homeostasis are associated with significant perinatal morbidity and mortality.[8, 9] Fluid administration in the nonpregnant state results in fluid transfer to the interstitium, a decrease in the lymphatic oncotic pressure (which decreases the forces moving fluid into the interstitium), and an increase in the plasma oncotic pressure. The interstitial fluid increase results in increased interstitial hydrostatic pressure, and increases the lymphatic flow rate severalfold.[3] Furthermore, the increase in interstitial fluid augments the hydrostatic forces that tend to move the fluid back into the intravascular system. Thus, after a short interval the balance is reestablished. The increase in hydrostatic pressures as a result of the volume alterations also produces an increase in urinary output, which returns volume to baseline. The interesting fact about fluid balance during pregnancy is that there is an alteration of the phenomena described above, with resulting fluid retention. Although there are many vascular changes in the renal circulation, the responses to fluid or salt challenges remain unchanged from the nonpregnant subjects.[10] Furthermore, many maternal diseases such as premature labor and preeclampsia are characterized by alterations in fluid retention or distribution.[8, 9]

Neither pulmonary wedge pressure,[5] nor venous pressure were altered during hypoproteinemia; therefore, we should expect a decrease in the total force retaining the fluid in the intravascular system. The index of pulmonary wedge pressure plus plasma oncotic pressure has been used to predict survival in a host of pathologic states including burns, sepsis, and cardiac failure[11]; this index has also been adopted for use in the management of preeclampsia.[12] The present findings of an increase in oncotic pressure gradient across the capillaries with hypoproteinemia, without a change in the hydrostatic pressure, would leave the pulmonary wedge pressure–oncotic pressure difference unchanged during hypoproteinemia.

The results of the foregoing experiment appear to conflict with the data obtained from Oian et al.[13] who determined the oncotic pressure differences across the capillaries in normal pregnancy and in the preeclamptic state. Our findings also differ from results obtained by Brace and Christian[14] who, with skin segments from pregnant animals, described a decrease in the difference in oncotic pressure across the capillaries in the guinea pig, an animal that displays fluid retention in pregnancy. There were no changes in oncotic pressure noted in sheep, a species that shows only modest changes in blood volume during pregnancy. A major factor that may explain the differences in results is that the aforementioned authors conducted the experiments in skin; however, skin contributes only a modest amount to total body lymphatic circulation. In contrast, our test were performed by obtaining estimates of whole body oncotic pressure differences.

The present finding of a decrease in the lymph/protein ratio of labeled albumin after hypoproteinemia suggests that a decrease in the permeability surface area may be the mechanism responsible for the maintenance of the plasma lymph oncotic pressure difference.

We interpreted the findings of maintained oncotic pressure differences during fluid infusion in a hypoproteinemic state as evidence that the bulk flow across the capillaries does not play a major role in the oncotic pressure difference. For instance, the decrease in lymph flow observed during hypoproteinemia indicates that the fluid transfer across the capillaries is decreased; therefore the convectives component of protein transfer would be decreased. When we increased the bulk flow across the capillaries by the fluid infusion, we found a maintenance of the oncotic pressure difference.

In conclusion, hypoproteinemia does produce an alteration in capillary permeability that protects the oncotic pressure differences. The present results also suggest that convective forces are less important than permeability in the transfer of protein. Therefore, to explain the edema observed during pregnancy it is necessary to postulate that there is an alteration in whole body capillary permeability.

REFERENCES

1. Hytten FE. Water storage in normal pregnancy. Int J Gynaecol Obstet 1970;8:343-8.
2. Renkin EM. Some consequences of capillary permeability to macromolecules. Starling's hypothesis reconsidered. Am J Physiol 1986;250:H706-10.
3. Guyton AC. The lymphatic system, interstitial fluid dynamics, edema, and pulmonary edema. In: Medical physiology. 6th ed. Philadelphia: WB Saunders, 1981.
4. Gonick B. Intensive care monitoring of the critically ill pregnant patient. In: Creasy RK, Resnik R, eds. Maternal-fetal medicine: principles and practice. Philadelphia: WB Saunders, 1989.
5. Valenzuela GJ, Hewitt CW, Kramer GC, Do Y, Hseuh WA. Effects of sustained lymph drainage on cardiovascular function and thoracic duct lymph in sheep. Am J Physiol 1989;256:R867-74.

6. Valenzuela GJ, Hewit CH, Graham AD. Angiotensin II infusion increases thoracic duct lymph flow in chronically catheterized sheep. Am J Physiol 1987;252:R853-8.
7. Demling RH, Manohar M, Will JA. Relationship between the measured and calculated colloid osmotic pressure of plasma and lung lymph in the sheep. Lymphology 1980;13:18-23.
8. Longo LD, Hardesty JS. Maternal blood volume: measurement, hypothesis of control, and clinical considerations. Rev Perinat Med 1984;5:35-59.
9. Goodlin RC, Quaife MA, Dirksen JW. The significance, diagnosis, and treatment of maternal hypovolemia as associated with fetal/maternal illness. Semin Perinatol 1981; 5:163-174.
10. Ferris TF. Renal diseases. In: Burrow GN, Ferris TF, eds. Medical complications during pregnancy. Philadelphia: WB Saunders, 1988.
11. Demling RH, Harms B, Kramer G, Gunther R. Acute versus sustained hypoproteinemia and posttraumatic pulmonary edema. Surgery 1982;92:79-86.
12. Moise KJ, Cotton DB. Colloid osmotic pressure and pregnancy. In: Clark SL, Phelan JP, Cotton DB, eds. Critical care obstetrics. Oradell, New Jersey: Medical Economics Books, 1987.
13. Oian P, Maltau JM. Calculated capillary hydrostatic pressure in normal pregnancy and preeclampsia. AM J OBSTET GYNECOL 1987;157:102-6.
14. Brace RA, Christian JL. Transcapillary Starling pressure in the fetus, newborn, adult, and pregnant adult. Am J Physiol 1981;240:H843-7.

Bound volumes available to subscribers

Bound volumes of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY are available to subscribers (only) for the 1989 issues from the Publisher, at a cost of $56.00 ($79.00 international) for Vol. 160 (January-June) and Vol. 161 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue in the volume. The binding is durable buckram with the JOURNAL name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact The C.V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318, USA; phone (800) 325-4177, ext. 351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular JOURNAL subscription.

Treatment of oophorectomized guinea pigs with intrauterine 17β-estradiol pellets may modulate myometrial β-adrenergic receptor binding properties

Christos G. Hatjis, MD, David M. Grogan, BA, and Donald R. Koritnik, PhD
Winston-Salem, North Carolina, and Columbus, Ohio

Intrauterine 17β-estradiol pellets can induce an up-regulation of guinea pig myometrial β-adrenergic receptor density and *l*-isoproterenol–dependent adenylate cyclase activity. Does 17β-estradiol influence the ability of β-adrenergic receptors to form a "high affinity" state with *l*-isoproterenol, which is a necessary step for adenylate cyclase activation? Nonpregnant, oophorectomized guinea pigs received intrauterine pellets of either placebo, 17β-estradiol, progesterone, or 17β-estradiol plus progesterone for 1 week. 17β-Estradiol resulted in pharmacologic, whereas progesterone resulted in physiologic plasma 17β-estradiol and progesterone concentrations, respectively. The affinity of myometrial β-adrenergic receptors for *l*-isoproterenol was measured by percentage of inhibition of $-[^{125}I]$cyanopindolol binding. In all groups, the competition curves in the presence of magnesium chloride could be resolved into two affinity states of the β-adrenergic receptor, "high" and "low," respectively. The ratio of their dissociation constants was not influenced by hormonal treatment. However, the relative concentration of β-adrenergic receptors in the high affinity state was significantly higher in the 17β-estradiol–treated group than that in the control group. This correlates with the up-regulation in myometrial adenylate cyclase activity and suggests that myometrial β-adrenergic receptor–adenylate cyclase function may be modulated by 17β-estradiol. (Am J Obstet Gynecol 1989;161:1628-32.)

Key words: Sex steroids, myometrial β-adrenergic receptor affinity states, nonpregnant guinea pigs

Myometrial contractility is regulated by the interaction between the hormonal milieu and the function of several stimulatory and inhibitory membrane receptors.[1] A major class of membrane receptors that inhibits myometrial contractility is the β-adrenergic receptor. Stimulation of β-adrenergic receptors in human or animal myometrium by β-adrenergic receptor agonists results in an increase in adenylate cyclase activity and high levels of intracellular 3′5′-cyclic adenosine monophosphate. The latter is a second messenger that initiates a series of events leading to smooth muscle relaxation.

The β-adrenergic receptor–adenylate cyclase system consists of at least three separate proteins: β-adrenergic receptor, guanosine nucleotide regulatory protein, and the catalytic moiety of the enzyme.[2] We[3] and others[2] have shown that the interactions between β-adrenergic receptor agonists (but not antagonists) and β-adrenergic receptor in mammalian tissues facilitates the binding of magnesium to guanosine nucleotide regulatory protein, resulting in the formation of a stable high affinity state of the receptor. From a functional standpoint there apears to be a direct relationship between the ability to form the high affinity state of the β-adrenergic receptor and the ability to activate adenylate cyclase.[2]

In pregnant guinea pigs at ≥0.9 of term gestation (term, 65 days), there is an up-regulation of the ability of myometrial β-adrenergic receptor to form this high affinity state.[3] We have also reported that in oophorectomized guinea pigs, myometrial β-adrenergic receptor concentration increases after exposure to local 17β-estradiol pellets.[4] Finally, we have shown that myometrial β-adrenergic receptor dependent adenylate cyclase stimulation is higher in oophorectomized guinea pigs after exposure to intrauterine 17β-estradiol pellets, as compared with placebo-treated controls or animals exposed to progesterone pellets alone.[5] Thus we have asked the question whether 17β-estradiol intrauterine pellets may influence the ability of the β-adrenergic receptor to form a high affinity state with *l*-isoproterenol, a necessary step for adenylate cyclase activation. To answer this question

From the Department of Obstetrics and Gynecology, Bowman Gray School of Medicine of Wake Forest University, and the Division of Perinatology, Riverside Methodist Hospitals.
Supported in part by United Cerebral Palsy Grant No. R-356-87 and National Institutes of Health Grant No. R23-HD20796.
Presented at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.
Reprint requests: Christos G. Hatjis, MD, Director of Perinatology, Riverside Methodist Hospitals, 3535 Olentangy River Road, Columbus, OH 43214.
6/6/15656

we have performed a series of competition studies in which we have examined the degree of inhibition of $-[^{125}I]$cyanopindolol binding by *l*-isoproterenol in the presence of magnesium chloride with or without guanosine triphosphate in myometrial membranes obtained from oophorectomized guinea pigs treated with either 17β-estradiol, progesterone, 17β-estradiol plus progesterone, or placebo intrauterine pellets.

Material and methods

Material. All chemicals were of reagent grade and of the highest purity available. $-[^{125}I]$cyanopindolol (2.2 Ci/μmol), a specific β-adrenergic receptor antagonist, was purchased from New England Nuclear (Boston, Mass.) on a monthly basis.

Animals. Nonpregnant Hartley nulligravid guinea pigs that weighed 600 to 700 gm were obtained from Perfection Breeders (Douglasville, Pa.). They were housed in the Animal Resources Department facilities (14 hours light, 10 hours darkness per day).

Animal surgery. Details of animal surgery have been previously described in detail.[4] Briefly, under anesthesia induced by ketamine-xylazine bilateral oophorectomies were performed. A total of 14 days later, under anesthesia, hysterotomies were performed and intrauterine pellets of 17β-estradiol (0.75 mg), progesterone (15 mg), 17β-estradiol plus progesterone, or placebo were inserted into the uterine cavities of each horn. Animals were distributed randomly to each of the treatment groups. After 7 days of treatment, the animals were anesthetized again and were killed.

Tissue collection. Details of tissue collection were as previously described.[4] Uterine tissue obtained at death was frozen in liquid nitrogen and stored at −70° C until its use. Freezing did not have any significant effects on β-adrenergic receptor binding characteristics.

Tissue preparation. Details of tissue preparation have been previously described.[3,4] Briefly, uteri were allowed to thaw in ice-cold buffer A containing 0.005 mol/L ethylenediaminetetraacetic acid (EDTA), 0.025 mol/L sucrose, 0.9% sodium chloride, and 0.02 mol/L tris(hydroxymethyl)-aminomethane hydrochloride (Tris HCl), pH 7.5. The uterus was minced with scissors and sonicated with a Brinkmann Polytron homogenizer (Brinkmann Instruments, Westbury, N.Y.). After filtration, the homogenate was centrifuged at 80,000 *g* (15 minutes at 4° C). The supernatant was discarded and the pellet was resuspended in buffer B containing 0.9% sodium chloride, 0.02 mol/L Tris HCl, pH 7.5, and 10 mmol/L magnesium chloride. The suspension was recentrifuged as before. The final pellets were then resuspended in ice-cold buffer B.

Binding studies. An aliquot of the final pellet (5 to 45 μg of protein per tube) was incubated with $-[^{125}I]$cyanopindolol and appropriate drugs as previously described.[3] To determine whether hormonal treatment alters the relative affinities of β-adrenergic receptor to *l*-isoproterenol, a β-adrenergic receptor agonist, we performed a series of drug competition studies. We also examined the interaction between myometrial β-adrenergic receptor and *l*-propranolol, a β-adrenergic receptor antagonist. Binding studies were carried out at 35° C for 45 minutes with various concentrations of *l*-isoproterenol or *l*-propranolol in duplicate. $-[^{125}I]$cyanopindolol was present in concentrations of 8 to 15 pmol/L. The reaction was stopped by dilution with 5 ml of buffer B without magnesium chloride. Binding was assessed by rapid filtration through Whatman GF/C glass fiber filters. Radioactivity was determined in a γ-counter (80% efficiency).

Specific binding of $-[^{125}I]$cyanopindolol was defined as the amount bound in the absence of competing ligand minus the amount bound in the presence of 10 mol/L of DL-propranolol. Specific binding routinely represented ≥90% of the total $-[^{125}I]$cyanopindolol binding. Total $-[^{125}I]$cyanopindolol was <10% to 20% of total $-[^{125}I]$cyanopindolol added and did not change when guanosine triphosphate was added in the incubation.

Inhibition constants were calculated by fitting the data by a nonlinear, iterative, curve-fitting procedure with a generalized model for complex ligand-receptor systems (LIGAND) as initially described by Munson

Table I. Guinea pig uterine weights

Hormonal treatment	*Uterine weight (gm)*
Placebo (n = 3)	0.78 ± 0.01*†
Estradiol (n = 4)	1.99 ± 0.25*
Progesterone (n = 5)	1.27 ± 0.21
Estradiol plus progesterone (n = 4)	1.90 ± 0.12†

Values are mean ± SEM.
*p = 0.01 by Newman-Keuls.
†p = 0.01 by Newman-Keuls.

Table II. Guinea pig plasma estradiol and progesterone concentrations

Hormonal treatment	*Estradiol (pg/ml)**	*Progesterone (ng/ml)**
Placebo (n = 3)	[13.30 ± 0.30]^{a,b}	0.33 ± 0.03^{f,g}
Estradiol (n = 4)	221.83 ± 45.77^{a,c,d}	0.56 ± 0.09^{h,i}
Progesterone (n = 5)	[13.00 ± 0.00]^{c,e}	2.49 ± 0.41^{f,h}
Estradiol plus progesterone (n = 4)	308.49 ± 14.20^{b,d,e}	2.20 ± 0.25^{g,i}

Values are mean ± SEM. Values in brackets were assigned the lowest limit of assay sensitivity.
*Values with same superscript are significantly different. For estradiol, p = 0.01 for *a*, *b*, *c*, and *e*, and p = 0.05 for *d* by Newman-Keuls. For progesterone, p = 0.01 for *f*, *g*, *h*, and *i* by Newman-Keuls.

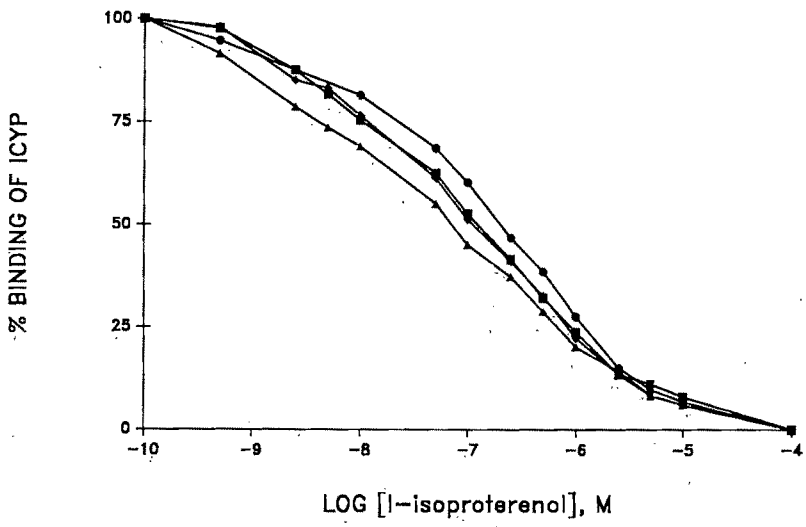


Fig. 1. Binding curves of competition of *l*-isoproterenol for −[^{125}I]cyanopindolol binding to myometrial membranes in presence of magnesium chloride (10 mmol/L) from nonpregnant, oophorectomized guinea pigs treated with different intrauterine pellets: *circle*, control (n = 3; SEM range, 0.51% to 6.13%); *triangle*, estradiol treated (n = 4; SEM range, 1.23% to 5.41%); *square*, progesterone treated (n = 5; SEM range, 0.46% to 1.88%); *diamond*, estradiol plus progesterone treated (n = 4, SEM range, 0.80% to 2.94%).

Table III. *l*-Isoproterenol binding parameters for β-adrenergic receptors in myometria of oophorectomized guinea pigs

	Magnesium chloride		*Magnesium chloride (10 mmol/L) plus guanosine triphosphate (250 μmol/L)*
Hormonal treatment	K_L (*μmol/L*)	K_H (*nmol/L*)	K_I (*μmol/L*)
Placebo (n = 3)	0.31 ± 0.11	3.28 ± 1.15	0.38 ± 0.10
Estradiol (n = 4)	0.31 ± 0.08	2.85 ± 0.89	0.37 ± 0.01 (n = 2)
Progesterone (n = 5)	0.23 ± 0.02	2.96 ± 0.54	0.41 ± 0.08 (n = 3)
Estradiol plus progesterone (n = 4)	0.30 ± 0.06	3.16 ± 0.81	0.37 ± 0.07 (n = 3)

Values are mean ± SEM.

and Rodbard.[7] This method provided estimates for the affinity of the ligands for the different states of the β-adrenergic receptor and for the relative proportions of these states. The dissociation constants were designated at K_H and K_L ; for the high and low affinity states, respectively. The corresponding β-adrenergic receptor population were designated as R_H and R_L · $R_T = R_H + R_L$.

Hormonal studies. When the animals were killed, systemic blood was obtained in heparinized syringes by intracardiac puncture. 17β-Estradiol and progesterone concentrations were measured as previously described.[4]

Protein determinations. Protein concentrations in the tubes were determined by the method of Bradford with bovine serum albumin as a standard.[8]

Statistical analysis. Parameters of interest were compared with analysis of variance (one- and two-way). For multiple comparisons, we used the Newman-Keuls test. The null hypothesis was that there was no significant difference in K_H, K_L, K_L/K_H and the proportion of the β-adrenergic receptor in the high affinity state (R_H/R_T) between placebo (control) animals, and animals treated with hormonal pellets. A p value of <0.05 was considered significant.

Results

All animals gained weight satisfactorily during the postoperative periods. After the placement of intrauterine pellets, the vaginal openings in the animals that received 17β-estradiol or 17β-estradiol plus progesterone pellets were noted to open within 24 to 48 hours of treatment. This is consistent with an estrogen-dependent effect.

At the time of death, uterine weights were determined in the treated animals. Table I shows uterine weights for each of the animal groups. There was a

significant increase in uterine weight for animals that received 17β-estradiol or 17β-estradiol plus progesterone pellets.

Hormonal treatment resulted in pharmacologic 17β-estradiol concentrations (Table II). In contrast, progesterone concentrations were in the physiologic range for nonpregnant animals.

Binding curves of the displacement of $-[^{125}I]$cyanopindolol by *l*-isoproterenol were obtained for each one of the animals studied. Fig. 1 shows binding curves from animals treated with placebo, 17β-estradiol, progesterone, and 17β-estradiol plus progesterone pellets in the presence of magnesium chloride. Each point on each curve represents the mean value of percent $-[^{125}I]$cyanopindolol binding at that concentration of *l*-isoproterenol derived from individual binding curves from each animal. In the presence of magnesium chloride a statistically better fit ($p < 0.05$) of the experimental data points was obtained when a two-site model was hypothesized instead of a one-site model. This was on the basis of the analysis of each of the binding curves by LIGAND (generalized model of complex ligand-receptor interaction).[7] Estimates of the dissociation constants for the high (K_H) and low (K_L) affinity states of β-adrenergic receptor, as well as an estimate of the relative percentage of the β-adrenergic receptor capable of forming the high affinity state, were thus obtained. The results from these calculations are shown in Tables III and IV. There were no significant differences with regard to K_H or K_L between groups. The ratio K_L/K_H was not different in the myometria obtained from animals in each of the experimental groups (Table IV). However, in the presence of magnesium chloride there was a significant increase in the percentage of the β-adrenergic receptor capable of forming the high affinity of the receptor in 17β-estradiol–treated animals as compared with placebo controls. Finally, when guanosine triphosphate was added to magnesium chloride, the best fit for the experimental data points was obtained with a one-site model (low affinity). In the presence of magnesium chloride and guanosine triphosphate, the calculated K_I was similar to the K_L.

Table V shows binding parameters for *l*-propranolol. In all cases, the experimental data fit better to a one-site model. There was no difference in K_I between animal groups in the presence of magnesium chloride with or without guanosine triphosphate.

Table IV. *l*-Isoproterenol–"derived" binding parameters for β-adrenergic receptors in myometria of oophorectomized guinea pigs

Hormonal treatment	K_L/K_H	R_H/R_T
Placebo ($n = 3$)	92.14 ± 2.11	0.40 ± 0.06*
Estradiol ($n = 4$)	140.48 ± 47.84	0.57 ± 0.07*
Progesterone ($n = 5$)	87.69 ± 13.78	0.45 ± 0.02
Estradiol plus progesterone ($n = 4$)	101.51 ± 16.13	0.50 ± 0.01

Mean ± SEM; $K_L = K_D$ for low affinity state and $K_H = K_D$ for high affinity state in the presence of 10 mmol/L magnesium chloride.

R_L and R_H corresponding β-adrenergic concentrations: $R_H + R_L = R_T$.

*$p = 0.05$.

Table V. *l*-Propranolol binding parameters for β-adrenergic receptors in myometria of oophorectomized guinea pigs

	Magnesium chloride (10 mmol/L)	*Magnesium chloride (10 mmol/L) plus guanosine triphosphate (250 μmol/L)*
Hormonal treatment	K_I *(nmol/L)*	K_I *(nmol/L)*
Placebo ($n = 3$)	0.74 ± 0.13	1.10 ± 0.38
Estradiol ($n = 3$)	0.66 ± 0.13	0.90 ± 0.34
Progesterone ($n = 5$)	0.54 ± 0.06	0.69 ± 0.13
Estradiol plus progesterone ($n = 4$)	0.43 ± 0.02	0.53 ± 0.04

Values are mean ± SEM.

Comment

In an attempt to further delineate the interaction between sex steroid hormones and myometrial β-adrenergic receptor function, we perfomred a series of experiments in which we studied the ability of the myometrial β-adrenergic receptor to form the high affinity state with *l*-isoproterenol in previously oophorectomized guinea pigs that subsequently were treated with intrauterine pellets that contained either 17β-estradiol, progesterone, 17β-estradiol plus progesterone, or placebo. In the absence of guanosine triphosphate but in the presence of magnesium chloride, myometrial β-adrenergic receptor from all guinea pigs interacted with *l*-isoproterenol and were able to form two affinity states, high and low, respectively. On the addition of guanosine triphosphate, β-adrenergic receptor were converted to the low affinity state. The above were observed in all animals regardless of the hormonal or placebo treatment. In contrast with the results obtained in our previous studies in pregnant and nonpregnant guinea pig myometria,[3] a significant pregnancy effect on K_H of *l*-isoproterenol was not seen in this series of experiments. Moreover, the ability to form a high affinity state of the β-adrenergic receptor as reflected in the increase of the K_L/K_H ratio was not detectable. The latter is in contrast to the results seen in pregnant animals at ≥0.9 of term gestation (term, 65 days).[3] However, we observed a modest but statistically significant 17β-estradiol–dependent increase in the relative concentration of β-adrenergic receptor capable for form-

ing the high affinity state for L-isoproterenol. This was not seen in the animals treated with 17β-estradiol plus progesterone or progesterone alone. Finally, *l*-propranolol recognized one site of myometrial β-adrenergic receptor of uniform affinity.

The K_L/K_H and R_H/R_T ratios might reflect similar attributes of the high affinity state of the myometrial β-adrenergic receptor. In this series of experiments and in our previously reported studies,[3] there is a dichotomy between the effects of hormonal treatment or pregnancy with regard to these two ratios. The reason for these apparently differing observations is not clear. It is plausible that the derived parameters might reflect different aspects of the interaction between the guanosine nucleotide regulatory protein/*l*-isoproterenol. However, they do provide an explanation for the up-regulation of *l*-isoproterenol dependent adenylate cyclase activity in guinea pig myometria of animals treated with 17β-estradiol pellets.[5]

It should be noted that the increase in R_H/R_T ratio was seen only with pharmacologic doses of 17β-estradiol. Interestingly, on addition of progesterone, this ratio was indistinguishable from that seen in the control (placebo-treated) animals. Clearly, this pharmacologic treatment with intrauterine pellets is different from the situation in normal guinea pig pregnancy in that the 17β-estradiol plasma concentrations are severalfold higher than the concentrations seen in normal pregnant animals and the progesterone concentrations are severalfold lower than those seen in pregnant guinea pigs at ≥0.7 gestation. Although there are some similarities in the properties of β-adrenergic receptor interaction with *l*-isoproterenol, one cannot extrapolate from the studies with hormonally treated animals and explain the observations seen in pregnant guinea pigs. Finally, given some inherent uncertainties in the determinations of R_H and R_L, these conclusions must await further studies in which the guanosine nucleotide regulatory protein concentrations are directly estimated. However, the experiments we report herein represent the neccesary first step toward that goal.

This is the first report in which the ability of myometrial β-adrenergic receptor to form a high affinity state with β-adrenergic receptor agonists has been reported in groups of animals treated with intrauterine 17β-estradiol and progesterone. A preliminary study in hormonally treated rabbits via a systemic route did not show a significant effect on this property.[3] In that regard, our observations confirm these studies. However, because the previous investigators did not report on hormonal concentrations, the differing results with regard to the R_H/R_T ratio cannot be readily explained.

From this series of experiments, we suggest that in nonpregnant, oophorectomized guinea pigs, myometrial β-adrenergic receptor interaction with *l*-isoproterenol may be subject to heterologous hormonal regulation by local 17β-estradiol. The effects of 17β-estradiol might be reversible by the addition of progesterone in physiologic doses. These experiments represent the first step toward the further characterization of the interaction of guanosine nucleotide regulatory protein and *l*-isoproterenol as a function of the animal's hormonal milieu at a molecular level.

REFERENCES

1. Riemer RK, Roberts JM. Endocrine modulation of myometrial response. In: Huszar G, ed. The physiology and biochemistry of the uterus in pregnancy and labor. Boca Raton, Florida: CRC Press, Inc., 1986:53-71.
2. Birnbaumer L, Codina J, Mattera R, et al. Regulation of hormone receptors and adenylyl cyclases by guanine nucleotide binding N proteins. Recent Prog Horm Res 1986;41:41-99.
3. Hatjis CG, Crews A. Regulation of guinea pig myometrial beta-adrenoreceptors by guanosine triphosphate and magnesium ion. Biol Reprod 1988;38:522-7.
4. Hatjis C, Koritnik D, Grogan D. Up-regulation of guinea pig myometrial β-adrenergic receptors by intrauterine estradiol and progesterone pellets. AM J OBSTET GYNECOL 1989;160:751-7.
5. Hatjis C, Koritnik D. Up-regulation of guinea pig myometrial adenylate cyclase activity by intrauterine estradiol and progesterone pellets. AM J OBSTET GYNECOL [In press].
6. Hatjis CG. β-Adrenergic-receptor and adenylate cyclase properties in pregnant and nonpregnant guinea pig myometrium. AM J OBSTET GYNECOL 1985;151:943-50.
7. Munson PJ, Rodbard D. Ligand: a versatile computerized approach for characterization of ligand-binding systems. Anal Biochem 1980;107:220-39.
8. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1986; 72:248-54.

Pregnancy-induced changes in the interaction of guinea pig myometrial β-adrenergic receptors with *l*-isoproterenol

Christos G. Hatjis, MD, and David M. Grogan, BA
Winston-Salem, North Carolina, and Columbus, Ohio

The ability of myometrial β-adrenergic receptors to form a "high-affinity state" with β-adrenergic receptor agonists might be greater in pregnant guinea pigs at ≥0.9 of gestation than in nonpregnant animals. To determine whether this difference is due to pregnancy in general or is associated only with late pregnancy and to determine whether it persists in the postpartum period, we studied the interaction of *l*-isoproterenol with β-adrenergic receptors in myometrial membranes obtained from nonpregnant (nulligravid) animals, pregnant (primigravid) animals at 0.3, 0.7, and 0.9 to 1.0 of gestation (term 65 days), and postpartum guinea pigs (2 to 3 days). The affinity of myometrial β-adrenergic receptors for *l*-isoproterenol was measured by percent inhibition of −[^{125}I]cyanopindolol binding. In the presence of magnesium chloride, the competition curves could be resolved into two affinity states of the β-adrenergic receptor, "high" and "low," respectively, in all groups. The ratio of the dissociation constant of the "low"-affinity state to that of the "high"-affinity state was significantly higher in pregnant guinea pigs at ≥0.9 of gestation than in nonpregnant or postpartum animals and in pregnant animals of earlier gestations. In the presence of guanosine triphosphate only one (low-affinity) state of the receptor was detectable. Thus it is only in pregnant guinea pigs at ≥0.9 of gestation that the ability of myometrial β-adrenergic receptors to form a high-affinity state is enhanced. (Am J Obstet Gynecol 1989;161:1633-6.)

Key words: Pregnancy and myometrial β-adrenergic receptor affinity states

The β-adrenergic receptor–adenylate cyclase system consists of at least three separate proteins: β-adrenergic receptor, guanosine nucleotide regulatory protein, and the catalytic moiety of the enzyme.[1] We[2] and others[1] have shown that the interaction between β-adrenergic receptor agonists (but not antagonists) and β-adrenergic receptor in mammalian tissues facilitates the binding of magnesium to guanosine nucleotide regulatory protein, resulting in the formation of a stable "high-affinity" state of the receptor. In turn, guanine nucleotides block or reverse the magnesium-dependent formation of the "high-affinity state" of the receptor for the agonists and convert the receptor to a "low-affinity state." From the operational standpoint, in the presence of β-adrenergic receptor agonists, magnesium, and guanosine triphosphate, guanosine nucleotide regulatory protein is activated and stimulates the catalytic moiety of the complex, resulting in the formation of cyclic adenosine 3′,5′-monophosphate. Thus there appears to be a direct relationship between the ability to form the high-affinity state of the β-adrenergic receptor and the ability to activate adenylate cyclase.

We have previously reported that myometrial β-adrenergic receptor–dependent adenylate cyclase stimulation is higher in guinea pigs in late pregnancy[3] (0.9 to 1.0 of gestation; term 65 days) than in nonpregnant or pregnant animals at 0.3 of gestation. Although there was an increase in the concentration of β-adrenergic receptor in this system with pregnancy, neither forskolin-stimulated adenylate cyclase activity,[4] a measure of the catalytic moiety of the system, nor sodium fluoride–dependent stimulation,[3] an indirect measure of guanosine nucleotide regulatory protein activation, appears to be modulated by pregnancy. More recently, we presented data that strongly suggested that the ability to form a high-affinity state of the β-adrenergic receptor was higher in pregnant animals at ≥0.9 of gestation than in nonpregnant controls.[2] We thus asked the question whether this differential regulation of the ability of the myometrial β-adrenergic receptor to form a high-affinity state with *l*-isoproterenol could be seen at an earlier point in gestation or was retained in the postpartum period. To answer the question, we performed a series of competition experiments in which we examined the degree of inhibition of −[^{125}I]cyanopindolol binding by *l*-isoproterenol in the presence of magnesium chloride with or without guanosine triphosphate.

From the Department of Obstetrics and Gynecology, Bowman Gray School of Medicine of Wake Forest University, and the Division of Perinatology, Riverside Methodist Hospitals.
This work was supported in part by United Cerebral Palsy grant R-356-87 and National Institutes of Health grant R23-HD20796.
Presented in part at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.
Reprint requests: Christos G. Hatjis, MD, Director of Perinatology, Riverside Methodist Hospitals, 3535 Olentangy River Road, Columbus, OH 43214.
6/6/15963

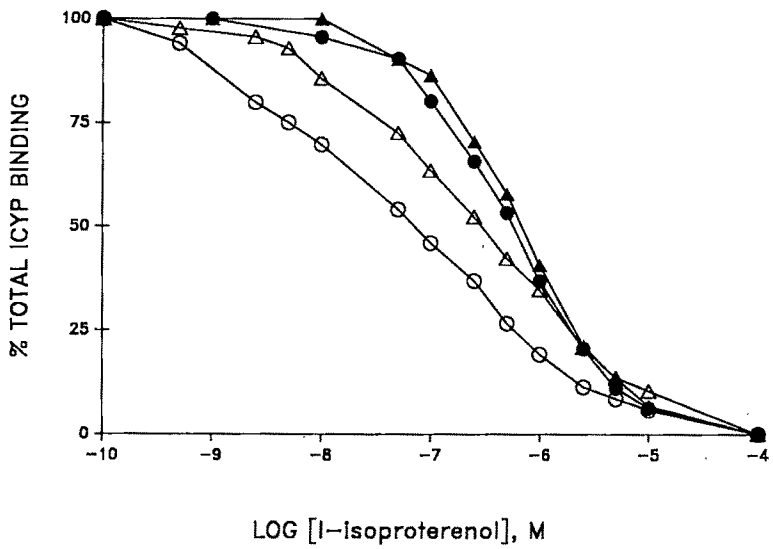


Fig. 1. Binding curves of competition of *l*-isoproterenol for $-[^{125}I]$cyanopindolol binding to myometrial membranes in the presence of magnesium chloride (10 mmol/L) *(open symbols)* or magnesium chloride with guanosine triphosphate (250 μmol/L) *(solid symbols)*. *Triangles*, Nonpregnant ($n = 5$); *circles*, pregnant, 0.9 of gestation ($n = 4$). Note the difference in the curves obtained in the presence of magnesium chloride only between the pregnant animals at ≥0.9 of gestation and the nonpregnant controls.

Table I. *l*-Isoproterenol binding parameters for β-adrenergic receptor in myometria from pregnant, nonpregnant, and postpartum guinea pigs

		Magnesium chloride, 10 mmol/L		
Reproductive state	*n*	K_L *(μmol/L)*	K_H *(nmol/L)*	K_I *(μmol/L) (with guanosine triphosphate, 250 μmol/L)*
Nonpregnant	5	0.590 ± 0.140	16.461 ± 7.139*	0.314 ± 0.048
Pregnant, 0.3 of gestation	5	0.278 ± 0.050	5.676 ± 1.237	0.323 ± 0.053
Pregnant, 0.7 of gestation	4	0.336 ± 0.091	5.461 ± 2.360	0.358 ± 0.042
Pregnant, ≥ 0.9 of gestation	5	0.263 ± 0.044	2.264 ± 0.432*	0.312 ± 0.045
Postpartum (2-3 days)	4	0.258 ± 0.070	3.815 ± 1.249	0.362 ± 0.076

Mean ± SEM. K_L, Dissociation constant for "low"-affinity state; K_H, dissociation constant for "high"-affinity state; K_I, dissociation constant for β-adrenergic receptor in the presence of magnesium chloride with guanosine triphosphate.

*$p = 0.05$ by Dunnett but $p = 0.1$ by Newman-Keuls when K_H values of nonpregnant and pregnant (≥0.9 of gestation) animals are compared.

Material and methods

Material. All chemicals were of reagent grade and of the highest purity available. $-[^{125}I]$cyanopindolol (2.2 Ci/mol), a specific β-adrenergic receptor antagonist, was purchased from New England Nuclear, Boston, on a monthly basis.

Animals. Nonpregnant (nulligravid) Hartley guinea pigs weighing 600 to 700 gm and time-dated pregnant (primigravid, nulliparous) guinea pigs at 0.3, 0.7, and 0.9 to 1.0 of gestation (term 65 days) were obtained from Perfection Breeders (Douglasville, Pa.). They were housed in the Animal Resources Department facilities (14 hours light and 10 hours darkness per day).

Tissue collection. Details of tissue collection were as previously described.[2-4] Uterine tissue, obtained at the time the animals were put to death, was frozen in liquid nitrogen and stored at −70° C until its use. Freezing did not have any significant effects on β-adrenergic receptor binding charcteristics.

Tissue preparation. Details of tissue preparation have been previously described.[2-4] Uteri were allowed to thaw in ice-cold buffer A containing 0.005 mol/L ethylenediaminetetraacetic acid (EDTA), 0.025 mol/L sucrose, 0.9% sodium chloride, and 0.02 mol/L tris(hydroxymethylaminomethane) hydrochloride, pH 7.5. The uterus was minced with scissors and sonicated with a Brinkman Polytron device. After filtration, the homogenate was centrifuged twice at 80,000 *g* (15 min-

utes at 4° C). The final pellets were resuspended in ice-cold buffer B containing 0.9% sodium chloride and 0.02 mol/L Tris-HCl, pH 7.5, and 10 mmol/L magnesium chloride.

Binding studies. An aliquot of the final pellet (5 to 45 μg of protein per tube) was incubated with $-[^{125}I]$cyanopindolol and appropriate drugs as previously described.[2] To determine whether pregnancy alters the relative affinities of β-adrenergic receptor to *l*-isoproterenol, a β-adrenergic receptor agonist, we performed a series of drug competition studies. Binding studies were carried out at 35° C for 45 minutes with various concentrations of *l*-isoproterenol in duplicate. $-[^{125}I]$cyanopindolol was present in concentrations of 8 to 15 pmol/L. The reaction was stopped by dilution and binding was assessed by rapid filtration through Whatman GF/C glass fiber filters. Radioactivity was determined in a γ-counter.

Specific binding of $-[^{125}I]$cyanopindolol was defined as the amount bound in the absence of competing ligand minus the amount bound in the presence of 10 mol/L of DL-propranolol. Specific binding routinely represented ≥90% of total $-[^{125}I]$cyanopindolol binding. Total $-[^{125}I]$cyanopindolol binding was < 10% to 20% of total $-[^{125}I]$cyanopindolol added.

Inhibition constants were calculated by fitting the data by a nonlinear, iterative, curve-fitting procedure according to a generalized model for complex ligand-receptor systems (LIGAND) as initially described by Munson and Rodbard.[5] This method provided estimates for the affinity of the ligands for the different states of the β-adrenergic receptor and for the relative proportions of these states. The dissociation constants were designated at K_H and K_L for the high- and low-affinity states, respectively. The corresponding β-adrenergic receptor populations were designated as R_H and R_L. $R_T = R_H + R_L$, where R_T is the total.

Protein determinations. Protein concentrations in the tubes were determined by the method of Bradford[6] with bovine serum albumin used as a standard.

Statistical analysis. Parameters of interest were compared with analysis of variance (one-way and two-way). For multiple comparisons we used nonparameteric tests. The null hypothesis was that there were no significant differences in K_H, K_L, K_L/K_H, and the proportion of the β-adrenergic receptor in the high-affinity state (R_H/R_T) among nonpregnant animals, pregnant animals at various points in gestation, and animals in the postpartum period. A p value of <0.05 was considered significant.

Results

Binding curves of the displacement of $-[^{125}I]$cyanopindolol by *l*-isoproterenol were obtained for each animal studied. Fig. 1 shows binding curves only from nonpregnant and pregnant (≥0.9 of gestation) animals in the presence of magnesium chloride with or without guanosine triphosphate. Similar curves were obtained from all other groups. Each point on each curve represents the mean value of the percent $-[^{125}I]$cyanopindolol binding at that concentration of *l*-isoproterenol derived from individual binding curves from each animal. In the presence of magnesium chloride but in the absence of guanosine triphosphate, a statistically better fit ($p < 0.05$) of the experimental data points was obtained when a two-site model was hypothesized instead of one. We thus obtained estimates of the dissociation constants for the high-affinity (K_H) and low-affinity (K_L) states of β-adrenergic receptor, as well as an estimate of the relative percentage of the β-adrenergic receptor capable of forming the high-affinity state. The results from the calculations are shown in Tables I and II. There was a trend indicating that K_H was significantly different between pregnant animals at ≥0.9 of gestation and nonpregnant controls (Table I). However, there was no statistical difference in the values of K_L between groups. In contrast to the above, when guanosine triphosphate was added, the best fit for the experimental data points was obtained with a one-site model (low affinity). As expected, in the presence of magnesium chloride and guanosine triphosphate, the K_I obtained was very similar to the K_L.

The ratio of K_L to K_H was significantly higher in the myometria obtained from animals at ≥0.9 of gestation than in those from the nonpregnant (nulligravid, nulliparous or primigravid, animals) or pregnant animals at 0.3 and 0.7 of gestation (Table II). Finally, although there was a trend for the percentage of the β-

Table II. *l*-Isoproterenol binding–derived parameters for β-adrenergic receptor in myometria from pregnant, nonpregnant, and postspartum guinea pigs

Reproductive state	K_L/K_H	R_H/R_T
Nonpregnant	49.256 ± 13.433	0.445 ± 0.050
Pregnant, 0.3 of gestation	54.413 ± 8.586	0.468 ± 0.027
Pregnant, 0.7 of gestation	73.485 ± 9.299	0.557 ± 0.023
Pregnant, 0.9 of gestation	119.536 ± 9.541*	0.542 ± 0.032
Postpartum (2-3 days)	75.637 ± 8.802	0.487 ± 0.043

Mean ± SEM. K_H, Dissociation constant for "high"-affinity state; K_L, dissociation constant for "low"-affinity state in the presence of magnesium chloride, 10 mmol/L. R_L and R_H are corresponding β-adrenergic receptor concentrations. $R_H + R_L = R_T$.

*Mean value of K_L/K_H for pregnant animals at 0.9 of gestation is significantly different from each of the other study groups by the Newman-Keuls test. There were no other significant differences.

adrenergic receptor capable of forming the high affinity of the receptor in the presence of magnesium chloride to be higher in the pregnant animals at 0.9 to 1.0 of gestation than in the nonpregnant or early pregnant groups, that difference did not achieve statistical significance.

Comment

This new series of experiments confirms our previously presented results[2] and describes new significant observations on the interaction between guinea pig myometrial β-adrenergic receptor and reproductive states and gestational age of the animals. In the absence of guanosine triphosphate but in the presence of magnesium chloride, guinea pig myometrial β-adrenergic receptor interacts with *l*-isoproterenol and is able to form two affinity states, "high" and "low" affinity, respectively. On the addition of guanosine triphosphate, β-adrenergic receptor is converted to the "low"-affinity state. These results were observed in all animals. However, the possibility of a significant effect of pregnancy on K_H of *l*-isoproterenol was seen only in the pregnant animals at ≥0.9 of gestation. Moreover, the ability to form a high-affinity state of β-adrenergic receptor, as reflected in the increase of the K_L/K_H ratio, is not observed until 0.9 to 1.0 of gestation since it is only in the latter group that this ratio is higher than that of the pregnant animals at 0.3 and 0.7 of gestation. Interestingly, this ratio returns to the nonpregnant level in the immediate postpartum period.

This is the first report where the ability of guinea pig myometrial β-adrenergic receptor to form a "high-affinity" state with a β-adrenergic receptor agonist has been reported at different times during pregnancy and the puerperium. These observations correlate well with our previous results concerning the up-regulation of β-adrenergic receptor–dependent adenylate cyclase activity in pregnant guinea pigs at ≥0.9 of gestation.[3]

The cause of this increase in the K_L/K_H ratio is not clear. However, several hypotheses can be advanced. First, an interaction between locally produced or systemic sex steroid hormones, such as estradiol or progesterone, could influence the interaction between β-adrenergic receptor agonists and myometrial β-adrenergic receptor. Preliminary observations in hormone-treated rabbits,[7] however, did not show a significant effect. Moreover, hormonal treatment of oophorectomized guinea pigs with local 17β-estradiol or progesterone did not result in any significant increase in the K_L/K_H ratio.[8] Thus the role of a sex steroid–induced β-adrenergic receptor modulation at the level of the guanosine nucleotide regulatory protein/*l*-isoproterenol interaction remains to be defined. Second, during pregnancy, the concentration of guinea pig myometrial norepinephrine decreases to an almost nondetectable level.[9] This prejunctional reduction of norepinephrine content can theoretically result ina postjunctional up-regulation of myometrial β-adrenergic receptor function. Finally, interaction of myometrial β-adrenergic receptor with other hormones specific to pregnancy (such as estriol) may be involved. Further work will be necessary to study these hypotheses.

Teleologically, a greater ability to form the high-affinity state of the myometrial β-adrenergic receptor and, consequently, an ability to stimulate adenylate cyclase activity to a greater extent may be extremely important in terms of achieving myometrial relaxation. This can be particularly relevant during the latter part of gestation when maximal uterine relaxation is necessary to accommodate the ever-enlarging fetal-placental volume. Why this change is detected only in animals who are about to go into labor is not understood. This process, however, is reversible in that in the postpartum period the K_L/K_H ratio returns to levels comparable to those seen in the nonpregnant animals or those of pregnant animals at 0.3 and 0.7 of gestation. Further experiments will be necessary to elucidate the factors controlling this reversible modulation of the interaction between the myometrial β-adrenergic receptor and β-adrenergic receptor agonists.

REFERENCES

1. Birnbaumer L, Codina J, Mattera R, et al. Regulation of hormone receptors and adenylyl cyclases by guanine nucleotide binding N proteins. Recent Prog Horm Res 1986; 41:41-99.
2. Hatjis CG, Crews A. Regulation of guinea pig myometrial beta-adrenoreceptors by guanosine triphosphate and magnesium ion. Biol Reprod 1988;38:522-7.
3. Hatjis CG. β-Adrenergic–receptor and adenylate cyclase properties in pregnant and nonpregnant guinea pig myometrium. Am J Obstet Gynecol 1985;151:943-50.
4. Hatjis CG. Forskolin: unique diterpene activator of adenylate cyclase in pregnant and nonpregnant guinea pig myometrial membranes. Am J Obstet Gynecol 1986;155: 1202-8.
5. Munson PJ, Rodbard D. Ligand: a versatile computerized approach for characterization of ligand-binding systems. Anal Biochem 1980;107:220-39.
6. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1986;72: 248-54.
7. Riemer RK, Roberts JM. Endocrine modulation of myometrial response. In: Huszar G, ed. The physiology and biochemistry of the uterus in pregnancy and labor. Boca Raton, Florida: CRC Press, 1986:53-71.
8. Hatjis CG, Grogan DM, Koritnik DR. Treatment of oophorectomized guinea pigs with intrauterine 17β-estradiol pellets may modulate myometrial β-adrenergic receptor binding properties. Am J Obstet Gynecol 1989;161:1628-32.
9. Thorbert G. Regional changes in structure and function of adrenergic nerves in guinea-pig uterus during pregnancy. Acta Physiol Scand 1978;79(suppl):1-32.

Vasopressin receptors in human pregnant myometrium and decidua: Interactions with oxytocin and vasopressin agonists and antagonists

Marina Ivanisevic, MD,* Oliver Behrens, MD, Hanns Helmer, MD, Keith Demarest, PhD, and Anna-Riitta Fuchs, DSc

New York, New York, and Raritan, New Jersey

Saturation analysis and competition experiments were performed to identify and characterize [^{3}H]arginine vasopressin binding to human myometrium and decidua in late pregnancy. [^{3}H]Arginine vasopressin bound with affinity similar to that of [^{3}H]oxytocin to both tissues (dissociation constant 1 to 2 nmol/L). The concentration of [^{3}H]arginine vasopressin binding sites was high, particularly in decidua, but in all instances was about 50% to 60% of [^{3}H]oxytocin binding. Analogs with selective oxytocic potency (4-threonine oxytocin, isotocin) had high affinity to both [^{3}H]arginine vasopressin and [^{3}H]oxytocin binding sites, as did analogs with both oxytocic and vasopressor activity (vasotocin). Analogs with selective antidiuretic activity (1-deamino-8-D-arginine vasopressin) showed drastically reduced affinity to [^{3}H]oxytocin binding sites and relatively low but significantly higher affinity to [^{3}H]arginine vasopressin binding sites. A new oxytocin antagonist (RW22164 or [1-deamino-2D-tyrosine-(O-ethyl)-4-threonine-8-ornithine]oxytocin) competitively bound to both binding sites. Its affinity to [^{3}H]oxytocin binding sites was greater than to [^{3}H]arginine vasopressin binding sites whereas the relative affinities of a predominantly vasopressor antagonist (Manning compound) were reversed, suggesting the presence of distinct receptors for oxytocin and arginine vasopressin in pregnant human myometrium and decidua. (Am J Obstet Gynecol 1989;161: 1637-43.)

Key words: Arginine vasopressin, oxytocin, receptor, human pregnant myometrium, decidua, oxytocin and vasopressin antagonists

Vasopressin is structurally closely related to oxytocin, but its physiologic actions are different and it is considered to have different target organs. In standard pharmacologic tests with rat uterus, arginine vasopressin has <5% of the oxytocic activity of oxytocin. However, in women, vasopressin is a powerful uterine stimulant; in nonpregnant women its potency surpasses that of oxytocin.[1, 2] The effects of vasopressin on the pregnant human uterus are not as well known. Embrey and Chassar Moir[3] reported in 1967 that in midpregnancy, vasopressin and oxytocin were about equipotent in stimulating human uterine contractions, but as pregnancy progressed, the uterus became increasingly sensitive to oxytocin whereas the responsiveness to vasopressin remained relatively unchanged. Theobald,[4] on the other hand, reported that the term pregnant human uterus was about equally responsive to oxytocin and vasopressin (antidiuretic hormone), and he successfully induced labor with antidiuretic hormone with infusion rates of 2 mU/min. Vasopressin is secreted in large amounts by the human fetus during labor[5]; therefore vasopressin may be of physiologic importance not only for fetal hemodynamics[6] but also for the stimulation of uterine contractions.

Vasopressin has been shown to bind to the nonpregnant human uterus,[7] and studies by Guillon et al.[8] suggested that distinct receptors for oxytocin and vasopressin were present in nonpregnant myometrium. While the importance of oxytocin receptors for the regulation of uterine contractility is well established,[9] little is known about vasopressin receptors in the pregnant human uterus. Since the presence of receptors is a prerequisite for hormone action, it is important to identify uterine arginine vasopressin receptors, as well as oxytocin receptors, to understand the role of neurohypophysial hormones in the physiology of the pregnant human uterus. In this study we have investigated the binding of arginine vasopressin to the pregnant uterus and studied the distribution of binding sites between myometrium and decidua, since we have previously shown that decidua is a target organ for oxytocin and has a high concentration of oxytocin receptors.[9] A fur-

From the Department of Obstetrics and Gynecology, Cornell University Medical College, and the R. W. Johnson Pharmaceutical Research Institute at Ortho.

This study was supported by a grant from Warner Lambert Foundation and the R. W. Johnson Foundation for Pharmaceutical Research and by a grant from Deutsche Forschungsgemeinschaft.

Presented at the Thirty-eighth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.

Reprint requests: A-R. Fuchs, DSc, Department of Obstetrics and Gynecology, Room S-412, Cornell University Medical College, 515 E. 71st St., New York, NY 10021.

**Recipient of a Fulbright Scholarship.*

6/6/16512

Table I. Pharmacologic activity of agonists used

Compound	Pharmacologic activity* (IU/μmol)		
	Oxytocic (rat uterus isolated)	*Vasopressor (rat blood pressure)*	*Antidiuretic (rat antidiuretic)*
Oxytocin selective			
4-Threonine-oxytocin	166	<0.01	0.002
Isotocin	145	0.06	0.18
Oxytocin	450	5	5
Vasopressor and antidiuretic			
Arg-vasotocin	120	255	260
Arg-vasopressin	17	400	430
Antidiuretic selective			
1-Deamnio-8-D-Arg-vasopressin	2	0.4	1230

*Data from Berde B, ed. Handbook of Experimental Pharmacology. XXIII. Neurohypophysial Hormones and Similar Peptides. Berlin: Springer-Verlag, 1968:802. The third international standard for oxytocic, vasopressor, and antidiuretic substances was used in all instances (source: Medical and Biological Research Laboratories, Sandoz AG, Basel, Switzerland).

Table II. K_d values for oxytocin and arginine vasopressin receptors (nanomoles per liter)

	Oxytocin		*Vasopressin*	
	Myometrium	*Decidua*	*Myometrium*	*Decidua*
Human (pregnant, near term)	1.3 ± 0.17	1.8 ± 0.11	1.6 ± 0.05	2.2 ± 0.57
Rat (pregnant, term)	2.6 ± 0.40[a]	2.3 ± 0.42[a]	6.2 ± 1.2[b]	10.4 ± 2.3[b]

Values with different superscripts (*a* and *b*) differ significantly ($n = 3$ in all instances).

ther purpose was to examine whether a newly developed oxytocin antagonist (RW22164 or [1-deamino-2D-tyrosine-(O-ethyl)-4-threonine-8-ornithine]oxytocin) (dTVT),[10] which competitively blocks the binding of oxytocin to the pregnant human uterus,[11] also blocks the binding of arginine vasopressin. For comparison, the pregnant rat uterus also was studied for the presence of arginine vasopressin receptors.

Material and methods

Subjects. Pregnant human uterine tissues were obtained from three patients in whom hysterectomy was performed in the thirty-sixth to thirty-eighth week of pregnancy for medical reasons (cervical cancer in one and total placenta previa causing intractable postpartum bleeding in the other two). Immediately after surgery the uteri were placed on ice and cut open longitudinally; the decidua parietalis was cut off with curved scissors. Pieces of myometrium were taken from the fundus and corpus. All tissues were wrapped in foil and frozen in dry ice and then kept at −70° C until prepared for receptor assay. The pregnant rats (Sprague-Dawley) were from the R. W. Johnson Pharmaceutical Research Institute at Ortho, Raritan, N.J. The rats were killed on the afternoon of day 22, the time that parturition begins in this strain of rats. The uteri were slit open, and the decidua was scraped off and frozen separately from the myometrium, which was cut free of mesometrium. The uteri and decidua from six to eight rats were pooled to obtain sufficient quantities of membranes.

Preparation of membranes. The preparation of crude plasma membrane fractions and the receptor assays were performed as described previously[7, 9] with minor modifications. Briefly, the frozen tissues were pulverized and then homogenized with five pulses of 5 seconds' duration with a Polytron homogenizer (Brinkmann Instruments, Westbury, N.Y.) followed by six up-and-down strokes with a glass tissue grinder (Kontes Co., Vineland, N.J.). The homogenization buffer consisted of 0.01 mol/L *tris*(hydroxymethyl)aminomethane hydrochloride buffer, pH 7.4, containing 0.5 mmol/L dithiothreitol and 1.5 mmol/L ethylenediaminetetraacetic acid (EDTA). Dissociation of endogenous oxytocin and vasopressin from their binding sites with EDTA permits measurement of the total number of binding sites, both unoccupied and occupied. The fraction sedimenting between 20,000 and 165,000 *g* was used for the binding studies. Further purification with sucrose gradient centrifugation did not significantly increase the binding so this was omitted. The fraction was washed twice in the same buffer and then suspended in Krebs-Ringer solution and stored frozen at −70° C in suitable aliquots. Protein concentration was measured by the method of Lowry et al. with bovine serum albumin used as standard.

Binding assays. Before assay aliquots of mem-

brane preparations were thawed and recentrifuged at 165,000 *g;* the pellets were suspended in the assay buffer with a Teflon pestle in a glass homogenizer. The assay buffer consisted of 0.05 mol/L mono (*tris*[hydroxymethyl]aminomethane) maleate buffer, pH 7.6, containing 5 mmol/L magnesium chloride and 0.1% gelatin. The total volume was 220 μl and the protein concentration was 0.225 to 0.45 mg/ml for human tissues and 0.9 to 1 mg/ml for rat tissues. The concentrations were chosen to be in the range where specific binding increases linearly with the protein concentration. Incubations were carried out at 22° C for 60 minutes and terminated by adding 5 ml of ice-cold buffer. Separation of bound from free protein was by rapid filtration through Whatman GF/F glass-fiber filters with a Millipore manifold (Millipore Corp., Bedford, Mass.). The filters were washed with 10 ml of buffer and dried. The amount of radioactivity retained on the filters was counted with 5 ml of Ecoscint (National Diagnostics, Manville, N.J.) in plastic minivials in a Beckman LS 350 apparatus (Beckman Instruments, Schiller Park, Ill.) with 40% efficiency.

Saturation analysis was performed with eight or nine different concentrations of labeled ligand (0.10 to 14 nmol/L); nonspecific binding was determined by adding 1.1 μmol/L unlabeled ligand. Displacement and competition analyses were carried out with 0.6 to 0.9 nmol/L labeled ligand in each tube and increasing amounts of either the unlabeled ligand or another analog or antagonist in the range of 0.9 to 200 nmol/L. All binding studies were carried out in parallel assays with [^{3}H]arginine vasopressin and [^{3}H]oxytocin and with the same membrane preparations.

Labeled and unlabeled ligands and analogs. [^{3}H]Arginine vasopressin and [^{3}H]oxytocin were obtained from New England Nuclear (Boston). Both were of high purity (97% to 99.5%); the specific activities were 67 Ci/mmol for [^{3}H]arginine vasopressin and 39 to 57 Ci/mmol for [^{3}H]oxytocin.

Unlabelled arginine vasopressin and oxytocin were obtained from Bachem Fine Chemicals, Torrance, Calif. The analogs used were 4-threonine-oxytocin (provided by Dr. Maurice Manning, Department of Biochemistry, Medical College of Ohio, Toledo), isotocin and vasotocin (Bachem Biosciences, Philadelphia) and (1-deamino-8-D-arginine) vasopressin (Sigma Chemical Co., St. Louis). The pharmacologic potencies of the agonists are shown in Table I. The antagonists were (1-β-mercapto-β,-β,-cyclopenta-methylene-propionic acid-2-Tyr(O-methyl)-8-Arg) vasopressin (Manning compound, Bachem Fine Chemicals, Torrance, Calif.) and dTVT (RW22164), provided by Ortho Pharmaceutical Corporation, Raritan, N.J. Manning compound is considered a highly specific blocker of vasopressin receptors of the VP_1 type, being more than

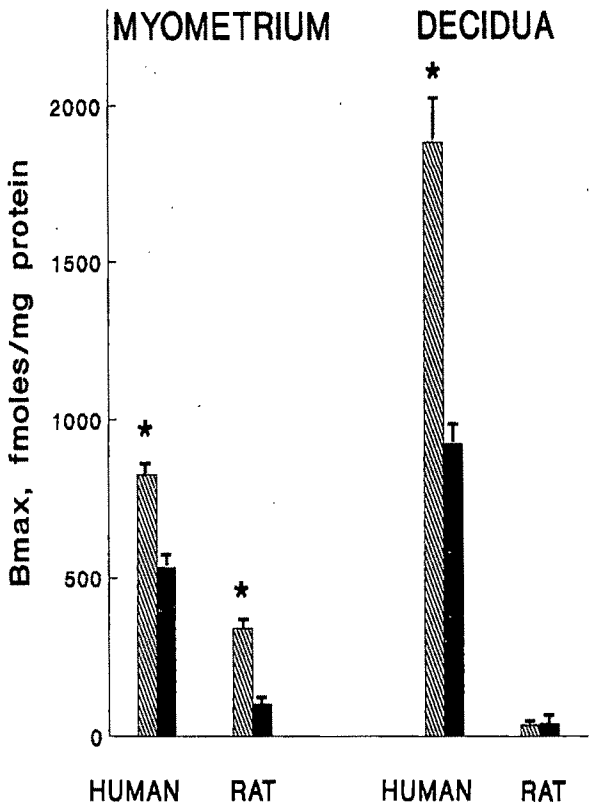


Fig. 1. Specific binding (B_{max}) of [^{3}H]oxytocin *(hatched columns)* and [^{3}H]arginine vasopressin *(solid columns)* to membrane preparations from three pregnant human uteri between 35 and 38 weeks of gestation and from pooled uteri of eight rats at term (day 22 of gestation). Saturation analyses were performed in duplicate with increasing concentrations of labeled ligands (0.1 to 14 nmol/L). Nonspecific binding was determined in the presence of 1.1 μmol/L unlabeled ligand. The concentration of protein was 0.25 to 0.45 mg/ml. The data were analyzed by an iterative nonlinear curve-fitting computer program to obtain the B_{max}, expressed as femtomoles bound per milligram of protein. *Asterisks* indicate significant differences between the two neuropeptides in each tissue. The B_{max} values for each neuropeptide were significantly higher in human than in rat uterine tissues. The B_{max} values were significantly higher in decidua than in myometrium for each neuropeptide in human uteri whereas the opposite was true in rats.

100 times more active in blocking vasopressor than oxytocic responses and having a weak antidiuretic activity.[12] dTVT is a highly specific oxytocin antagonist with a very weak diuretic activity and no vasopressor activity.[10] All peptides were stored as stock solutions in 0.25% acetic acid containing 0.1% gelatin and diluted with the assay buffer before each assay.

Data analysis and statistical analysis. Saturation analyses were performed in duplicate and the parameters presented (dissociation constant, K_d, and maximum binding, B_{max}) are means for two or three experiments. The binding data were analyzed by an iterative nonlinear curve-fitting program (Biosoft, Elsevier Sci-

Table III. Comparison of binding affinities of selected agonists to oxytocin and vasopressin receptors in pregnant human myometrium

Compound	K_i (nmol/L)	
	Oxytocin receptors	*Arg-vasopressin receptors*
4-Threonine-oxytocin	18.6 ± 1.96[a] (n = 3)	1.4 ± 0.33[b] (n = 2)
Isotocin	11.8 ± 1.21[a] (n = 3)	0.6 ± 0.3[b] (n = 2)
Oxytocin	1.3 ± 0.08[a] (n = 6)	0.6 ± 0.17[b] (n = 3)
Arg-vasotocin	2.3 ± 0.92 (n = 3)	0.7 ± 0.11 (n = 3)
Arg-vasopressin	9.0 ± 2.48 (n = 3)	3.8 ± 1.10 (n = 4)
1-Deamino-8-D-Arg-vasopressin	600.0 ± 44[a] (n = 3)	31.0 ± 6.0[b] (n = 2)

Values with different superscripts (*a* and *b*) for each analog differ significantly.

Table IV. Comparison of binding affinities of selected agonists to oxytocin and vasopressin receptors in pregnant human decidua

Compound	K_i (nmol/L)	
	Oxytocin receptors	*Arg-vasopressin receptors*
4-Threonine-oxytocin	13.7 (n = 1)	—
Isotocin	3.3 ± 0.80 (n = 3)	3.9 (n = 1)
Oxytocin	2.0 ± 0.40 (n = 5)	0.7 ± 0.14 (n = 2)
Arg-vasotocin	2.1 ± 0.98 (n = 3)	1.7 ± 0.40 (n = 2)
Arg-vasopressin	7.6 ± 1.27[a] (n = 3)	1.9 ± 0.87[b] (n = 3)

Values with different superscripts (*a* and *b*) for each analog differ significantly.

entific Publishers), which is an adaptation of the LIGAND program developed at the National Institutes of Health by Munson and Rodbard. Statistical analysis of the goodness of fit for one or two affinity binding sites is performed by the program, with the F statistic used to compare residual variances. The binding data from displacement and competition experiments were also analyzed by the program. The parameters presented are K_I (the dissociation constant calculated according to Cheung and Prusoff, with the Hill constant near unity) and B_{max}. The significance of differences between means was determined by Student's t test, and $p < 0.05$ was considered significant.

Results

All decidual and myometrial membrane preparations of the three pregnant human uteri bound [^{3}H]arginine vasopressin specifically. Like [^{3}H]oxytocin binding, the specific binding of [^{3}H]arginine vasopressin was time and temperature dependent, reaching equilibrium in about 45 minutes at 22° C (data not shown). Addition of EDTA at equilibrium resulted in an immediate dissociation of the specific bound [^{3}H]arginine vasopressin, like [^{3}H]oxytocin.

Saturation experiments. Analysis of the data obtained in saturation experiments indicated that only one saturable site of high affinity and low capacity for [^{3}H]arginine vasopressin binding was present in both the myometrium and the decidua of women in late pregnancy. The K_d for both decidual and myometrial membrane preparations was 1 to 2 nmol/L and similar to the K_d for [^{3}H]oxytocin binding, with no significant differences (Table II). The saturation binding data for [^{3}H]oxytocin also indicated the presence of a single saturable site in both decidua and myometrium, confirming previous observations.[9] The concentration of [^{3}H]arginine vasopressin binding sites, like [^{3}H]oxytocin binding sites, was significantly higher in decidual than in myometrial membrane preparations (Fig. 1). The concentration (B_{max}) for [^{3}H]arginine vasopressin in the myometrium was 533 ± 27 fmol/mg protein and the decidual concentration was 924 ± 55 fmol/mg; for [^{3}H]oxytocin the corresponding values were 826 ± 37 and 1885 ± 130 fmol/mg (values are mean ± SE, n = 3).

Rat uterine membranes showed significantly lower affinity for [^{3}H]arginine vasopressin than for [^{3}H]oxytocin; this applied to both the myometrial and decidual membrane preparations (Table II). The concentration of [^{3}H]arginine vasopressin binding sites was much lower in rat uteri than in human uteri, both in myometrium and especially in decidua (Fig. 1). Myometrial receptor concentrations were significantly lower for arginine vasopressin than for oxytocin in rat uteri at term (arginine vasopressin, 100 ± 15 fmol/mg; oxytocin, 340 ± 27 fmol/mg) whereas decidual receptor concentrations were similar (oxytocin, 40 ± 5 fmol/mg; arginine vasopressin, 49 ± 9 fmol/mg). All values are mean ± SE, n = 3 (Fig. 1).

Displacement and competition experiments. In human uterine membrane preparations, displacement of [^{3}H]arginine vasopressin and [^{3}H]oxytocin by the unlabeled peptides arginine vasopressin and oxytocin also indicated interaction with a single population of binding sites for both peptides (Fig. 2). Although a curvilinear Scatchard plot was sometimes obtained at high unlabeled ligand concentrations, LIGAND analysis of the binding data resulted in the identification of only one saturable site of high affinity for both neuropeptides. The B_{max} values obtained from displacement experiments were similar to those obtained from saturation experiments, with no statistical differences (arginine vasopressin in myometrium: K_i = 3.8 ± 1.1

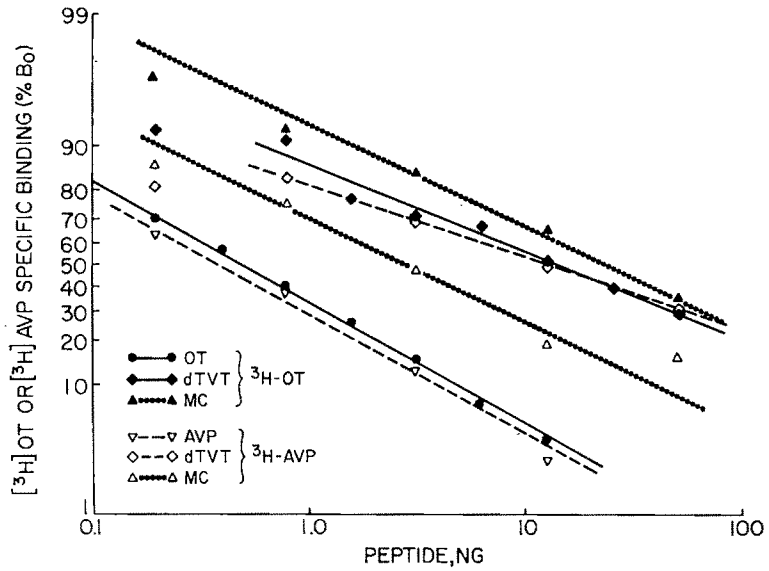


Fig. 2. Displacement and competition curves for [^{3}H]arginine vasopressin and [^{3}H]oxytocin by the oxytocin antagonist dTVT and the vasopressor antagonist Manning compound after logit-log transformation of the data. Experiments were performed in the presence of a constant amount of labeled ligand (0.6 to 0.8 nmol/L). The specific binding without added competitors is taken as 100% binding and the specific binding in the presence of increasing amounts of unlabeled ligand or analogs is expressed as a percent of this. Membranes were from myometrium of human pregnancy (0.45 μg per tube). Values are means of four to six experiments with each analog. For clarity the standard error bars are omitted; standard errors ranged from 1.2% to 14.3% (7.8% on the average).

nmol/L, B_{max} = 630 ± 55 fmol/mg; arginine vasopressin in decidua: K_i = 1.9 ± 0.4 nmol/L, B_{max} = 890 ± 75 fmol/mg; oxytocin in myometrium: K_i = 1.3 ± 0.2 nmol/L, B_{max} = 750 ± 50 fmol/mg; oxytocin in decidua: K_i = 2.0 ± 0.2 nmol/L, B_{max} = 1650 ± 180 fmol/mg).

Competition by oxytocin for [^{3}H]arginine vasopressin occurred with equal affinity as that for [^{3}H]oxytocin in both human myometrium (K_i = 0.6 nmol/L) and decidua (K_i = 0.7 nmol/L) while arginine vasopressin competed for [^{3}H]oxytocin with significantly lower affinity than that for [^{3}H]arginine vasopressin (K_i = 9.0 nmol/L for myometrium and K_i = 7.6 nmol/L for decidua, respectively) (Tables III and IV).

Competition for [^{3}H]arginine vasopressin and [^{3}H]oxytocin binding by selected analogs and antagonists with well-defined pharmacologic profiles showed many similarities between the two binding sites but also significant differences. The K_i values for the agonists are shown in Tables III and IV for human myometrium and decidua, respectively, and in Table V the corresponding values for the antagonists are shown. To facilitate comparison between the affinity profiles of oxytocin and arginine vasopressin binding sites in the two tissues, the relative affinities (K_i arginine vasopressin/K_i analog or K_i oxytocin/K_i analog) of all analogs are arranged in order of decreasing potency in Table VI, the analog with the greatest potency being listed as number 1.

Table V. Comparison of binding affinities of selected antagonists to oxytocin and arg-vasopressin receptors in pregnant human myometrium and decidua

Compound	*K_i (nmol/L)*	
	Oxytocin receptors	*Arg-vasopressin receptors*
Myometrium dTVT	37.0 ± 1.7[a] (n = 5)	18.7 ± 7.4[b] (n = 4)
Manning compound	68.4 ± 13.0[a] (n = 4)	9.9 ± 0.7[b] (n = 4)
Decidua dTVT	25.2 ± 6.1 (n = 4)	21.1 ± 8.6 (n = 3)
Manning compound	48.6 ± 12.1[a] (n = 2)	8.7 ± 4.6[b] (n = 4)

Values with different superscripts (*a* and *b*) for each analog differ significantly.

The affinity profiles of both binding sites were similar, but particularly striking was the great affinity of all analogs with high oxytocic potency to both binding sites; the affinity of isotocin and 4-threonine-oxytocin to myometrial [^{3}H]arginine vasopressin binding sites was even greater than to the [^{3}H]oxytocin binding sites. A difference between the two binding sites was noted in their affinities toward arginine vasopressin and (1-deamino-8-D-arginine) vasopressin, the analogs with the most selective antidiuretic activities; both had significantly greater affinity for the [^{3}H]arginine vaso-

Table VI. Order of relative affinities of selected agonists and antagonists to [^{3}H] arg-vasopressin and [^{3}H] oxytocin binding sites in myometrium and decidua of human pregnancy

Order of decreasing affinity	*Oxytocin*		*Vasopressin*	
	Myometrium	*Decidua*	*Myometrium*	*Decidua*
Agonists				
1	IT	AVT	OT	OT
2	AVT	OT	AVT	AVT
3	OT	AVP	IT	IT
4	4-Thr-OT	—	AVP	AVP
5	AVP	IT	4-Thr-OT	4-Thr-OT
6	DDAVP	—	DDAVP	—
Antagonists				
1	dTVT	dTVT	MC	MC
2	MC	MC	dTVT	dTVT

IT, Isotocin; *AVT*, Arg-vasotocin; *OT*, oxytocin; *AVP*, Arg-vasopressin; *4-Thr-OT*, 4-threonine-oxytocin; *DDAVP*, 1-deamino-8-D-Arg-vasopressin; *MC*, Manning compound.

pressin than for the [^{3}H]oxytocin binding site (Tables III and IV). Another significant difference was observed between the relative affinities of the two antagonists, the oxytocin antagonist, dTVT, and the predominantly vasopressor antagonist, MC, for the [^{3}H]arginine vasopressin and [^{3}H]oxytocin binding sites (Tables V and VI), which were reversed for the two binding sites. This was observed in both decidua and myometrium (see also Fig. 2). Decidual binding sites for the two peptides showed generally qualitatively similar affinities when compared with the myometrial binding sites.

There was no significant correlation between the K_i values for the [^{3}H]arginine vasopressin binding sites and the relative antidiuretic or vasopressor potencies of the analogs, as reported in the literature. The high affinity of arginine vasotocin to both [^{3}H]arginine vasopressin and [^{3}H]oxytocin binding sites is noteworthy.

Comment

The results of this study demonstrate the presence of saturable high-affinity binding sites for both arginine vasopressin and oxytocin in human myometrium in late pregnancy. This is in agreement with Theobald's findings[4] that at term, vasopressin is as effective as oxytocin in eliciting uterine contractions. The analysis of the binding data with the iterative nonlinear curve-fitting program indicated in both instances the presence of a single class of homogeneous receptors. It is not possible on the basis of our results to prove definitely that the receptors for arginine vasopressin and oxytocin are distinct. However, the differences in binding capacity in the face of similar affinities and the differences in the relative affinities of the oxytocin and vasopressor antagonists and certain agonists for the [^{3}H]arginine vasopressin and [^{3}H]oxytocin binding sites suggest that a distinct receptor population is present in human myometrium and decidua for each neurohypophysial peptide. Guillon et al.[5] arrived at the same conclusion with respect to nonpregnant human myometrium. In contrast to our results, they found vasopressin to bind to two classes of binding sites, one with high affinity (K_d 2.2 nmol/L) and low capacity (B_{max} about 200 fmol/mg protein) and another with low affinity (K_d 655 ± 209 nmol/L) and high capacity (B_{max} about 6000 fmol/mg). This may be due to the use of the less potent [^{3}H]lysine vasopressin instead of [^{3}H]arginine vasopressin or to differences between the nonpregnant and pregnant tissues.

In contrast to our findings in human myometrium of pregnancy, the concentration of vasopressin binding sites in nonpregnant myometrium was considerably higher than that of oxytocin binding sites,[8] in harmony with the known effects of the two neuropeptides on uterine activity.[1-3] The arginine vasopressin binding sites in nonpregnant human myometrium differed from those in the pregnant human uterus also with respect to the relative affinities of various agonists. In nonpregnant myometrium the relative affinities of the analogs correlated well with their vasopressor activity,[8] whereas no significant correlation was found in pregnant myometrium or decidua. Thus the dynamic changes occurring during pregnancy in the human uterus in regard to neurohypophysial hormone binding are not only quantitative but also qualitative.

The much lower concentrations and lower affinity of arginine vasopressin binding sites in the pregnant rat myometrium in comparison to human myometrium is in keeping with the pharmacologic potency of arginine vasopressin measured in the uterus of this species.

The high concentration of both oxytocin and arginine vasopressin receptors in the human decidua is quite remarkable, and this tissue must therefore be considered a target organ for both neurohypophysial hormones. The function of oxytocin receptors in endometrium and decidua is believed to be stimulation of

arachidonic acid metabolism,[13, 14] probably by stimulating phosphoinositol turnover and phospholipase C.[15, 16] The cellular responses elicited by arginine vasopressin receptor occupancy in decidua are unknown at this time. Since one of the renal actions of arginine vasopressin is stimulation of prostaglandin E_2 synthesis, arginine vasopressin may have the same function in decidua, as also suggested by Schrey et al.[15]

The relative affinities of the various agonists to the arginine vasopressin binding sites and the lack of effect of arginine vasopressin on uterine cyclic adenosine monophosphate levels indicate that the uterine receptor is different from the adenyl cyclase–associated VP_2-subtype receptor that mediates the antidiuretic action of arginine vasopressin. On the basis of the order of potency of the oxytocic, vasopressor, and antidiuretic analogs in competing for the uterine [^{3}H]arginine vasopressin binding sites, they also differ from the VP_1-subtype receptor found in vascular smooth muscle and hepatocytes. The uterine vasopressin receptors may therefore represent a new subtype or perhaps a vasotocin receptor.

Of great clinical interest is the fact that the putative oxytocin antagonist competed with about equal affinity with both oxytocin and arginine vasopressin binding sites in myometrium and decidua. An oxytocin antagonist has pharmacologically minimal side effects and offers great promise as a new tocolytic agent, as suggested by preliminary clinical trials.[17]

REFERENCES

1. Coutinho EM, Lopes ACV. Response of the nonpregnant uterus to vasopressin as an index of ovarian function. Am J Obstet Gynecol 1968;102:479.
2. Coutinho EM, Filho AJ, Xavier R, Fuchs A-R, Fuchs F. Effect of ethanol on the response of the non-pregnant human uterus to oxytocin and vasopressin. J Obstet Gynaecol Br Commonw 1970;77:164.
3. Embrey MP, Chassar Moir J. A comparison of the oxytocic effects of synthetic vasopressin and oxytocin. J Obstet Gynaecol Br Commonw 1967;74:648.
4. Theobald GW. The synthesis of divergent observations concerning oxytocin. In: Caldeyro-Barcia R, Heller H, eds. Oxytocin. Oxford: Pergamon Press, 1961:212-28.
5. Chard T, Boyd NRH, Edwards CRW, Hudson CN. Release of oxytocin and vasopressin by the human foetus during labour. Nature 1971;223:352.
6. Pohjavuori M, Fyhrqvist F. Vasopressin, ACTH and neonatal haemodynamics. Acta Paediatr Scand 1983;85 (suppl):79.
7. Fuchs AR, Fuchs F, Husslein P, Soloff MS. Oxytocin receptors in nonpregnant human uterus. J Clin Endocrinol Metab 1985;60:37.
8. Guillon G, Balestre MN, Roberts JM, Bottari SP. Oxytocin and vasopressin: distinct receptors in myometrium. J Clin Endocrinol Metab 1987;64:1129.
9. Fuchs AR, Fuchs F, Husslein P, Soloff MS. Oxytocin receptors in pregnant human uterus. Am J Obstet Gynecol 1984;150:734.
10. Hahn DW, Demarest KT, Ericson E, Homin RE, Capetola RJ, McGuire JL. Evaluation of 1-deamino-[D-Tyr (O-ethyl)^2,Thr^4,Orn^8] vasotocin, an oxytocin antagonist, in animal models of uterine contractility and preterm labor: a new tocolytic agent. Am J Obstet Gynecol 1987;157: 977.
11. Fuchs AR, Vangsted A, Ivanisevic M, Demarest K. Oxytocin antagonist (dTVT) and oxytocin receptors in myometrium and decidua. Am J Perinatol 1989;6:205.
12. Manning M, Sawyer WH. Design and uses of selective agonistic and antagonistic analogs of the neuropeptides oxytocin and vasopressin. Trends Neurosci 1984;7:6.
13. Roberts JS, McCracken JA, Gavagan JE, Soloff MS. Oxytocin stimulated release of prostaglandin $F_{2\alpha}$ from ovine endometrium in vitro: correlation with estrous cycle and oxytocin receptor binding. Endocrinology 1976;99:1107.
14. Fuchs AR, Husslein P, Fuchs F. Oxytocin and the initiation of human parturition. II. Stimulation of prostaglandin production in human decidua by oxytocin. Am J Obstet Gynecol 1981;141:694.
15. Schrey MP, Read AM, Steer PJ. Oxytocin and vasopressin stimulate inositol phosphate production in human gestational myometrial and decidual cells. Biosci Rep 1986;6: 613.
16. Flint APF, Leat WMF, Sheldrick EL, Stewart HJ. Stimulation of phosphoinositide hydrolysis by oxytocin and the mechanism by which oxytocin controls prostaglandin synthesis in the ovine endometrium. Biochem J 1986;237: 797.
17. Andersen LF, Lyndrup J, Akerlund M, Melin P. Oxytocin receptor blockade: a new principle in the treatment of preterm labor. Am J Perinatol 1989;6:196.

The antagonistic effect of oxytocin and relaxin on rat uterine segment contractility

Laura T. Goldsmith, PhD, Joan H. Skurnick, PhD,[a] Andrea S. Wojtczuk, MS, Martin Linden, PhD,[b] Mary Jeanne Kuhar, MD, and Gerson Weiss, MD

Newark, New Jersey

To test the interaction of various hormones on the myometrium, the following experiments were done studying in vitro contractile activity of uterine segments from immature rats. The rats were divided into two groups: group 1 animals were treated with estrogen (n = 9) and group 2 animals were treated with both estrogen and progesterone (n = 11). Uteri from animals in each group were removed and segments were maintained in a temperature- and pH-controlled organ bath. After baseline contractions were established, uterine segments were treated with either oxytocin and then relaxin, or relaxin and then oxytocin. The dose of relaxin used, 20 ng/ml, was previously shown to be effective in inhibiting uterine contractions of animals treated with either estrogen or estrogen plus progesterone. The dose of oxytocin, 2.5 mIU/ml, was the maximal effective dose shown not to produce prolonged tetany. Estrogen plus progesterone treatment increased the frequency of contractions and resulted in contractions of greater duration of the maximal contractile force, as compared with treatment with estrogen alone. Oxytocin caused a stimulation of contractions in relaxin-inhibited uterine strips. Relaxin decreased the hypertonic contractions produced by oxytocin treatment, resulting in contractions similar to baseline. These data demonstrate that oxytocin and relaxin are directly antagonistic in their effects on uterine contractility. This suggests that labor may occur as a result of increased sensitivity to oxytocin or a decreased sensitivity to relaxin. (AM J OBSTET GYNECOL 1989;161:1644-9.)

Key words: Relaxin, oxytocin, myometrium, uterine contractions, labor

The inhibition of uterine activity during pregnancy and the initiation of labor depend, in part, on the interaction of various hormones on the myometrium.[1] Uterine activity is the sum of the dynamic balance between inhibitory and stimulatory factors. Labor occurs when the stimulatory factors become dominant.[2, 3]

The purpose of this study was to determine the interaction of an inhibitory agent (relaxin) and a stimulatory agent (oxytocin) on uterine contractions, and to determine whether progesterone modifies this interaction. The model system used was selected to avoid the interaction of other steroid or peptide factors that have uterine contractile activity.

Material and methods

The methods used in these studies were similar to those previously used to examine the effect of relaxin on rat uterine contractility in vitro.[4-7] Care and use of the experimental animals followed guidelines approved by the American Association for Accreditation of Laboratory Animal Care–accredited animal care facility at New Jersey Medical School. Immature female Long Evans rats, 33 to 36 days old, were given daily subcutaneous injections of 10.0 μg of estradiol benzoate (Sigma Chemical Co., St. Louis, Mo.) in sesame oil for 3 consecutive days. Exposure to estrogen, which increases the negativity of the cell resting potential and enhances the conduction of excitation throughout the muscle, is required for spontaneous uterine contractile activity. On the last 2 days of treatment with estradiol benzoate, one group of animals (n = 11) received 2 mg of progesterone (Sigma Chemical Co., St. Louis, Mo.) in sesame oil and another group of rats (n = 9) received only vehicle. On the fourth day, the animals were killed by cervical dislocation and the uteri were isolated, removed, and dissected free of extrauterine tissue. The cervical halves of the uterine horns were tied at each end with cotton threads, suspended in a double-jacketed organ bath, and immersed in 10 ml of Ringer-Locke solution (154 mM sodium chloride, 5.6 mM potassium chloride, 2.8 mM calcium chloride, 6.0 mM sodium bicarbonate, and 5.6 mM dextrose), pH 7.4, preheated to 37° C. One end of the uterine horn segment was attached to a hook at the bottom of the bath, and the other was attached to an isometric smooth muscle transducer of a Grass 7D polygraph (Grass Instrument Co., Quincy, Mass.). An initial tension of 1 gm was applied to the muscle.

A mixture of 95% oxygen and 5% carbon dioxide

From the Departments of Obstetrics and Gynecology and Preventive Medicine and Community Health,[a] University of Medicine and Dentistry of New Jersey–New Jersey Medical School, and New Jersey Institute of Technology.[b]

Supported by National Institutes of Health Grant No. HD 22338.

Presented at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.

Reprint requests: Dr. Gerson Weiss, Department of Obstetrics and Gynecology, New Jersey Medical School, 185 South Orange Ave., Newark, NJ 07103.

6/6/15969

was then continuously bubbled through the Ringer-Locke solution. After the uterine segments equilibrated, they were allowed to establish baseline contractions for 15 minutes. The chart speed was set at 50 mm/min. This rapid paper speed was used to allow observation and quantitation of alterations in waveform including duration of peak force, cycle length, and area under the contraction curve in addition to evaluation of amplitude. In the few cases in which segments did not contract at a frequency of once per minute, they were stimulated electrically with 5 V for 10 seconds every minute at 60 Hz, with the use of a direct-current stimulator. This was accomplished by placing one electrode in the bath with the other electrode touching the muscle at its fixed end. In those cases in which electrically stimulated cycles were preceded by intrinsic cycles, waveforms of the stimulated cycles closely resembled intrinsic waveforms.

Treatments were applied as described in Table I. To clearly delineate the interaction of relaxin and oxytocin, two sets of in vitro experiments were performed for both groups of animals: (1) those in which uterine segments were given relaxin first and then oxytocin, and (2) those in which uterine segments were given oxytocin and then relaxin. The dose of porcine relaxin (purified porcine relaxin CMa', 3000 guinea pig units/mg, kindly provided by Dr. Bernard G. Steinetz) used (20 ng/ml) was previously determined to inhibit contractions in uterine segments treated with estrogen and in those treated with estrogen plus progesterone.[5] The dose of oxytocin (Pitocin, synthetic oxytocin, 99.5% purity, Parke-Davis, Morris Plains, N.J.) used (2.5 mIU/ml) had been shown to be the maximal effective dose that did not produce prolonged tetany.

Several parameters of the initial baseline contraction pattern including cycle length, duration of peak force, amplitude, and frequency of contractions of uterine segments from animals treated with estrogen ($n = 9$) versus estrogen plus progesterone ($n = 11$) were evaluated and compared. The cycle length is defined as the time from the onset of one contraction to the onset of the next contraction. The duration of peak force is defined as the time from the onset of the contraction to the onset of the relaxation phase. The contractions recorded in a 15-minute period (generally eight contraction cycles) during baseline and hormone-treated contractions were analyzed. Statistical analysis of mean cycle length, frequency, and duration of peak force of these contractions was done with the use of the Student t test for a small sample size.[8]

Area under the curves of the initial baseline contractions and relaxin-treated and oxytocin-treated contractions were also assessed. These measurements were determined by the use of a MOP-3 morphometric image analyzer (Carl Zeiss Inc., Thornwood, N.Y.). Each contraction was traced with a magnetic optical pen;

Table I. Experimental paradigm

	In vitro	
	First agent added	*Second agent added*
Estrogen pretreatment in vivo	Relaxin	Oxytocin
	Oxytocin	Relaxin
Estrogen and progesterone pretreatment in vivo	Relaxin	Oxytocin
	Oxytocin	Relaxin

Table II. Effect of progesterone on contractions of estrogen-treated uteri

	Estrogen only ($n = 9$)	*Estrogen and progesterone ($n = 11$)*
Mean cycle length (min/cycle)	1.68	1.26*
Frequency (cycles/min)	0.59	0.79*
Duration of peak force (min)	0.30	0.44*

*$p < 0.05$.

duplicate tracings were performed for each contraction to ensure reproducibility. Reproducibility in area under the curve measurements with this method was 99%. The contractions recorded in a 15-minute period (generally eight contraction cycles) during baseline and hormone-treated contractions were traced. The data were analyzed by paired Student t test and repeated measures analysis of variance.[8] For all statistical analyses used in these studies, 0.05 was taken as the criterion of statistical significance. Numbers of experiments performed were chosen to ensure an adequate statistical evaluation.

Results

Exposure to progesterone in vivo resulted in a decrease in the contraction cycle length, an increase in the frequency of contractions, and an increase in the duration of the peak force of contractions of rat uterine segments in vitro, as shown in Table II. The mean cycle length of the initial baseline contractions of uterine segments from rats treated with both estrogen and progesterone, 1.26 min/cycle ($n = 11$) was significantly shorter ($p < 0.05$) than the mean cycle length of the initial baseline contractions of uterine segments of rats treated with estrogen and vehicle, 1.68 min/cycle ($n = 9$). The frequency of contractions, the reciprocal of the mean cycle length, was significantly greater in uterine segments of animals treated with both estrogen and progesterone (0.79 cycles/min) than the frequency of contractions of uterine segments of animals treated with estrogen and vehicle (0.59 cycles/min).

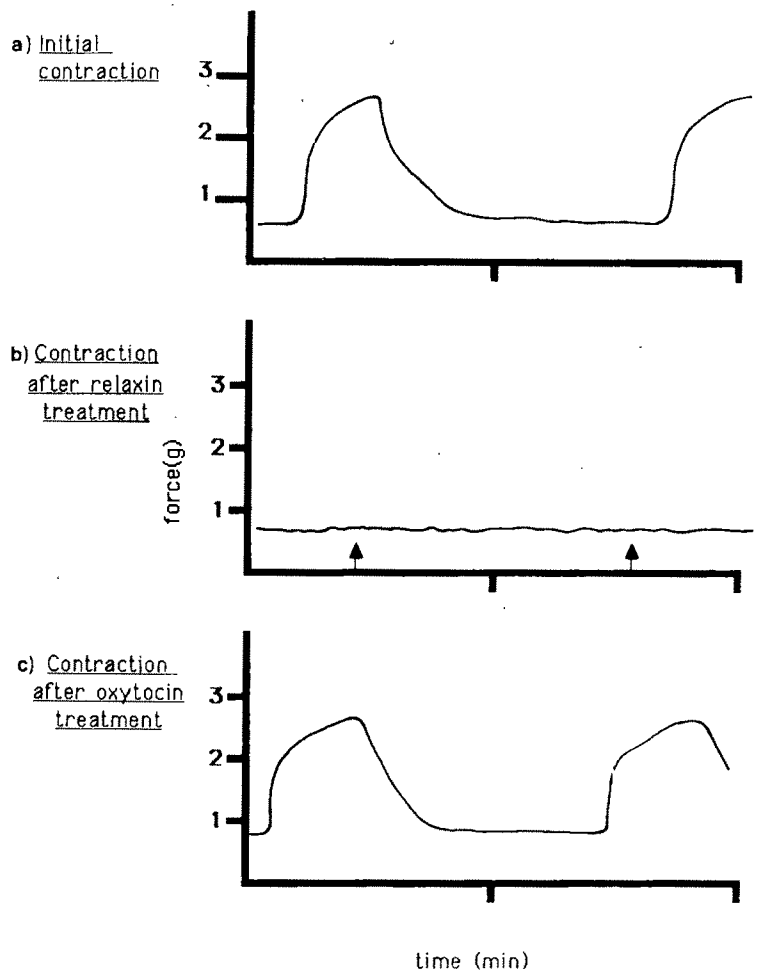


Fig. 1. Contractions of uterine segment from estrogen-treated rat. Panel **a** denotes initial baseline contraction; panel **b** denotes contraction pattern after addition of relaxin to baseline contraction; panel **c** denotes contraction pattern after addition of oxytocin to relaxin-treated tissue. *Vertical arrow* indicates electrical stimulation. Each subdivision on X axis (time) represents 1 minute.

Although contraction amplitude was similar in both groups, the duration of the peak force of the initial baseline contractions of uterine segments from rats treated with estrogen plus progesterone (0.44 min) was significantly longer ($p < 0.05$) than that of uterine segments of rats treated only with estrogen (0.30 min).

In addition, treatment with progesterone altered the shape of the contraction curve. A typical contraction curve of the estrogen-only group can be seen in Fig. 1, *a*. A typical curve of the group with estrogen plus progesterone can be seen in Fig. 2, *a*. Although differences in mean area under the curves were observed for baseline contractions of uterine segments from the two groups of rats, these differences did not achieve statistical significance (364 mm^2 for estrogen, $n = 7$; 541 mm^2 for estrogen plus progesterone, $n = 11, p = 0.16$).

In all uterine segments studied, those from both estrogen-treated and estrogen plus progesterone–treated rats, relaxin markedly decreased the amplitude or totally eliminated uterine contractions. As was expected from these results, a statistically significant decrease in mean area under the curves of contractions of relaxin-treated segments, as compared with baseline initial contractions, was shown (36 mm^2, $n = 4$, $p = 0.009$ for estrogen and 214 mm^2, $n = 4, p = 0.018$ for estrogen plus progesterone). In those cases in which relaxin totally eliminated contractions, electrical stimulation was employed but was unable to produce contractions in these relaxin-treated uterine segments (Fig. 1, *b*). Oxytocin restored contractions in relaxin-treated uterine segments, both in segments from rats treated with estrogen and rats treated with estrogen plus progesterone (Fig. 1, *c*). Oxytocin significantly increased mean area under the curve measurements of contractions of relaxin-treated segments to 394 mm^2, $n = 4$ ($p = 0.012$) for estrogen-treated

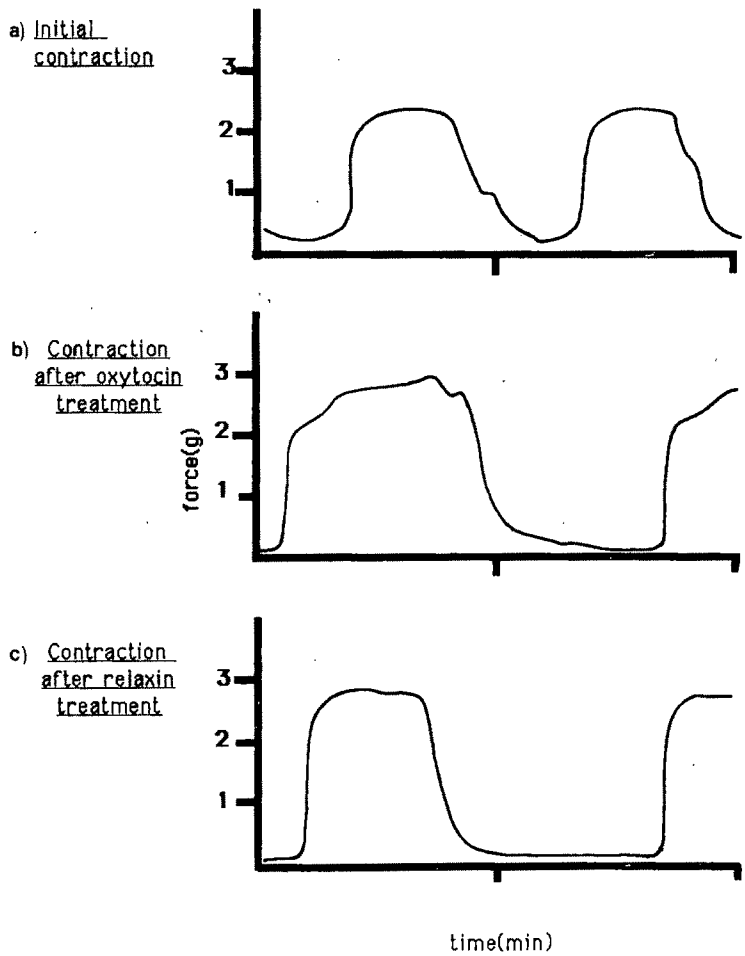


Fig. 2. Contractions of uterine segment from estrogen plus progesterone–treated rat. Panel **a** denotes initial baseline contraction; panel **b** denotes contraction pattern after addition of oxytocin to baseline contraction; panel **c** denotes contraction pattern after addition of relaxin to oxytocin-stimulated contraction. Each subdivision on X axis (time) represents 1 minute.

rats and to 474 mm², $n = 4$ ($p = 0.007$) for estrogen plus progesterone–treated rats. These areas were similar to that of baseline contractions.

In segments from estrogen-treated and estrogen plus progesterone–treated rats, oxytocin stimulated the force of contractions, as compared with initial baseline contractions. With prior exposure of the muscle segment to progesterone, oxytocin was more likely to produce a prolonged or tetanic contraction. Mean area under the curve measurements of contractions of uterine segments from estrogen-treated rats treated with oxytocin were increased above that of baseline to 495 mm², $n = 5$; yet this was not statistically significant ($p = 0.076$). Exposure to progesterone resulted in significantly increased mean area under the curve measurements of 1133 mm², $n = 7$ ($p = 0.015$). Whereas oxytocin stimulated all muscle segments, progesterone may have increased uterine sensitivity to oxytocin (Fig. 2, *b*). In segments from rats treated with estrogen and rats treated with estrogen plus progesterone, relaxin decreased the hypertonic contractions produced by oxytocin. Relaxin added to oxytocin-treated segments resulted in contractions similar to baseline (Fig. 2, *c*). Relaxin decreased mean area under the curve measurements in oxytocin-treated uterine segments from estrogen-treated animals to 299 mm², $n = 5$ ($p = 0.031$) and in those from animals treated with estrogen plus progesterone to 840 mm², $n = 7$ ($p = 0.056$).

Comment

The control of uterine activity is complex and involves a variety of hormonal and nonhormonal factors. The ultimate understanding of how the uterus acts during any physiologic state depends on an understanding of the effect of these various agents as individual substances and how uterine activity may be affected by these substances when they act together. A uterine tissue system that has not previously been exposed to any known or potential stimulatory or inhib-

itory hormones can be used to study the effects of various substances on uterine activity and generate clean and uncompromised results. The model used in this study is a simple and easily reproducible model to study the actions and interaction of various hormones on uterine activity without influence of unaccountable substances. It is not, however, a model of hormone action on human or pregnancy uterus, but rather a model of hormone action on a previously nonstimulated virgin uterus. The immature rat, not having been exposed to estrogen, has a nonreactive uterus. This is different from the uterus of mature ovariectomized animals, which is contractile and has been exposed to various hormonal and nonhormonal factors that can affect and bias results. The doses of hormones used in this study were chosen to give sensitive responses when used individually. Each dose was chosen separately on the basis of detailed experimental dose-response curves performed with this system before the experiments presented here. Electrical stimulation was used to dependably ensure contractions. The failure of this stimulus to cause contractions in the presence of relaxin demonstrates that relaxin suppresses conduction.

The use of rapid paper speed allows observation and evaluation of various parameters of the contraction patterns including alterations in waveform, cycle length, and duration of peak force of contractions not detected when a slower paper speed is used. A slower paper speed, which is most generally used in studies of uterine contractility, allows only observation of contraction amplitude. Thus this study is a more sensitive look at the alterations in contractions that occur under the influence of these hormones than that of looking at amplitude alone. We were able to demonstrate the effect of progesterone on these parameters, and can conclude that, in this system, progesterone increases the frequency and duration of peak force of uterine contractions. Studies of progesterone effects on contraction waveform and duration of peak force have not been previously done. Previous studies have demonstrated that progesterone synergizes with relaxin to decrease the amplitude of contractions.[4, 5] Further studies are needed to determine the interaction of relaxin and progesterone on the additional parameters studied here.

Our current observations demonstrate that a combination of progesterone and estrogen resulted in more prolonged contractions than those of estrogen alone in immature rat uterine segments, on the basis of duration of peak force. Progesterone also increased contraction frequency. In some experimental systems, progesterone has been shown to inhibit uterine activity.[9] However, progesterone is not a tocolytic agent[10]; it cannot be used to stop labor. The effect of progesterone in an experimental system may depend on conditions and dosage. This is also true of other steroid hormones; estrogen stimulates uterine contractions in immature rats but suppresses contractions in ovariectomized animals.[11] Use of the system described here was an attempt to eliminate nonspecific conditional and biologic effects and test the substances in question in as clean a system as possible. Progesterone has been shown to block estrogen stimulation of myometrial oxytocin receptors.[12] This effect may not have occurred in this system because the effect of oxytocin was not decreased by treatment of the rats with progesterone; rather, the stimulation of contractility by oxytocin appeared to be magnified by progesterone. Again, this difference may be due to conditions and dosage effects. Alternatively, it is possible that other mechanisms are responsible for the results presented here, mechanisms with workings that are not related to the concentration of the receptor. The results of the studies presented here clearly demonstrate that relaxin and oxytocin have antagonistic effects. These results should clarify some ambiguities in the literature regarding this issue. For example, Porter[10] has presented data that appear to demonstrate that relaxin inhibits the frequency of oxytocin-stimulated uterine contractions in the ovariectomized rat, yet the author states that relaxin had no effect. That relaxin and oxytocin have antagonistic effects suggests that labor can, in part, result from a probable increased sensitivity to oxytocin or a decreased sensitivity to relaxin. Additional studies with this model system can determine the effects of a combination of agents on uterine contractility with additional parameters of contractility able to be assessed. This information may provide insight beyond that provided for by studies of single agents on uterine contractility as is prevalent in the current literature.

REFERENCES

1. Liggins GC. The paracrine system controlling human parturition. In: Jaffe RB, Dell Acqua S, eds. The endocrine physiology of pregnancy and the peripartal period. New York: Raven Press, 1985 vol 21:205-22.
2. Garfield RE. Control of myometrial function in preterm versus term labor. Clin Obstet Gynecol 1984;27:572-91.
3. Haszar G, Roberts JM. Biochemistry and pharmacology of the myometrium and labor: regulation at the cellular and molecular levels. AM J OBSTET GYNECOL 1982; 142:225-37.
4. Sarosi P, Schmidt C, Essig M, Steinetz BG, Weiss G. The effect of relaxin and progesterone on rat uterine contractions. AM J OBSTET GYNECOL 1983;145:402-5.
5. Brenner SH, Lessing JB, Weiss G. The effect of in vivo progesterone administration on relaxin-inhibited rat uterus contractions. AM J OBSTET GYNECOL 1984;148: 946-50.
6. Lessing JB, Brenner SH, Weiss G. Effect of prolactin and relaxin on in vitro rat uterine contractions and prolactin interaction with relaxin. Obstet Gynecol 1984;64:97-100.
7. Ginsburg FW, Rosenberg CR, Schwartz M, Colon JM, Goldsmith LT. The effect of relaxin on calcium fluxes in the rat uterus. AM J OBSTET GYNECOL 1988;159:1395-401.

8. Winer BJ. Statistical principles in experimental design. 2nd ed. New York: McGraw-Hill, 1971.
9. Saldivar JT Jr., Melton CE Jr. Effects in vivo and in vitro of sex steroids on rat myometrium. Am J Physiol 1966; 111:835-43.
10. Porter DG. The myometrium and the relaxin enigma. Anim Reprod Sci 1979;2:77-96.
11. Downing SJ, Lye SJ, Bradshaw JMC, Porter DG. Rat myometrial activity in vivo: effects of estradiol-17 β and progesterone in relation to the concentration of cytoplasmic progesterone receptors. J Endocrinol 1978;78:103-17.
12. Fuchs A-R, Periyasamy S, Alexandrova M, Soloff MS. Correlation between oxytocin receptor concentration and responsiveness to oxytocin in pregnant rat myometrium: effects of ovarian steroids. Endocrinology 1983;113: 742-9.

Decreased β-carotene tissue levels in uterine leiomyomas and cancers of reproductive and nonreproductive organs

Prabhudas R. Palan, PhD, Magdy Mikhail, MD, and Seymour L. Romney, MD
Bronx, New York

The dietary importance of β-carotene as a factor in health maintenance has recently attracted considerable interest. Previously, total carotene content was estimated in a limited number of human tissues by means of spectrophotometric methods. In this study the levels of β-carotene were measured by high-pressure liquid chromatography in tissue samples of uterine leiomyomas and adjacent normal myometrium obtained at hysterectomy from uteri of 18 patients. β-Carotene concentration was significantly ($p = 0.0013$) lower in fibroid tissue than in normal myometrium. In addition, levels of β-carotene were assayed in tissue samples of cancers of the cervix, endometrium, ovary, breast, colon, lung, liver, and rectum and were compared with levels of respective adjacent normal sites. The concentrations of β-carotene were found to be lower in all cancer tissues. The decreased levels of β-carotene suggest that β-carotene deficiency may have a role in the cause and/or pathogenesis of leiomyomas and cancers of the organs that were investigated. The mechanism of action, however, remains unknown. (Am J Obstet Gynecol 1989;161:1649-52.)

Key words: β-Carotene, human tissue, leiomyoma, cancer

Increased attention is being focused on the role of essential micronutrients in the cause and possibly prevention of human cancer, as well as in the maintenance of health. The biologic functions of β-carotene (provitamin A) and vitamin A are currently of considerable interest.[1, 2] Numerous epidemiologic studies, linking diet and nutrition to cancer incidence and mortality, suggest a possible antitumor property of β-carotene, independent of its provitamin A activity.[3, 4] The antitumor implication is largely based on studies investigating the relationship between serum β-carotene levels and the incidence of specific cancers.[5-7]

From the Department of Obstetrics and Gynecology, Albert Einstein College of Medicine.
Supported in part by the Friends of Gynecologic Cancer Research of the Albert Einstein College of Medicine and by a Grant-in-Aid from Hoffmann-La Roche Inc.
Presented in part at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.
Reprint requests: Prabhudas R. Palan, PhD, Department of Obstetrics and Gynecology, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY 10461.
6/6/16913

A question of mechanistic interest is whether β-carotene exerts an independent influence through direct interactions at specific target tissues. In animal systems, dietary β-carotene supplementation exerts a preventive action on the spontaneous incidence of experimental tumors.[8, 9] Suda et al.[10] demonstrated that β-carotene, when applied topically, significantly inhibits oral carcinogenesis in an initiation-promotion hamster buccal pouch system. Tissue β-carotene levels in cancers of the uterine cervix, endometrium, ovary, and breast in humans have not been previously reported. We have investigated the tissue levels of β-carotene in coded tissue samples obtained from surgically excised specimens of each of these organ sites. In addition, the levels of tissue β-carotene in a spectrum of commonly encountered carcinomas of the colon, lung, liver, and rectum also were determined.

Material and methods

Collection of tissue samples. The study protocol was approved by the institutional review board. Staff pathologists of the Weiler Hospital of the Albert Einstein College of Medicine provided coded (a) normal, (b)

Table I. β-Carotene concentrations in uterine leiomyomas and normal myometrium from the same uteri

Subject	Age (yr)	β-Carotene (ng/gm tissue)	
		Normal myometrium (n = 18)	Leiomyoma (n = 8)
A. G.	48	39.1	ND*
A. S.	45	17.5	7.6
F. M.	47	105.0	23.0
C. F.	38	57.4	ND
J. A.	53	35.6	50.0
F. Z.	48	37.0	ND
H. J.	44	85.0	ND
J. M.	25	64.0	ND
C. C.	37	171.0	ND
S. F.	39	52.3	22.6
F. S.	60	45.0	ND
E. R.	49	44.0	38.0
P. O.	47	106.0	31.0
W. H.	45	43.0	ND
M. C.	49	121.3	ND
O. B.	44	25.0	ND
E. S.	48	16.8	34.4
J. S.	60	86.0	76.0
Mean ± SD		64.0 ± 11.0	35.3 ± 20.6

*ND, Not detectable by high-pressure liquid chromatography method.

benign tumor, and (c) malignant tumor tissue samples within 2 hours of surgical excision of the involved organs and after removal of appropriate tissue specimens for pathologic assessment. Normal myometrium and fibroid tissues were obtained at hysterectomy from the same uteri of patients treated for benign gynecologic conditions. Tissue samples of various carcinomas, i.e., uterine cervix, endometrium, ovary, breast, lung, liver, colon, and rectum, were submitted with corresponding adjacent normal, noncancerous tissues obtained from the same patients. Before surgery, no special dietary restrictions were imposed on any of the patients.

Each tissue section of the surgical specimen, corresponding to the tissue samples released for biochemical analysis, was marked with india ink and processed for histologic-histopathologic interpretation by staff pathologists. Thus each subject served as her own control. All the β-carotene analyses were carried out without any knowledge of the source of the sample or the patient's clinical status.

Tissue extraction. Under the assigned code numbers, tumor and normal tissue specimens were collected immediately on surgical removal, transported to the laboratory in ice-cold 0.05 mol/L Tris buffer, pH 7.2, and stored in dark containers at −80° C until analyzed. Handling, processing, and assaying of all materials were done under subdued or gold light.

Samples of tissue (0.5 gm) were washed free of blood and homogenized with a Polytron PT homogenizer. An aliquot of the whole homogenate was saponified with 6% ethanolic potassium hydroxide for 20 minutes at 60° C in the dark and extracted with hexane (containing 0.025% butylated hydroxytoluene). Sample extracts were dried under nitrogen, redissolved in 300 μl of ethanol containing 0.025% butylated hydroxytoluene, and analyzed.

Analysis of β-carotene by high-pressure liquid chromotography. Briefly, 40 μl of the ethanolic extract was injected onto a C_{18} column (5 μm) and eluted with acetonitrile/methylene chloride/methanol (75:20:5) at a flow rate of 2 ml/min, with monitoring at 450 nm. Results were calculated by use of the peak area of the compound over the internal standard. The recovery after single extraction of β-carotene was about 95%. The coefficient of variation was <10% in all cases.

Statistical analysis. On completion of the β-carotene assays, the study code was broken and a comparative analysis was carried out for any association between β-carotene tissue levels and histologic or histopathologic diagnoses of the tissue specimens obtained from the same patients. The sign test of related samples (matched pairs of observations) was applied to determine whether the distribution of changes in the pathologic groups was significant. Because of the small sample size of tissues from various organ sites, the sign test was performed for all cancer sites combined.

Results

A total of 68 samples, consisting of 34 tumor samples and an equal number of normal tissue samples, were assayed. The surgical indications for removal of the various organs were: uterine leiomyoma, 18 patients; cancer of the uterine cervix, 5; cancer of the endometrium, ovary, breast, and colon, 2 each; and cancer of the lung, liver, and rectum, 1 each.

Leiomyomas and normal myometrium. A comparison of the β-carotene concentrations in extracts of normal myometrium with the levels in fibroid tissues of the 18 subjects is presented in Table I. The calculated mean β-carotene level of 18 normal myometrium samples was 64.0 ng/gm tissue whereas the mean level of β-carotene in eight fibroid tissue samples was 35.3 ng/gm tissue. β-Carotene was not detectable (<20 ng/gm) in the remaining 10 of the 18 fibroid tissue samples. When paired, in 16 of the 18 myometrium tissues, the fibroid specimens had lower β-carotene concentrations than normal specimens. When pair comparisons were analyzed by the sign test, the difference was significant ($p = 0.0013$).

Carcinomas versus adjacent normal tissues. Among the 16 patients with various carcinomas of the cervix, endometrium, ovary, breast, lung, liver, colon, and rectum, a wide range of β-carotene levels (i.e., 70 to 855 ng/gm wet tissue) was measured in the normal tissues

Table II. Tissue β-carotene levels in various cancers and adjacent normal sites in humans

Subject	Age (yr)	Organ	β-Carotene (ng/gm tissue)	
			Normal	Cancer
		Reproductive		
W. P.	42	Cervix	320	ND*
G. S.	50	Cervix	360	ND
M .C.	51	Cervix	504	ND
S. B.	68	Cervix	172	ND
G. F.	53	Cervix	360	ND
R. R.	62	Endometrium	236	ND
P. L.	39	Endometrium	381	ND
M. S.	23	Ovary	320	72
D. P.	31	Ovary	658	ND
		Nonreproductive		
E. B.	44	Breast	681	374
G. K.	65	Breast	140	129
E. S.	70	Colon	75	39
I. S.	69	Colon	70	ND
W. S.	57	Lung	401	ND
J. P.	53	Liver	855	95
J. S.	59	Rectum	150	145

**ND,* Not detectable by high-pressure liquid chromatography method.

adjacent to cancer sites in the same excised surgical specimen. In contrast, β-carotene content was either reduced ($n = 6$) or nondetectable ($n = 10$) in cancer tissues of the various organs that were assayed compared with those of normal tissues (Table II). When pair comparisons were analyzed for all cancer sites combined by the sign test, the difference was highly significant ($p = 0.0004$).

Comment

This is the first study to report comparative β-carotene levels in normal and cancer tissue sites of uterine cervix, endometrium, myometrium, ovary, breast, colon, lung, liver, and rectum derived from surgically excised human specimens. The assays were measured by a specific and sensitive analytic high-pressure liquid chromatography method. Tumor tissue from these various organ sites and adjacent normal tissue were sampled and released for β-carotene analyses by staff pathologists under assigned code numbers. The analyses were carried out as unknowns without any knowledge of origin of the tissue or the patient's clinical status. The histologic or histopathologic nature of the tissue submitted was determined microscopically. β-Carotene was detected in all of the normal tissues in variable amounts. Tissue concentrations were significantly lower in the benign leiomyomas when compared with the amounts present in adjacent normal myometrium. The results of this pilot study revealed variable amounts of β-carotene in normal tissues, whereas lower or nondetectable β-carotene levels were measured in fibroid and different cancer tissues. In this study blood samples were not obtained from the patients. At present, simultaneous measurements of β-carotene levels in blood and tissues are being carried out to investigate the cause/effect relationship of our findings.

Human adipose tissue and the liver are important sites for storage and deposition of carotenoids. Most previous reports used nonsensitive spectrophotometric methods for the determination of total carotenoids or β-carotene in limited kinds of human tissues.[11, 12] The present findings are additionally important because the β-carotene levels in human female reproductive organs, as well as in the breast, colon, lung, liver, and rectum, were determined by a sensitive high-pressure liquid chromatography method. Our β-carotene values are comparatively lower than those reported for tissues of other human organs (10.8 μg carotene per gram adrenal, 4.7 μg carotene per gram testis, 150 μg per gram liver).[11-13] Spectrophotometric analyses of tissue extracts of the total carotene content are not as sensitive. The human uterus, ovary, breast, colon, lung, and rectum are not primary storage sites for β-carotene. Recently it was reported that the bovine ovarian corpus luteum can hydrolyze β-carotene suggesting that the ovary may store or use β-carotene as a source of retinol.[14]

The renewed interest in β-carotene, per se, in addition to its recognized provitamin A activity, is in its postulated role as a potential cancer protective agent.[1, 15] However, definitive evidence in support of this hypothesis in the pathogenesis of human neoplasms and possible mechanistic leads of a role for this micronutrient in cancer protection has thus far not been elucidated. β-Carotene has been reported to inhibit chemically induced tumor growth.[16, 17] More than

any other carotene, β-carotene is a free-radical trap and efficient quencher of singlet oxygen. Singlet oxygen is a known reactive form of oxygen that is both mutagenic and effective in causing lipid peroxidation.[18]

The human diet contains many natural mutagens and carcinogens that may act through the generation of oxygen radicals.[19] The dietary consumption of natural antioxidants, such as vitamins C and E, as well as β-carotene, may enhance the host's ability to detoxify such carcinogens. Exposure to other potential carcinogens, including human papillomavirus, may induce increased essential micronutrient requirements of tissues and an altered epithelial metabolism. While tissue availability of β-carotene may not be related directly to dietary intake, the decreased levels of β-carotene in leiomyomas and cancer tissues investigated suggest that a deficiency of tissue β-carotene may be a confounding variable in the multifactorial etiology of both benign and malignant neoplasms.

These findings focus attention on the possible metabolic interactions of β-carotene in commonly encountered gynecologic neoplasms, as well as malignant tumors of the breast, colon, lung, liver, and rectum. Basic studies are needed to elucidate the potential importance of β-carotene, possibly other carotenoids, and the synergistic interaction of other essential micronutrients in the etiology of human cancer and the role of nutrition in its prevention.

We thank Dr. Boyce Bennett of the Weiler Hospital of the Albert Einstein College of Medicine and his staff for providing surgical specimens and histopathologic diagnoses.

REFERENCES

1. Peto R, Doll R, Buckley JD, Sporn MB. Can dietary β-carotene materially reduce human cancer rates? Nature 1981;290:201-8.
2. Stahelin HB. Vitamins and cancer. Recent Results Cancer Res 1988;108:227-34.
3. Ziegler RG, Mason TJ, Stemhagen A, et al. Carotenoid intake, vegetable, and the risk of lung cancer among white men in New Jersey. Am J Epidemiol 1986;123:1080-93.
4. Paganini-Hill A, Chao A, Ross RK, Henderson BE. Vitamin A, beta-carotene, and the risk of cancer: a prospective study. JNCI 1987;79:443-8.
5. Wald NJ, Boreham J, Hayward JL, Bulbrook RD. Plasma retinol, β-carotene and vitamin E levels in relation to the future risk of breast cancer. Br J Cancer 1984;49:321-4.
6. Nomura AMY, Stemmermann GN, Heilbrun LK, Salkeld RM, Vuilleumier JP. Serum vitamin levels and the risk of cancer specific sites in men of Japanese ancestry in Hawaii. Cancer Res 1985;45:2369-72.
7. Menkes MS, Comstock GW, Vuilleumier JP, Helsing KJ, Ridder AA, Brookmeyer R. Serum beta-carotene, vitamins A and E, selenium, and the risk of lung cancer. N Engl J Med 1986;315:1250-4.
8. Mathews-Roth MM. Antitumor activity of β-carotene, canthaxanthin and phytoene. Oncology 1982;39:33-7.
9. Temple NJ, Basu TK. Protective effect of β-carotene against colon tumors in mice. JNCI 1987;78:1211-4.
10. Suda R, Schwartz J, Shklar G. Inhibition of experimental oral carcinogenesis by topical beta carotene. Carcinogenesis 1986;7:711-5.
11. Raica N Jr, Scott J, Lowry L, Sauberlich HE. Vitamin A concentration in human tissue collected from five areas in the United States. Am J Clin Nutr 1972;25:291-6.
12. Mathews-Roth MM, Abraham AA, Gabuzda TG. β-Carotene content of certain organs from patients receiving high doses of β-carotene. Clin Chem 1976;22:922-4.
13. Dagadu JM. Distribution of carotene, and vitamin A in liver, pancreas and body fat of Ghanians. Br J Nutr 1967;21:453-6.
14. Sklan D. Carotene cleavage activity in the corpus luteum of cattle. Int J Vitam Nutr Res 1983;53:23-6.
15. Temple NJ, Basu TK. Does beta carotene prevent cancer? A critical appraisal. Nutr Res 1988;8:685-701.
16. Alam BS, Alam SQ. The effect of different levels of dietary β-carotene on DMBA-induced salivary gland tumors. Nutr Cancer 1987;9:93-101.
17. Peng A, Rundhaug JE, Yoshizawa CN, Bertram JS. β-Carotene and canthaxanthin inhibit chemically and physically induced neoplastic transformation in 10T 1/2 cells. Carcinogenesis 1988;9:1533-9.
18. Machlin LT, Bendich A. Free radical tissue damage: protective role of antioxidant nutrients. FASEB J 1987;1:441-5.
19. Ames BN. Dietary carcinogens and anticarcinogens. Science 1983;221:1256-64.

Compensatory ovarian hypertrophy occurs by a mechanism distinct from compensatory growth in the regenerating liver

Ronald D. Alvarez, MD, William E. Grizzle, MD, PhD, Lori J. Smith, and Donald M. Miller, MD, PhD

Birmingham, Alabama

The mechanism by which compensatory ovarian growth occurs is complex and not completely understood. To compare the molecular events in compensatory ovarian growth with those known to occur in other compensatory growth processes such as the regenerating liver, the temporal pattern of proto-oncogene expression and dexoyribonucleic acid synthesis was investigated in rat ovarian tissue after unilateral castration. One hundred fifty female rats were subjected to either a left hemioophorectomy or a sham oophorectomy. Twenty-four rats from each group were put to death at 3 and 14 days after the initial procedure and the ovaries were weighed. There was a mean compensatory weight increase in the right ovaries of the hemioophorectomy group of 7.9% at 3 days and 22.5% at 14 days. The temporal pattern of proto-oncogene expression was determined by removing the right ovary from six rats in each group at 4, 8, 12, 24, 36, and 48 hours after the initial procedure. The ovaries were paired into three samples in each group for each time point and the ribonucleic acid was extracted. Dot blot hybridization was performed on each ribonucleic acid sample with radiolabeled complementary dexoyribonucleic acid probes for the proto-oncogenes c-*myc*, c-HA-*ras*, and c-*fos*. There was no significant increase in proto-oncogene expression in the right ovaries of the hemioophorectomy group when compared with the right ovaries of the sham oophorectomy group. The temporal pattern of dexoyribonucleic acid synthesis was determined by removing the right ovary from three rats in each group at 8, 12, 24, 36, and 48 hours after the initial procedure. Each rat had been injected intraperitoneally with [^{3}H]thymidine 2 hours before the right oophorectomy. The specific activity of dexoyribonucleic acid extracted from each ovarian sample did not demonstrate a significant increase in ovarian dexoyribonucleic acid synthesis after hemioophorectomy or any significant difference in dexoyribonucleic acid synthesis between the hemioophorectomy and the sham oophorectomy groups. This report concludes that compensatory ovarian growth occurs by a mechanism distinct from compensatory growth in the regenerating liver. (Am J Obstet Gynecol 1989;161:1653-7.)

Key words: Compensatory ovarian growth

Compensatory ovarian growth has been demonstrated to occur in the rat and other animal models after unilateral castration.[1, 2] It has been hypothesized by some investigators that compensatory ovarian growth occurs as a result of an increase in gonadotropin secretion stimulated by declining levels of ovarian steroids or by declining levels of an "inhibin-like" factor.[3-7] Others have postulated a functional role of the peripheral autonomic nervous system in compensatory ovarian growth.[8-10]

In other compensatory growth processes, cellular proliferation is preceded by an increase in proto-oncogene expression. For example, increased expression of the proto-oncogenes c-*myc*, c-Ha-*ras*, and c-*fos* precedes an increase in dexoyribonucleic acid (DNA) synthesis and cell proliferation in the regenerating liver after partial hepatectomy.[11-16] No study to date has reported the molecular events in compensatory ovarian growth or compared these events with the molecular events in regenerating liver. It is the purpose of this study to do so by investigating the temporal pattern of proto-oncogene expression and DNA synthesis in rat ovarian tissue after unilateral castration.

From the Departments of Obstetrics and Gynecology, Pathology, and Internal Medicine, The University of Alabama at Birmingham.

Presented at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.

Reprint requests: Ronald D. Alvarez, MD, OHB 550, University Station, University of Alabama at Birmingham, Birmingham, AL 35294.

6/6/16507

Material and methods

Animals. Fischer rats (weight range 106 to 177 gm) were housed under controlled conditions of temperature, humidity, and light (lights on 12 hours and lights off 12 hours). All animals were fed water and standard rat chow as desired. Institutional guidelines for the care and experimental use of animals were strictly adhered to.

Determination of compensatory growth. Twelve female rats were subjected to a left hemioophorectomy and 12 female rats were subjected to a sham oophorectomy both through a ventral midline abdominal in-

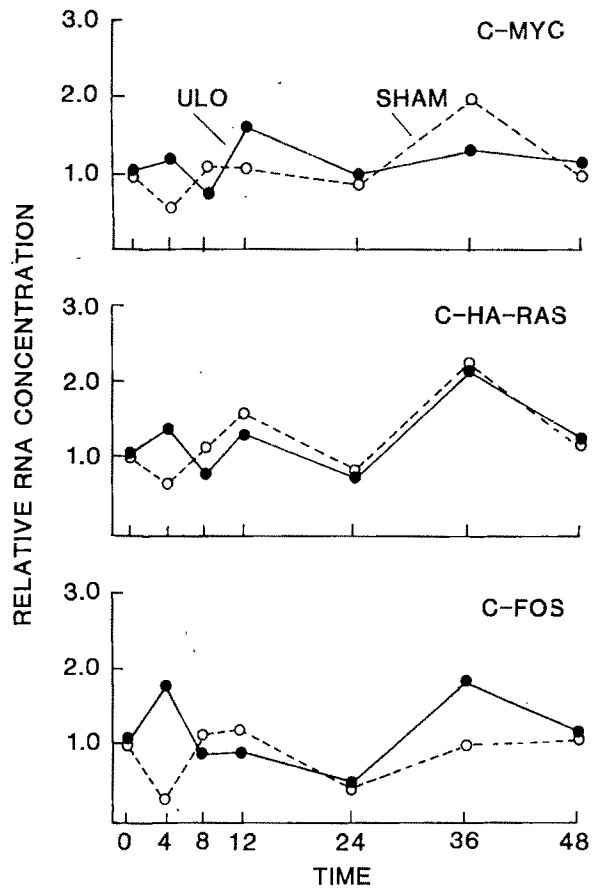


Fig. 1. Mean fold increases in proto-oncogene expression in the hemioophorectomy *(ULO)* and sham oophorectomy *(SHAM)* groups. The right ovaries were excised from each group at various intervals between 4 and 48 hours after the initial procedure. RNA extracted from ovarian samples was analyzed by dot hybridization to radiolabeled cDNA probes for the proto-oncogenes c-*myc*, c-HA-*ras*, and c-*fos*.

cision with the animals under ketamine anesthesia. Encapsulating fibroadipose tissue was trimmed from the excised ovaries and the ovaries were weighed individually. At 3 and 14 days after the initial procedure, the remaining ovaries were excised from six rats in each group, trimmed of fibroadipose tissue, and individually weighed.

The amount of compensatory ovarian growth was calculated by the formula:

$$\text{Compensatory growth (\%)} = \frac{R_o - R_s}{R_s} \times 100$$

where R_o is the mean weight of the right ovaries in the hemioophorectomy group and R_s is the mean weight of the right ovaries in the sham oophorectomy group.

The experiment was repeated in 24 additional female rats, and the mean compensatory ovarian growth was determined from the results of the two experiments.

Determination of proto-oncogene expression. Thirty-six female rats were subjected to a left hemioophorectomy and 36 were subjected to a sham oophorectomy. At 4, 8, 12, 24, 36, and 48 hours after the initial surgery, six rats from each group were put to death. The right ovaries were excised and paired into three samples in both study groups for each time point and immediately frozen in liquid nitrogen.

Total cellular ribonucleic acid (RNA) was isolated from each sample by the method described by Chomczynski and Sacchi.[17] RNA was extracted from pulverized frozen tissue homogenized in a guanidium thiocyanate solution. After phenol-chloroform extraction the RNA was precipitated with isopropanol and quantitated by absorption spectrophotometry. RNA samples from each time point in both the hemioophorectomy group and the sham oophorectomy group were analyzed by dot hybridization.[18] Each RNA sample was assayed by hybridization to complementary DNA (cDNA) probes for the c-*myc*, c-Ha-*ras*, and c-*fos* proto-oncogenes. The cDNA insert for each probe was purified by preparative gel electrophoresis and radiolabeled by nick translation.[19] Autoradiograms of each filter were analyzed by laser densitometry, and the mean increase in proto-oncogene expression was plotted for both the hemioophorectomy and the sham oophorectomy group.

Determination of DNA synthesis. Fifteen female rats were subjected to a left oophorectomy and 15 underwent a sham oophorectomy. At 6, 10, 22, 34, and 46 hours after the initial procedure, three rats from each group were injected intraperitoneally with 200 mCi of [^{3}H]thymidine diluted in 1 ml of saline solution. Two hours later the right ovaries were excised from each rat and immediately frozen in liquid nitrogen.

Cellular DNA was isolated from each frozen ovary specimen by proteinase K digestion and phenol extraction.[20] The rate of DNA synthesis in the hemioophorectomy group and in the sham oophorectomy group was determined by measuring the specific activity of each DNA sample and by calculating the mean specific activity for each time point.

Results

The mean compensatory weight increase in the right ovaries of the rats who had undergone left hemioophorectomy was 7.9% at 3 days and 22.5% at 14 days. This degree of compensatory ovarian growth is in agreement with that seen by previous investigators.[1, 2, 5, 7] This compensatory growth is less dramatic than that seen in the regenerating liver, which has a 300% to 500% increase in mass within 7 to 14 days after partial hepatectomy.[11]

As demonstrated in Fig. 1, there is no significant difference in the level of expression of the proto-oncogenes c-*myc*, c-HA-*ras*, and c-*fos* between the right

ovaries of the hemioophorectomized rats and the right ovaries of the sham oophorectomized group. This is in clear contradistinction to the observations in regenerating liver. Previous studies conducted in our laboratory have demonstrated a sevenfold to eightfold increase in c-*myc* expression within 3 hours after partial hepatectomy (Campbell VW, Rousell J, Rigsby D, et al. Selective inhibition of c-myc expression by mithramycin prevents hepatocyte proliferation in regenerating liver. Unpublished data). Others have reported as much as a fifteenfold increase in c-*myc* expression during the same time period.[12-14] The c-*myc* expression returns to baseline, only to rise again to a less pronounced level of expression between 8 and 24 hours after partial hepatectomy (Campbell VW, Rousell J, Rigsby D, et al. Selective inhibition of c-myc expression by mithramycin prevents hepatocyte proliferation in regenerating liver. Unpublished data). The c-Ha-*ras* expression has been demonstrated by our previous studies[1] and by others[12, 15, 16] to increase 200% to 300% within 48 hours after partial hepatectomy. Fig. 2 graphically compares our observations of the pattern of c-*myc* and c-Ha-*ras* expression in compensatory ovarian growth and in regenerating liver. In similar studies, c-*fos* levels have been shown to be at least fourfold above normal 30 minutes after partial hepatectomy, only to decline by 2 hours.[13] A second twofold increase in c-*fos* expression occurs at 8 hours after partial hepatectomy.

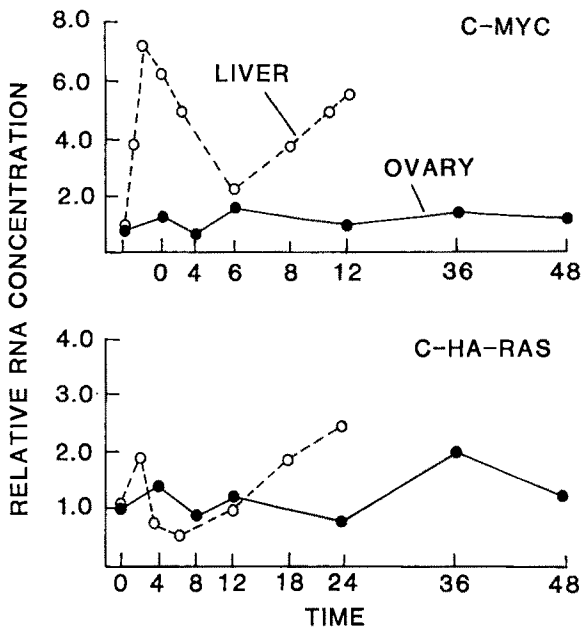


Fig. 2. Mean fold increase in proto-oncogene expression in regenerating liver and compensatory ovarian growth. In previous studies (Campbell VW, Rousell J, Rigsby D, et al. Selective inhibition of c-myc expression by mithramycin prevents hepatocyte proliferation in regenerating liver. Unpublished data) RNA extracted from regenerating liver within 24 hours after partial hepatectomy was analyzed by dot hybridization to radiolabeled cDNA probes for the proto-oncogenes c-*myc* and c-HA-*ras*.

As shown in Fig. 3, there is no significant increase in ovarian DNA synthesis after hemioophorectomy. The level of DNA synthesis in the hemioophorectomy and the sham oophorectomy groups is not significantly different at any time during the first 48 postoperative hours. In contrast, there is a dramatic burst of DNA synthesis in the regenerating liver that occurs 20 to 24 hours after partial hepatectomy.[21] Our previous studies (Campbell VW, Rousell J, Rigsby D, et al. Selective inhibition of c-myc expression by mithramycin prevents hepatocyte proliferation in regenerating liver. Unpublished data) have demonstrated peak [^{3}H]thymidine incorporation into regenerating hepatocytes approximately 20 hours after partial hepatectomy. The pattern of DNA synthesis in the regenerating liver is compared with that in compensatory ovarian growth in Fig. 4.

Comment

In this investigation the molecular events surrounding compensatory ovarian growth have been compared with those that occur during liver regeneration. The results of these experiments indicate that the early events of compensatory ovarian growth are quite different from those of liver regeneration. The distinction between these types of compensatory growth may reflect two basic mechanisms by which different organs respond to injury. In spite of an increase in mass of the remaining ovary, there appears to be no increase in the expression of the c-*myc*, c-Ha-*ras*, or c-*fos* proto-oncogenes and no increase in DNA synthesis during compensatory ovarian growth.

The apparent dissimilarities in compensatory growth between these two organ systems raise several key issues. First, does the magnitude of compensatory growth explain the differences in the molecular events during compensatory growth in these two systems? The regenerating liver approaches its original weight by 7 days after partial hepatectomy.[11] However, by 14 days after hemioophorectomy, compensatory growth in the contralateral ovary is only slightly >20%. Increased proto-oncogene expression may have evolved as a mechanism to initiate more rapid growth in more vital organ systems. The magnitude of compensatory ovarian growth may explain the differences in this process when compared with that in regenerating liver, although the complete absence of even a proportional increase in proto-oncogene expression and in DNA synthesis in compensatory ovarian growth argues against this.

Second, although there appears to be no increase in expression of the proto-oncogenes c-*myc*, c-Ha-*ras*, and c-*fos*, our results do not exclude the possibility that other

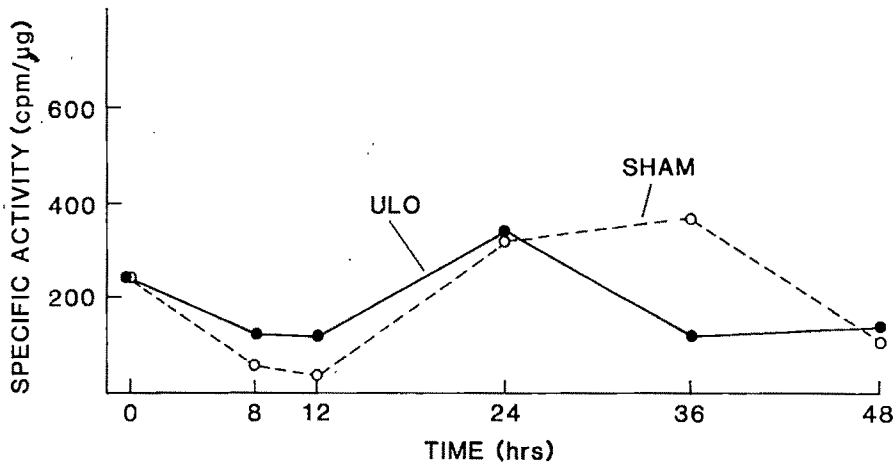


Fig. 3. DNA synthesis in the hemioophorectomy *(ULO)* and sham ovariectomy *(SHAM)* groups. Each rat was injected intraperitoneally with 200 mCi of [^{3}H]thymidine at various intervals between 6 and 46 hours after the initial procedure. The right ovaries were excised 2 hours after [^{3}H]thymidine injection and DNA was extracted. The level of DNA synthesis was determined by calculating the mean specific activity for each time point.

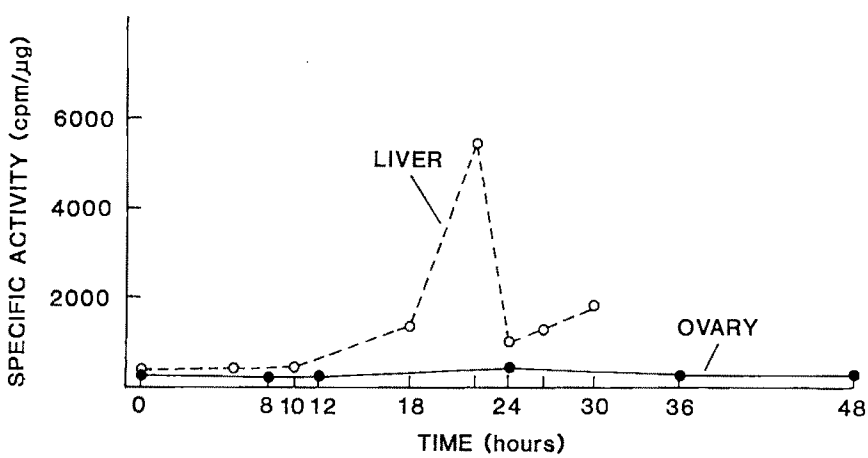


Fig. 4. DNA synthesis in regenerating liver and compensatory ovarian growth. In previous studies (Campbell VW, Rousell J, Rigsby D, et al. Selective inhibition of c-myc expression by mithramycin prevents hepatocyte proliferation in regenerating liver. Unpublished data) DNA was extracted from regenerating liver 1 hour after intraperitoneal injection of [^{3}H]thymidine at various intervals between 6 and 30 hours after partial hepatectomy. The level of DNA synthesis was determined by measuring the specific activity of each sample at each time point.

proto-oncogenes may be involved in the compensatory ovarian growth process. Many of the known proto-oncogenes have been documented to have increased expression in both normal and abnormal (neoplastic) cell transformation processes.[22-25] Although increased c-*myc* expression has provided an accurate marker for cellular proliferation in other growth processes, the results of this experiment do not exclude the possibility of a more ovary-specific proto-oncogene that may initiate a slower growth mechanism in the compensatory ovarian growth process.

Last, are hypertrophic, rather than hyperplastic, cell processes involved in the early events of compensatory ovarian growth? If hyperplasia plays a major role in compensatory growth, cell divison and DNA synthesis should occur. Contrary to the events in the regenerating liver, there appears to be no measureable increase in DNA synthesis in the remaining ovary within the

first 48 hours after hemioophorectomy according to the results of this study. The molecular distinction between hypertrophic and hyperplastic changes is unclear, but it would appear from our data that should hypertrophic changes occur in compensatory ovarian growth, they do so by a pathway different from the hyperplastic changes in regenerating liver.

REFERENCES

1. Arai H. On the cause of the hypertrophy of the surviving ovary after semi-spaying (albino rat) and on the number of ova on it. Am J Anat 1920;28:59-79.
2. Hatai S. The effect of castration, spaying or semi-spaying on the weight of the central nervous system and of the hypophysis of the albino rat; also the effect of semi-spaying on the remaining ovary. J Exp Zool 1913;15:297-314.
3. Ramirez VD, Sawyer CH. A sex difference in the rat pituitary FSH response to unilateral gonadectomy as revealed by plasma RIA. Endocrinology 1974;94:475-82.
4. Welschen R, Dullaart J, de Jong FH. Interrelationships between circulating levels of estradiol-17β progesterone, FSH, and LH immediately after unilateral ovariectomy in the cyclic rat. Biol Reprod 1978;18:421-7.
5. Benson B, Sorrentino S, Evans JS. Increase in serum FSH following unilateral ovariectomy in the rat. Endocrinology 1969;84:369-74.
6. Butcher RL. Changes in gonadotropins and steroids associated with unilateral ovariectomy of the rat. Endocrinology 1977;101:830-40.
7. Edgren RA, Parlow AF, Peterson DL, Jones RC. On the mechanism of ovarian hypertrophy following hemicastration in rats. Endocrinology 1965;76:97-102.
8. Burden HW, Lawrence IE, Smith CP, et al. The effects of vagotomy on compensatory ovarian hypertrophy and follicular activation after unilateral ovariectomy. Anat Rec 1986;214:61-6.
9. Burden HW, Lawrence IE. The effect of denervation on compensatory ovarian hypertrophy. Neuroendocrinology 1977;23:368-78.
10. Curry TE, Lawrence IE, Burden HW. Effect of ovarian sympathectomy on follicular development during compensatory ovarian hypertrophy in the guinea-pig. J Reprod Fertil 1984;71:39-44.
11. Bucher NLR, Malt RA. Regeneration of liver and kidney. Boston: Little, Brown, 1971.
12. Makino R, Hayoshi K, Sugimura T. C-myc transcript is induced in rat liver at a very early stage of regeneration or by cycloheximide treatment. Nature 1984;310:697-8.
13. Thompson NL, Mead JE, Brown L, Goyette M, Shank PR, Fausto N. Sequential protooncogene expression during rat liver regeneration. Cancer Res 1986;46:3111-7.
14. Goyette M, Petropoulos CJ, Shank PR, Fausto N. Regulated transcription of c-Ki-ras and c-myc during compensatory growth of rat liver. Mol Cell Biol1984:4:1493-8.
15. Goyette M, Petropoulos CJ, Shank PR, Fausto N. Expression of a cellular oncogene during liver regeneration. Science 1983;219:510-2.
16. Fausto N, Shank PR. Oncogene expression in liver regeneration and hepatocarcinogenesis. Hepatology 1983; 3:1016-23.
17. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidium throcyanate-phenol-chloroform extraction. Anal Biochem 1987;162:156-9.
18. Thomas PS. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc Natl Acad Sci USA 1980;77:5201-5.
19. Rigby PW, Dreckmann M, Rhodes C, Berg P. Labeling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. J Mol Biol 1977;113:237-51.
20. Blin N, Stafford DW. Isolation of high molecular-weight DNA. Nucleic Acids Res 1976;3:2303-10.
21. Gresham JW. Morphologic study of dexoyribonucleic acid synthesis and cell proliferation in regenerating rat liver: autoradiography with [^{3}H]-thymidine. Cancer Res 1962; 22:842-9.
22. Bishop JM: Cellular oncogenes and retroviruses. Ann Rev Biochem 1983;52:301-54.
23. Cooper GM. Cellular transforming genes. Science 1982; 217:801-11.
24. Bishop JM. Oncogenes. Sci Am 1982;246:80-92.
25. Weinberg RA. A molecular basis of cancer. Sci Am 1983;249:126-43.

Assessment of epidermal growth factor in the healing process of clean full-thickness skin wounds

Alfredo J. Jijon, MD, Donald G. Gallup, MD, M. Ali Behzadian, PhD, and William P. Metheny, PhD

Augusta, Georgia

Epidermal growth factor is a potent stimulant of epithelialization. However, the usefulness of topical applications of epidermal growth factor in accelerating wound healing in full-thickness skin wounds with a large panniculus adiposus has not been clear. Four full-thickness skin incisions were made in the back of 10 female pigs that treated twice a day for 14 days with 2 ml of epidermal growth factor (300 ng/ml) or 2 ml of Ringer's lactate solution in a single-blind, randomized fashion. Two pigs received only epidermal growth factor, two pigs received only Ringer's lactate solution, and six pigs were treated with both solutions. The original skin plug was weighed to ensure similarity of groups. Photographs and measurements of each incision were taken every 7 days. The mean surface areas of the incisions treated with epidermal growth factor were 8.45, 7.50, and 2.30 cm^2; in the incisions treated with Ringer's lactate solution the measurements were 8.42, 8.16, and 2.37 cm^2 on observation days 1, 7, and 14, respectively. Although a trend toward a faster healing rate was noted in the incisions treated with epidermal growth factor, this difference was not statistically significant. With the doses and the time interval used between treatments, minimal benefit was obtained with epidermal growth factor when compared with Ringer's lactate solution. (Am J Obstet Gynecol 1989;161:1658-62.)

Key words: Epidermal growth factor, wound healing

The different properties of epidermal growth factor have been established. It is a potent stimulant of epithelialization both in vivo and in vitro. It has been noted to increase the thickness of the epidermis, increase the epithelial cell proliferation and keratinization,[1] and accelerate wound contraction.[2] Thus its use generally results in decreased wound contracture and scarring.[3] Epidermal growth factor also decreases the thickness of the skin by decreasing the amount of fat,[4] and stimulates protein synthesis.[5] Most studies on the wound-healing properties of epidermal growth factor have been done in split- or partial-thickness skin wounds in animals that have a small panniculus adiposus.[3, 5, 6-8]

Our study was designed to evaluate the in vivo effectiveness of epidermal growth factor in accelerating epithelial regeneration in full-thickness skin wounds in an experimental animal model with a large panniculus adiposus to determine the potential application of epidermal growth factor in superficial abdominal wound breakdown. If wound healing is accelerated, the use of epidermal growth factor could potentially decrease hospitalization time, costs, and discomfort for affected patients. Its application could be useful in patients with infection, hypoproteinemia, and diabetes, not uncommon conditions occurring in some patients in our specialty.

From the Department of Obstetrics and Gynecology, Medical College of Georgia.

Presented at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.

Reprint requests: Donald G. Gallup, MD, Section of Gynecologic Oncology, CK-166, Medical College of Georgia, August, GA 30912.

6/6/15662

Material and methods

Ten female pigs of approximately 2 to 3 months of age served as animal models. Pigs were chosen because of their large panniculus adiposus. Four full-thickness skin incisions, including the panniculus carnosus, were made with a sterile knife in the back of each animal, two on the right side and two on the left side. Each incision was 5 cm long and 1.5 cm wide. The cuts were made with a sterile technique and with the animals under general anesthesia. Permission for this study was obtained from the Committee on Animal Use for Research and Education at the Medical College of Georgia; all guidelines for the care and use of animals at our institution were followed. The tissue removed was weighed. Each wound was assigned a number, measured, and photographed from a standard distance of 50 cm.

Two solutions were prepared; one contained 300 ng of epidermal growth factor per milliliter of Ringer's lactate solution, and the other contained only Ringer's lactate solution. A laboratory assistant, blinded to the intent of the study, marked the two solutions as red or green.

The ten pigs were divided into three groups: group 1, two pigs with eight incisions that were treated only with green solution; group 2, two pigs with eight incisions treated only with red solution; group 3, six pigs in which the incisions on the left received the green solution and the incisions on the right received the red solution. Thus a total number of 40 incisions were studied; 20 were treated with the green solution and 20 with the red solution. Treatments of the incisions were done twice a day after the pigs were sedated. Wet-to-dry dressing changes with 2 ml of either the green or the red solution were applied. Each pig was caged separately and supplied with regular food and water. The duration of the project was 14 days.

Measurements of the incisions were done every 7 days with the pigs under heavy sedation, for a total of three measurements of each incision. The maximum and minimum length, depth, and width were obtained, and from these values a maximum, minimum, and mean area and volume were calculated.

Each incision was photographed every 7 days with a ruler placed on its side to more accurately measure the surface of the wound. Color slides were obtained. Each individual slide was projected onto sheets of standardized batches of paper from exactly the same distance so that the length of the ruler was always kept constant. The margins of each wound picture were traced, cut, and weighed.

As a sideline to this experiment, we compared the correlations among the three different measurements used to assess wound healing. We examined these correlations to determine whether all three measurements are necessary for future studies in this line of investigation. A strong correlation (>0.80) would suggest that only one assessment method is necessary.

Before the analysis the code was broken, and it was established that the green solution contained the epidermal growth factor and the red solution contained the placebo.

The results were analyzed with an independent *t* test and a repeated-measures analysis of variance test with a single covariate, and the Pearson Product-Moment correlation coefficient. The α-value was set at 0.05 with a one-tailed test. We considered that the sample size of 20 incisions per condition would provide sufficient power to detect significant differences, judging from the large effect size (>0.08) yet low power (<0.30) as a result of small samples observed in previous studies.[2, 3, 6-9]

Results

During each observation means and standard deviations were calculated for each of the three variables: surface area, wound volume, and weight of the tracing paper. To ensure that the individual values did not make a difference, we used the weight of the skin plug as covariate in our analysis.

Table I. Initial values (mean ± SD) of wounds per treatment condition

Assessment measurements	*Ringer's lactate solution and epidermal growth factor*	*Ringer's lactate solution and placebo*
Skin plug (gm)	3.43 ± 0.86	3.64 ± 0.68
Surface area (cm^2)	8.45 ± 0.41	8.41 ± 0.48
Wound area (cm^3)	3.67 ± 0.98	4.06 ± 0.93
Weight of paper tracing (gm)	0.82 ± 0.07	0.84 ± 0.08

The weight of the skin plug removed at the beginning of the experiment was nearly equivalent for both groups, and the values were not statistically different according to an independent *t* test. The values of the two groups with regard to surface area, wound volume, and the weight of paper tracing were roughly equivalent, and no statistically significant differences were found between them (Table I).

We subsequently performed three separate analyses and used the weight of the skin plug removed from each pig at the beginning of the experiment as a control for each. In the first analyses we compared the mean surface area for the 3 days of observation. On observation day 1 (initiation of the study) the mean surface area was 8.45 cm^2 in the epidermal growth factor group and 8.41 cm^2 in the placebo group. On observation day 7 the area was 7.50 cm^2 (±2.1) and 8.16 cm^2 (±2.16) in the epidermal growth factor and placebo groups, respectively. On observation day 14 the area was 2.30 cm^2 (±1.15) and 2.37 cm^2 (±1.18) in the epidermal growth factor and placebo groups respectively (Fig. 1).

An identical analysis was conducted for the weight of the paper tracing. On observation day 1 the weight of the paper tracing of the projected slides of the incisions was 0.82 gm, and 0.84 mg in the epidermal growth factor and placebo groups, respectively. On observation day 7 the weight was 0.75 gm (±0.26) and 0.80 gm (±0.23) in the epidermal growth factor and placebo groups, respectively. On observation day 14 the weight was 0.24 gm (±0.11) and 0.25 gm (±0.12) in the epidermal growth factor and placebo groups, respectively (Fig. 2).

In the third analysis the mean wound volume only was compared during the first two observations because it was thought that at the end of the experiment all incisions could have a depth of zero. On observation day 1 the wound volume was 3.67 cm^3 and 4.06 cm^3 in the epidermal growth factor and placebo groups, respectively. On observation day 7 the volume was

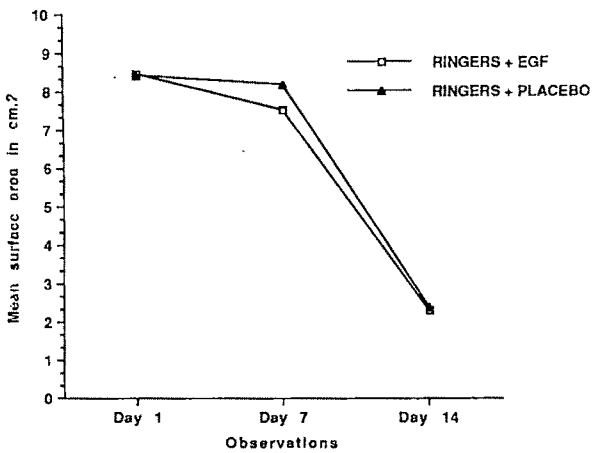


Fig. 1. Plotted comparison of two treatments for wound healing measured by mean surface area in square centimeters at three observation points.

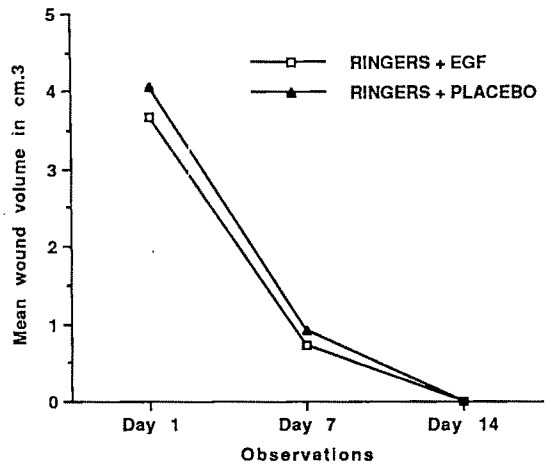


Fig. 3. Plotted comparison of two treatments for wound healing measured by mean volume in cubic centimeters at three observation points.

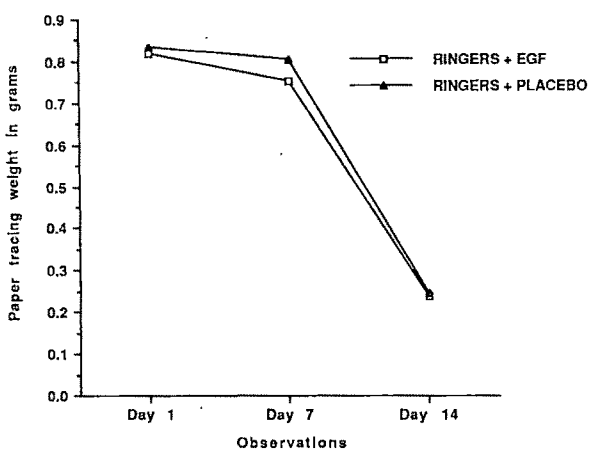


Fig. 2. Plotted comparison of two treatments for wound healing measured by mean weight in grams from paper tracings of surface area photographs at three observation points.

0.72 cm^3 (± 1.28) and 0.92 cm^3 (± 1.33) in the epidermal growth factor and placebo groups, respectively. On observation day 14 both groups of incisions had a volume of 0 (Fig. 3).

Although a faster healing rate was noted in incisions treated with epidermal growth factor compared with those treated with placebo, in none of the three analyses was this difference found to be statistically significant. Nevertheless, all three analyses demonstrated a time effect in the healing rate, independent of group. Most healing occurred during the first week.

The incisions were then analyzed according to the initial three groups of treatment: group 1, those incisions made in the two pigs that only received epidermal growth factor; group 2, those incisions made in the two pigs that only received placebo; and group 3, those incisions made in the six pigs that received both epidermal growth factor and placebo. This group was divided for the purpose of the analysis into two groups: group 3.1, incisions on the left side of the pigs that received only epidermal growth factor; group 3.2, incisions on the right side of the pigs that received only placebo.

In the study of the incisions by treatment groupings, we noted that the groups were statistically equivalent (see Figs. 4 and 5) at the onset of the experiment. However, by observation day 7 the incisions in both groups 1 and 2 had healed faster than those in both groups 3.1 and 3.2. The groups were roughly equivalent by observation day 14, since no statistically significant differences appeared. These results indicate that incisions on animals treated with only one solution, either epidermal growth factor or placebo, healed faster than incisions on animals that received epidermal growth factor on the left side and placebo on the right side.

As a third component of this study, we examined the correlations among the three different methods used to assess wound healing. The correlation of 0.92 between surface area and mean wound volume indicates that these two methods are equivalent. Surface area also correlated significantly with the weight of the paper tracing measure ($r = 0.75$). The paper tracing measure showed a weaker relationship with mean wound volume ($r = 0.65$).

Comment

Controversy still exists concerning the actual benefit of epidermal growth factor in accelerating wound healing. Some have found epidermal growth factor to be useful,[2, 3, 5, 6, 9] whereas others have found no significant effect.[7, 8] Because of this controversy we used three different assessment methods of wound healing to eval-

uate epidermal growth factor in the pig model. Thus confirmation of our values was attained. Analyses of the correlation of the different assessment methods showed that surface area provides the optimal method for assessing wound healing.

Many animals tend to lick their open wounds. This observation and the documented presence of epidermal growth factor in the saliva led Niall et al.[6] to evaluate its use in open granulating wounds. They noted that topical applications of epidermal growth factor enhanced wound closure and demonstrated that the healing rate was faster in both intact or castrated male rats than in female rats. They postulated an androgen-dependent cell sensitivity to epidermal growth factor but noted that such sensitivity does not require the continuous presence of androgens for its expression. Hutson et al.[2] demonstrated that mice caged in groups healed faster than those caged separately; however, sialoadenectomized mice caged in groups did not. To avoid the potential androgenic influence on the activity of epidermal growth factor, we used only female pigs. Each pig was caged separately to avoid licking of the incisions.

Franklin and Lynch[3] performed full-thickness incisions in rabbits' ears and demonstrated that the wound defects treated with epidermal growth factor filled sooner with more cellular elements, thus leaving less space for collapse by wound contracture. Brown et al.[9] also found that epidermal growth factor significantly accelerates epidermal regeneration in partial-thickness skin burns of miniature pigs. Greaves et al.[7] were unable to see any effect in otherwise healthy skin wounds of five human volunteers. Thornton et al.[8] used once-a-day treatments in partial-thickness skin wounds and obtained similar results. We chose twice-a-day treatments, a regimen frequently used in wound breakdowns in our patients.

Epidermal growth factor has two types of cellular receptors. The high-affinity receptors seem to play the major role for epidermal growth factor stimulation of cell division, and these receptors are stimulated at low concentrations of epidermal growth factor. Low-affinity receptors are stimulated with higher concentrations of epidermal growth factor, a process of down-regulation of the receptors is produced, and an inhibitory effect of cell division is seen.[10, 11] Behzadian et al.[11] demonstrated that blocking low-affinity receptors by a specific monoclonal antibody does not decrease the cellular response to epidermal growth factor and the subsequent stimulation of deoxyribonucleic acid synthesis. They concluded that low concentrations of epidermal growth factor (100 to 200 ng/ml), which mainly stimulate high-affinity receptors, are ideal for cellular proliferation. Our choice of 300 ng/ml was based on this information. However, we recognize that the topical

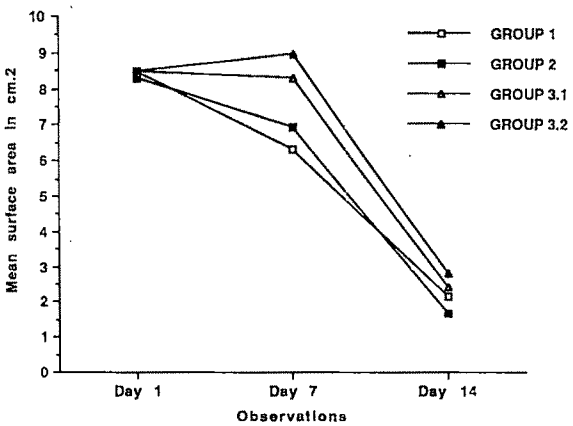


Fig. 4. Plotted comparison of two treatments, grouped by condition within subject, for wound healing measured by mean surface area in centimeters at three observation points: group 1, incisions treated with epidermal growth factor only; group 2, incisions treated with placebo only; group 3.1, incisions on left side treated with epidermal growth factor; group 3.2, incisions on right side treated with placebo.

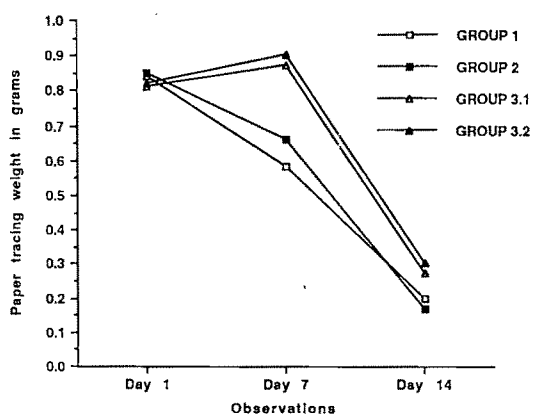


Fig. 5. Plotted comparison of two treatments, grouped by condition within subject, for wound healing measured by mean weight in grams from paper tracing of surface area photographs at three observation points: Group 1, incisions treated with epidermal growth factor only; group 2, incisions treated with placebo only; group 3.1, incisions on left side treated with epidermal growth factor; group 3.2, incisions on right side treated with placebo.

application of the ligand on a live animal could never be as precise and uniform as noted in in vitro studies. We applied 2.0 ml of the solution to each wound, since this amount completely moistened the gauze.

When the analysis was done by groups of treatment, pigs with half of their incisions treated with either epidermal growth factor or placebo were statistically different from those pigs with all four of their incisions treated with either epidermal growth factor or placebo. The healing rate was faster when only one solution was used on the same animal. However, no significant dif-

ference was noted between the incisions on the animals that received either only one solution or both solutions. These results suggest that a systemic regulation of epidermal growth factor may be present.

In summary, a trend toward faster healing in the incisions treated with epidermal growth factor was noted. The benefit of using epidermal growth factor to accelerate the healing of full-thickness skin wounds in the pig model was not demonstrated in this study. Further research is needed to assess the potential of epidermal growth factor in wound healing.

REFERENCES

1. Carpenter G, Cohen S. Epidermal growth factor. Annu Rev Biochem 1979;48:193-216.
2. Hutson JM, Niall M, Evans D, et al. Effects of salivary glands on wound contraction in mice. Nature 1979; 279:793-5.
3. Franklin JD, Lynch JB. Effects of topical applications of epidermal growth factor on wound healing. An experimental study on rabbit ears. Plast Reconstr Surg 1979; 64:766-70.
4. Serrero G. EGF inhibits the differentiation of adipocyte precursors in primary cultures. Biochem Biophys Res Commun 1987;146:194-202.
5. Hiramatsu, M, Hatakeyama K, Minami N, et al. Increase in collagen synthesis of cotton pellet granuloma in rats by epidermal growth factor. Jpn J Pharmacol 1982;32:198-201.
6. Niall M, Graeme BR, O'Brien BMcC. The effect of epidermal growth factor in wound healing in mice. J Surg Res 1982;33:164-9.
7. Greaves MW. Lack of effect of topically applied epidermal growth factor (EGF) on epidermal growth in man in vivo. Clin Exp Dermatol 1980;5:101-3.
8. Thornton JW, Hess CA, Cassingham V, et al. Epidermal growth factor in the healing of second degree burns: a controlled animal study. Burns 1981;8:156-60.
9. Brown GI, Curtsinger L, Brightwell JR, et al. Enhancement of epidermal regeneration by biosynthetic epidermal growth factor. J Exp Med 1986;163:1319-4.
10. King AC, Cuatrecasas P. Resolution of high and low affinity epidermal growth factor receptors. J Biol Chem 1982;257:3053-60.
11. Behzadian MA, Shimizu N. Monoclonal antibody that immunoreacts with a subclass of human receptors for epidermal growth factor. Cell Struct Funct 1985;10:219.

Immunocytochemical study of *ras* and *myc* proto-oncogene polypeptide expression in the human menstrual cycle

Lawrence D. Odom, MD,[a] J. Michael Barrett, PhD,[b] Cooley G. Pantazis, MD,[c] Leland D. Stoddard, MD,[c] and Paul G. McDonough, MD[a]

Augusta, Georgia

The human endometrium is a unique and dynamic tissue model system characterized by cyclic processes of cellular proliferation, differentiation, and menstrual desquamation. Both the glandular epithelial and stromal mesenchymal components work in synchronous response to the mitogenetic effect of estradiol and the antimitogenetic effect of progesterone. Mechanisms whereby estradiol and progesterone exert their effects are not completely understood. This study was undertaken to evaluate the expression and localization of the polypeptide products of the *ras* and *myc* proto-oncogenes sequentially during the menstrual cycle. Sixteen endometrial biopsy specimens were evaluated. Immunocytochemical quantitation of *ras* and *myc* expression was done by use of color image analysis (CoreScan). There was no cyclic variation in the *ras* polypeptide product, but the expression of *myc* polypeptide was low in the secretory phase and high in the proliferative phase. (AM J OBSTET GYNECOL 1989;161:1663-8.)

Key words: Proto-oncogene, *ras*, *myc*, endometrium, menstrual cycle

It is well established that the glandular and stromal endometrial components contain target cell responders to steroid hormones produced sequentially by the dominant ovarian follicle and the corpus luteum. Endometrial proliferation occurs in response to estradiol production, a factor of granulosa cell activity. Estradiol, while remaining in the nuclei of target cells, stimulates the synthesis of deoxyribonucleic acid (DNA) resulting in a wave of mitosis. With the secretion of progesterone, there is a cessation of mitotic activity and the glandular cells differentiate.

Despite continued progress in the understanding of the physiologic mechanisms involved in proliferation of the endometrium and control of such proliferation, additional questions have been raised. It has been demonstrated that murine uterine epithelium is dependent on the presence of homologous stroma for proper response to estrogen stimulation.[1] Additionally, the presence of immunoreactive epidermal growth factor (EGF) in the uterus,[2] the rapid increase in EGF messenger ribonucleic acid (mRNA)[3] and EGF receptor[4] after estrogen, and the growth-promoting effect of EGF in uterine cell culture[5] support the hypothesis that autocrine or paracrine growth factors may be important in the uterine response to estrogen.

The mRNA levels of genes known to be regulated by sex steroids are not altered until ≥1 hour after steroid administration, although the steroid receptor complex has attained an intranuclear location within minutes. A possibility considered by Fink et al.[6] is that steroid-induced gene regulation might involve early regulatory genes whose transcription would be rapidly modulated by steroid receptor binding and whose protein products would in turn regulate the transcription of the structural genes.

To further investigate endometrial physiologic characteristics, a study was undertaken to evaluate the expression of two proto-oncogene (*ras* and *myc*) polypeptide products in the proliferative and secretory phases of the human menstrual cycle.

Material and methods

Fifteen endometrial biopsy specimens were chosen on the basis of a normal menstrual and conception history, as well as absence of pathologic changes at the time of laparoscopic sterilization. One biopsy specimen was obtained before total vaginal hysterectomy for procidentia. No associated abnormalities could be determined at the time of surgery. There was no history of recent exogenous hormonal exposure in any of the study subjects. Dates of the formalin-fixed, paraffin-embedded biopsy specimens were assigned by one author (L. D. S.) without prior knowledge of the clinical history. Histomorphologic criteria used to establish dates of the 4 μm thick sections were based fundamentally on the study by Noyes et al.[7] The menstrual

From the Section of Reproductive Endocrinology, Department of Obstetrics and Gynecology,[a] the Department of Anatomy,[b] and the Department of Pathology,[c] Medical College of Georgia.

Presented in part at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.

Reprint requests: Lawrence D. Odom, MD, Section of Reproductive Endocrinology, Department of Obstetrics and Gynecology, Medical College of Georgia, Augusta, GA 30912-3360.

6/6/15955

Table I. Percentage of immunoreaction product for average of three areas scanned

	myc Gland	*myc Stroma*	*ras Gland*	*ras Stroma*
Stage 1	0.66	0.45	0.20	0.12
Stage 2	0.4	0.16	0.31	0.44
Stage 3	0.3	0.40	0.19	0.61
	0.3	0.07	0.39	0.15
	0.6	0.55	0.31	0.19
	0.6	0.46	0.71	0.46
	0.6	0.25	0.45	0.35
	0.5	0.12	0.71	0.35
	0.7	0.40	0.44	0.23
Stage 4	0.1	0.10	0.36	0.30
	0.2	0.25	0.22	0.10
	0.2	0.08	0.26	0.14
Stage 5	0.17	0.04	0.08	0.07
	0.03	0.03	0.45	0.22
	0.33	0.06	0.45	0.24
Stage 6	0.01	0.02	0.09	0.21

Stages 1 to 3 are proliferative; stages 4 to 6 are secretory.

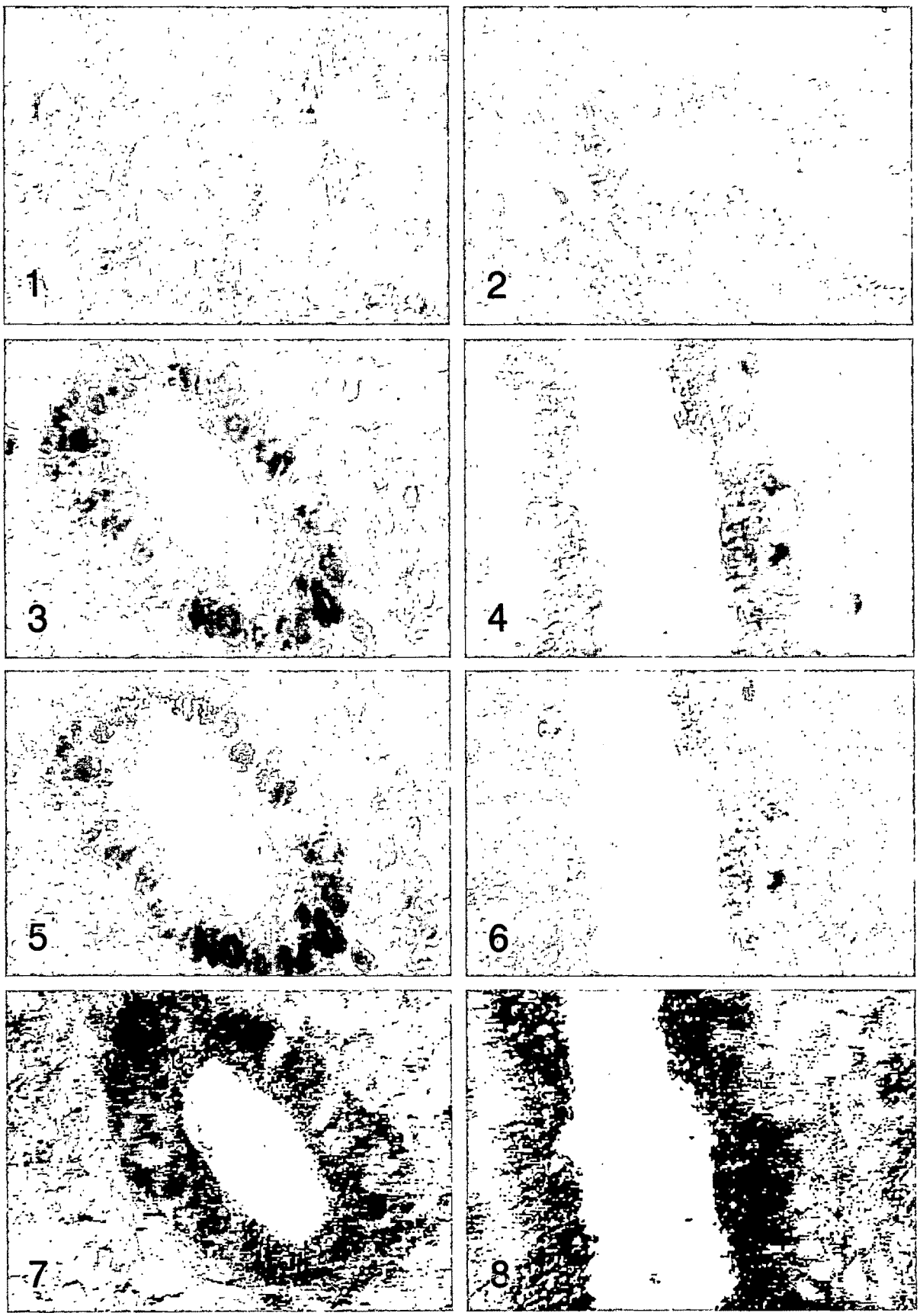
cycle was divided into six stages. These were early, mid, and late proliferative and secretory designations. These stages were categorized 1 through 6. There was one patient in category 1, one patient in category 2, seven patients in category 3, three patients in category 4, three patients in category 5, and one patient in category 6. For statistical evaluation, these categories were grouped 1 to 3 ($n = 9$) and 4 to 6 ($n = 7$).

For this study an indirect peroxidase-antiperoxidase method described previously[8] was modified. In situ hybridization was performed with the use of the CRL Universal immunoperoxidase staining kit. Sections were deparaffinized in xylene (two changes, 5 minutes each). Rehydration was performed with a graded series of ethanols (100% alcohol, two changes, 3 minutes each; 95% alcohol, two changes, 3 minutes each; 70% alcohol, 3 minutes). The slides were then placed in a final bath of 0.05 mol/L Tris buffer plus 1.5% sodium chloride, pH 7.6. Steps in the method are as follows: step 1, blocking agent—3% hydrogen peroxide (10 minutes); step 2, blocking agent—normal serum (40 minutes); step 3, primary antibody—dilution RAP-5 1:1000 and OP-10 1:10 (18 hours)[9]; step 4, linking reagent (20 minutes); step 5, labeling reagent (peroxidase-antiperoxidase immunoenzyme complex). The complex was visualized by the addition of a chromogenic substrate, amino-ethylcarbazole. All sections were counterstained with hematoxylin and mounted with Permount.

To evaluate the presence of *ras* proto-oncogene (c-*ras*) polypeptide product, the monoclonal antibody RAP-5 was chosen. This monoclonal antibody was supplied by the Tissue Type Culture at the National Institutes of Health (by Dr. Jean Simpson). It was developed against a synthetic peptide immunogen reflecting positions 10 to 17 of the human *ras* protein product from the T-24 bladder carcinoma cell line. RAP-5 has demonstrated reactivity with cellular and point-mutated forms of the polypeptide product (p21) from all three members of the *ras* oncogene family.[10-12] The presence of *myc* proto-oncogene (c-*myc*) polypeptide product was demonstrated with the assistance of the monoclonal antibody OP-10 purchased from Oncogene Science. This monoclonal antibody was developed by using a synthetic peptide corresponding to residues 405 to 432 of the human c-*myc* polypeptide (p62) and was characterized by Evan et al.[13] Positive controls consisted of a T-24 bladder carcinoma cell line for the RAP-5 monoclonal antibody and a Burkitt lymphoma specimen for the OP-10 monoclonal antibody. Negative controls in both instances were provided by omitting the primary antibody application.

The immunoreactive product was quantitated with CoreScan Color Image System software (Delta Tech-

Fig. 1. c-*ras* immunocytochemical reaction product—late proliferative endometrium.
Fig. 2. c-*ras* immunocytochemical reaction product—postovulation day 5 endometrium.
Fig. 3. c-*myc* immunocytochemical reaction product—late proliferative endometrium.
Fig. 4. c-*myc* immunocytochemical reaction product—postovulation day 5.
Fig. 5. c-*myc*—late proliferative endometrium—64-color band.
Fig. 6. c-*myc*—postovulation day 5 endometrium—64-color band.
Fig. 7. c-*myc*—late proliferative endometrium—computerized localization of reaction product.
Fig. 8. c-*myc*—postovulation day 5 endometrium—computerized localization of reaction product.



Figs. 1 to 8. For legends see opposite page.

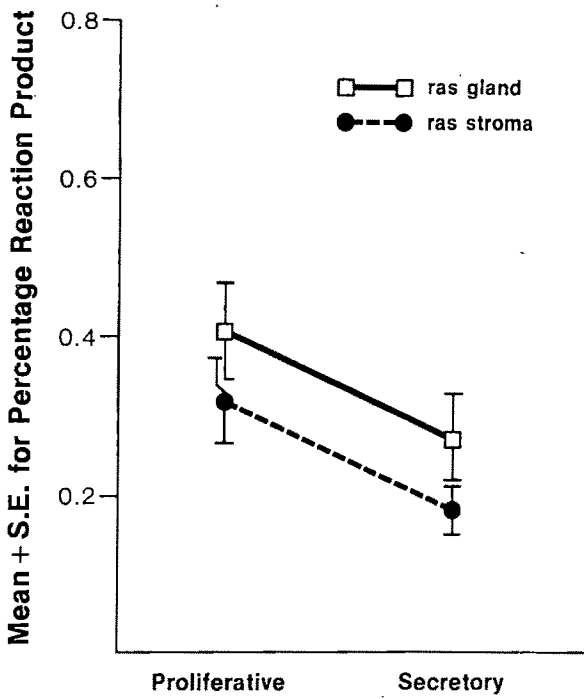


Fig. 9. Immunocytochemical reaction product *(ras)*—statistical analysis.

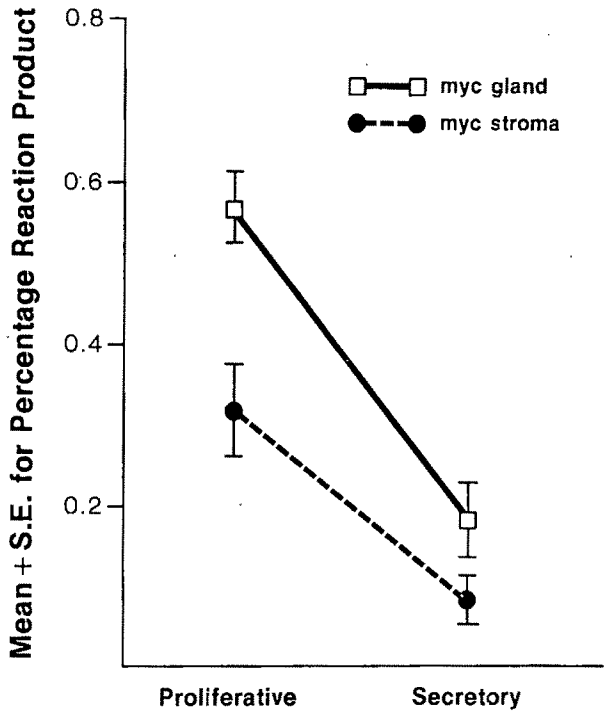


Fig. 10. Immunocytochemical reaction product *(myc)*—statistical analysis.

nologies, Inc., Augusta, Ga.). The system includes a color video camera mounted on a Zeiss Axiophot microscope. The video is cabled to an AT & T True Vision Image Capture Board (ICB) installed in an IBM-PC computer. The output from the ICB monitor is directed to a Sony color monitor. A software program called CoreScan was used to perform color video analysis.[14] Three representative glandular structures and stromal areas were chosen from each slide by two investigators (C. G. P. and L. D. O.) for digitized analysis.

Since the categories were grouped 1 to 3 (n = 9) and 4 to 6 (n = 7), statistical analysis was carried out with hypothesis testing, the difference between population means and Student's t test. To assume equality of population variances, the sample variance ratios were evaluated on the basis of the F distribution.

This study was presented before the Human Assurance Committee of the Medical College of Georgia and met with its approval.

Results

The values obtained in Table I represent reaction product expressed as a percentage of the total area scanned averaged over the three fields (×40). The polypeptide products, c-*ras* p21 and c-*myc* p62, were found to be expressed in both the proliferative and secretory phases of the normal menstrual cycle (Figs. 1, 2, 3, and 4, respectively). Reaction product was identified in both the glandular epithelial and stromal compartments of the endometrium. The *ras* monoclonal antibody identified reaction product was localized exclusively to the cytoplasm (plasma membrane) as expected (Figs. 1 and 2). The *myc* monoclonal antibody–identified reaction product was in both the nucleus and the cytoplasm. In this study a nuclear distribution for c-*myc* was visually apparent in the actively dividing cells (proliferative phase) (Fig. 3). In differentiated cells (secretory phase) the c-*myc* distribution was primarily cytoplasmic in location (Fig. 4). Figs. 5 (c-*myc* late proliferative phase) and 6 (c-*myc* postovulation day 5) represent computerized banding with a reduction to 64 colors. Figs. 7 and 8 are representative of the same two biopsy specimens with computerized localization of the immunocytochemical reaction product.

Statistical analysis (α = 0.05) of the sample data indicated no statistically significant difference between proliferative and secretory expression of the c-*ras* peptide product in either the glandular epithelium (significance level p = 0.14) or stromal compartments (significance level p = 0.06) (Fig. 9). However, there was evidence that the c-*myc* peptide product was expressed to a lesser degree in both the glandular (significance level p = 0.00003) and stromal compartments (significance level p = 0.005) of the secretory phase when compared with the proliferative phase (Fig. 10). The progressive loss of c-*myc* expression was seen through-

out the secretory cycle and followed a temporal relationship with the postovulation day status.

Comment

Cellular oncogenes are present in all normal cells. Current evidence suggests that most oncogenes code for proteins that are involved in the regulation of cell division and/or differentiation.[15] The *ras* genes were first isolated as the transforming genes of Harvey and Kirsten sarcoma viruses. At least three different *ras* genes (Harvey-*ras*, Kirsten-*ras*, and N-*ras*) exist in mammals. All three genes have a similar genetic structure and code for a protein product of 188 or 189 amino acids with a molecular weight of 21,000 d. The cellular location of the p21 polypeptide is the inner surface of the plasma membrane. The specific cellular function of *ras* p21 is unclear; however, it has been shown to bind guanosine 5′-diphosphate and triphosphate, to have guanosine triphosphatase activity, and to exhibit autokinase activity. Its properties suggest that *ras* p21 may function as a signal transducer, similar to the G proteins.[10, 12] The human c-*myc* proto-oncogene is a cellular gene homologous with the 3′ sequence of the transforming gene of avian myelocytomatosis virus MC29. The protein product of the c-*myc* gene has a molecular weight of 62,000 d and is believed to be composed of 439 amino acids. Like the c-*ras* p21, the action of c-*myc* p62 in normal cells is not entirely known. However, unlike p21, active c-*myc* p62 is localized in the nucleus.[13] Studzinski et al.[16] have shown that the addition of polyclonal or monoclonal antibodies against human c-*myc* protein to nuclei isolated from several types of human cells reversibly inhibited DNA synthesis and DNA polymerase activity but not the transcription of RNA. This suggests that c-*myc* encodes a polypeptide product that participates in DNA replication.

With the rat uterus as a model, Murphy et al.[17] confirmed that mitogenetic stimulation resulted in a rapid accumulation of *myc* proto-oncogene mRNA. In this case the mitogen was estradiol. The administration of estrogen had no significant effect on the abundance of c-*myc* transcripts in the kidney, liver, or lung. The avian oviduct study performed by Fink et al.[6] revealed a tissue-specific and rapid down-regulation of c-*myc* mRNA by progesterone. They concluded that the reduction of c-*myc* gene expression in target cells might represent one mechanism whereby progesterone inhibits estrogen-induced mitogenetic events.

As stated previously, active c-*myc* p62 polypeptide seems to be localized in the nucleus. However, additional data suggest that c-*myc* transcripts are exported from the nucleus in quiescent cells.[18] In a study by Jack et al.,[19] a nuclear distribution of c-*myc* p62 was seen in the actively dividing cells of the bone marrow and in some tumors. However, in most normal cells the localization was cytoplasmic. It was suggested that localization of the polypeptide in vivo may differ in cells that are dividing compared with those that are arrested or terminally differentiated.

To our knowledge this report represents the first to evaluate the expression of the *ras* and *myc* proto-oncogenes serially in the human menstrual cycle. With the use of monoclonal antibodies, there has been confirmation of *ras* and *myc* polypeptide products in normal endometrial tissue. On the basis of our data, the expression of the *ras* polypeptide product during the menstrual cycle is not cyclic. Since it is not certainly known that the *ras* p21 polypeptide is involved in signal transduction, these findings do not rule out an autocrine or paracrine effect in the endometrium. On the other hand, the lack of change in p21 expression may be due simply to the sensitivity of the testing procedure or the fact that guanosine triphosphate binding is an on-again/off-again phenomenon leaving a constant amount of p21 polypeptide at the plasma membrane's inner surface.

Our data add to the growing evidence that *myc* p62 polypeptide expression is influenced by steroidal mitogenetic effect (estradiol), as well as steroidal antimitogenesis (progesterone). Furthermore, we have made observations suggesting the transportation of the c-*myc* p62 polypeptide from the nucleus to the cytoplasm in differentiated cells.

We extend our appreciation to Mr. William Wansley for his technical expertise in slide preparation and to Mr. Rollie Harp of the Research Computing and Statistical Department of the Medical College of Georgia for his contributions to statistical analysis.

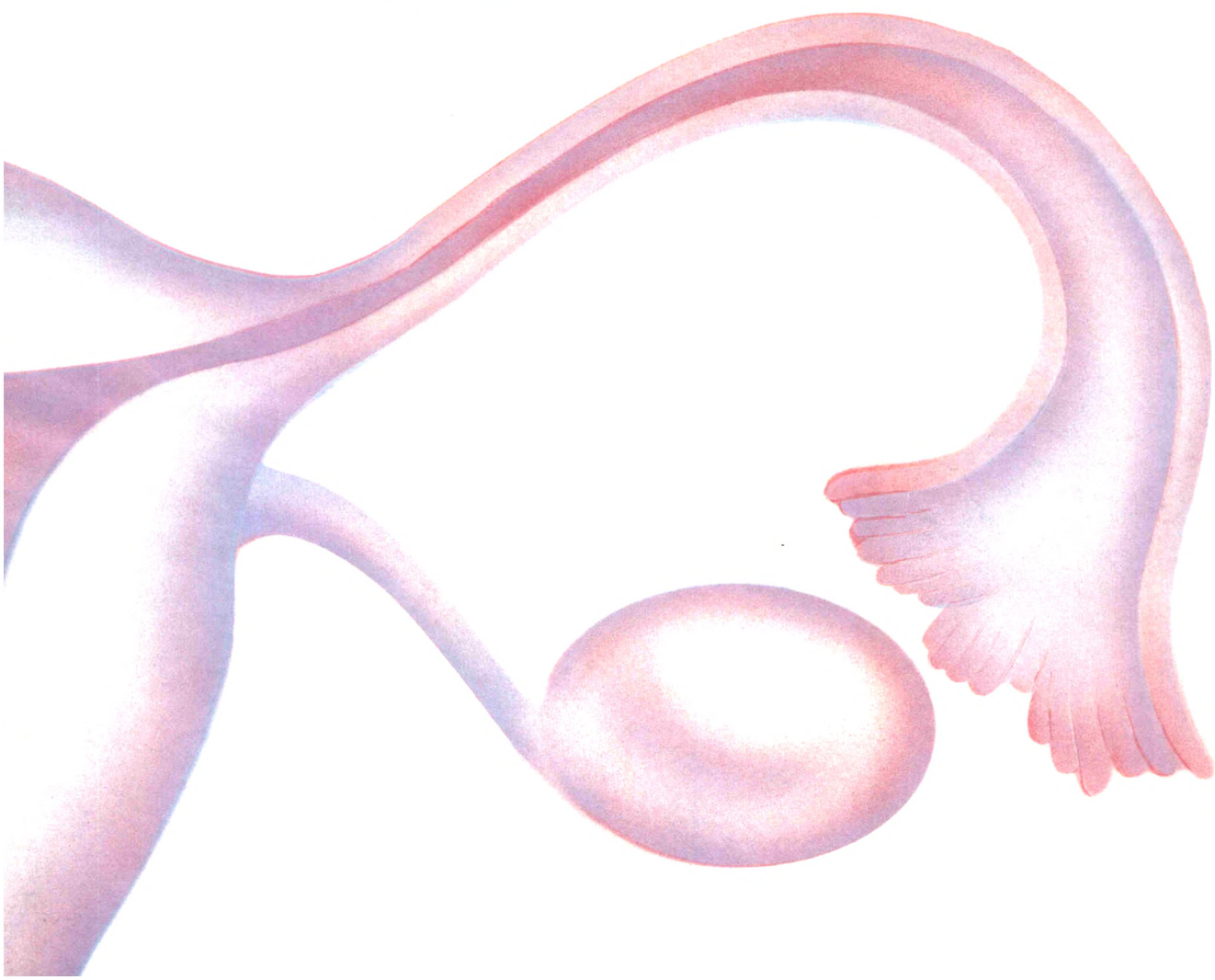
REFERENCES

1. Cooke PS, Uchima FDA, Fujii DK, Bern HA, Cunha GR. Restoration of normal morphology and estrogen responsiveness in cultured vaginal and uterine epithelia transplanted with stroma. Proc Natl Acad Sci USA 1986; 83:2109-13.
2. Y Imai. Epidermal growth factor in the rat uterine luminal fluid. In: Proceedings of the sixty-fourth annual meeting of the Endocrine Society. Endocrine Society, 1982:162.
3. DiAugustine RP, Lannon DE, McLachlan M. Sex steroid hormones rapidly increase uterine epidermal growth factor (EGF). In: Proceedings of the sixty-seventh annual meeting of the Endocrine Society. Endocrine Society, 1985:1074.
4. Mukku VR, Stancel GM. Regulation of epidermal growth factor receptor by estrogen. J Biol Chem 1985;60: 9820-4.
5. Tomooka Y, DiAugustine RP, McLachlan JA. Proliferation of mouse uterine epithelial cells in vitro. Endocrinology 1986;118:1011-8.
6. Fink KL, Wieben ED, Woloschak GE, Spelsberg TC. Rapid regulation of c-*myc* proto-oncogene expression by progesterone in the avian oviduct. Proc Natl Acad Sci USA 1988;85:1796-800.
7. Noyes RW, Hertig AT, Rock J. Dating the endometrial biopsy. Fertil Steril 1950;1:3-25.

8. Sternberger LA. Immunocytochemistry, 3rd ed. New York: Churchill Livingstone, 1986:90-209.
9. Furth ME, Aldrich TH, Cordon-Cardo C. Expression of *ras* proto-oncogene proteins in normal human tissue. Oncogene 1987;1:47-58.
10. Hand PH, Thor A, Wunderlich D, Muraro R, Caruso A, Schlom J. Monoclonal antibodies of predefined specificity detect activated *ras* gene expression in human mammary and colon carcinomas. Proc Natl Acad Sci USA 1984; 81:5227-31.
11. Thor A, Hand PH, Wunderlich D, Caruso A, Muraro R, Schlom J. Monoclonal antibodies define differential *ras* gene expression in malignant and benign colonic diseases. Nature 1984;311:562-5.
12. Thor A, Ohuchi N, Hand PH, et al. Biology of disease: *ras* gene alterations and enhanced levels of *ras* p21 expression in a spectrum of benign and malignant human mammary tissues. Lab Invest 1986;55:603-15.
13. Evan GI, Lewis GK, Ramsay G, Bishop JM. Isolation of monoclonal antibodies specific for human c-*myc* proto-oncogene product. Mol Cell Biol 1985;5:3610-6.
14. Samuels V, Barrett JM, Bockman S, Pantazis CG, Allen MB Jr. Immunocytochemical study of transforming growth factor expression in benign and malignant gliomas. Am J Pathol 1989;134:895-902.
15. Garrett CT. Oncogenes: critical review. Clin Chim Acta 1986;156:1-40.
16. Studzinski GP, Brelvi ZS, Feldman SC, Watt RA. Participation of c-*myc* protein in DNA synthesis of human cells. Science 1986;234:467-70.
17. Murphy LJ, Murphy LC, Friesen HG. Estrogen induction of N-*myc* and c-*myc* proto-oncogene expression in the rat uterus. Endocrinology 1987;120:1882-8.
18. Campisi J, Gray HE, Pardee AB, Dean M, Sonenshein GE. Cell-cycle control of c-*myc* but not c-*ras* expression is lost following chemical transformation. Cell 1984;36: 241-7.
19. Jack AS, Kerr IB, Evan G, Lee FD. The distribution of the c-*myc* oncogene product in malignant lymphomas and various normal tissues as demonstrated by immunocytochemistry. Br J Cancer 1986;53:713-9.

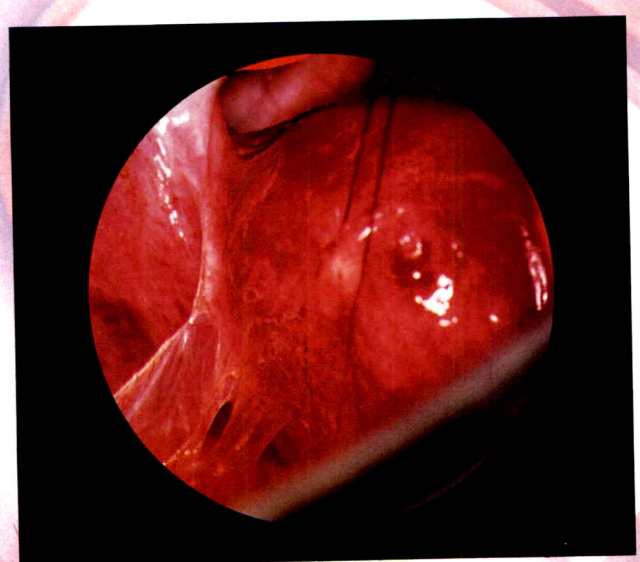
Surgical breakthrough

To reduce adhesions later...



Intercede now

Introducing the first proven adhesio



Sidewall adhesion prior to lysis.

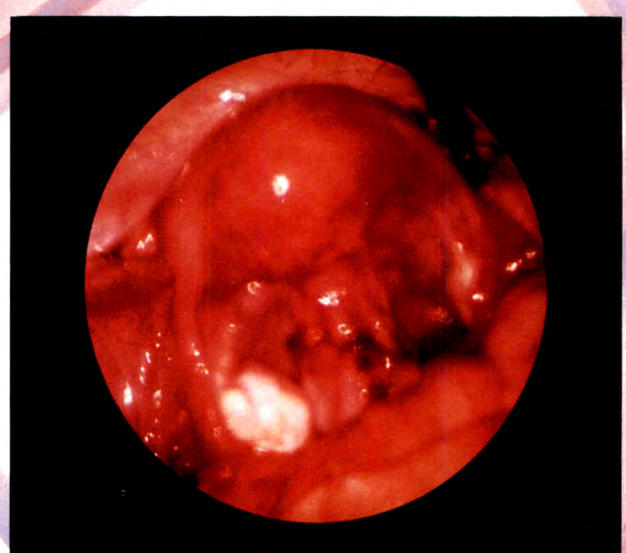
Without INTERCEED Barrier, adhesions were prevented in only 24% of the pelvic sidewalls (N = 32) treated with state-of-the-art surgery alone (134 patients).

The first surgical adjuvant specifically indicated to reduce postoperative adhesions.

- **Effective adhesion control** may help preserve fertility[1-4]
- **Twice as effective** as state-of-the-art surgery alone, when used in conjunction with meticulous surgical techniques
- **Minimal tissue reaction**–significantly less than other absorbable products
- **No complications**–the INTERCEED Barrier was completely absorbed within 28 days . . . no adverse reactions were reported

rrier for gynecologic pelvic surgery

With INTERCEED Barrier, 51% of the pelvic sidewalls (N = 68) treated with state-of-the-art surgery were completely free of adhesions (134 patients).



Evaluation at second-look laparoscopy.

NEW

INTERCEED* (TC7)

Absorbable Adhesion Barrier

(OXIDIZED REGENERATED CELLULOSE)

Johnson & Johnson

PATIENT CARE, INC.

*Trademark of Johnson & Johnson Patient Care, Inc.

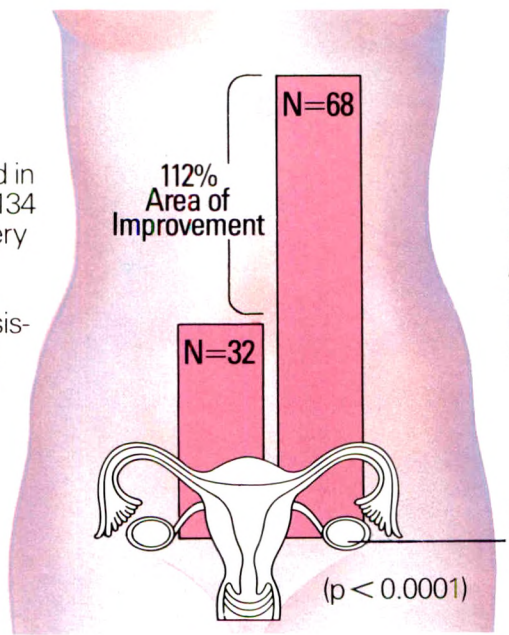
Landmark study demonstrates:

INTERCEED*(TC7) Absorbable Adhesion Barrier helps reduce adhesion formation

Twice as effective in preventing adhesion formation

Study design

INTERCEED Barrier was evaluated in a controlled multicenter study[5] of 134 women undergoing elective surgery to correct bilateral pelvic-sidewall adhesions. After microsurgery via laparotomy (with hemostasis consistent with microsurgical principles), application of INTERCEED Barrier was randomized to the left or ri side, with the contralateral side serving as a control site. *Thus, patient was, in effect, her own control.* Results established the utility of INTERCEED Barrier as adjuvant to gynecologic surger



INTERCEED Barrier prevented th formation of adhesions in twice many sites as surgery alone.

NEW

INTERCEED* (TC7)

Absorbable Adhesion Barrier

(OXIDIZED REGENERATED CELLULOSE)

Brief Summary for INTERCEED* (TC7) Absorbable Adhesion Barrier (Oxidized Regenerated Cellulose). Before prescribing, please see full prescribing information.

INDICATIONS: INTERCEED* (TC7) Absorbable Adhesion Barrier is indicated as an adjuvant in gynecologic pelvic surgery for reducing the incidence of postoperative pelvic adhesions after hemostasis is achieved consistent with microsurgical principles.

CONTRAINDICATIONS: The use of INTERCEED Barrier is contraindicated in the presence of frank infection. INTERCEED Barrier is not indicated as a hemostatic agent. Appropriate means of achieving hemostasis must be employed.

WARNINGS: INTERCEED Barrier must not be used in cases in which complete hemostasis has not been established. The effectiveness of INTERCEED Barrier is reduced when saturated with blood.

INTERCEED Barrier is supplied sterile. As the material is not compatible with autoclaving or ethylene oxide sterilization, INTERCEED Barrier must not be resterilized.

Although none have been reported, foreign body reactions may occur in some patients.

PRECAUTIONS: The safety and effectiveness of using INTERCEED Barrier in combination with other adhesion prevention treatment have not been clinically established.

Ectopic pregnancies have been associated with fertility surgery of the female reproductive tract. No adequate and well controlled studies have been conducted in women who become pregnant within the first month after exposure to INTERCEED Barrier. No data exist to establish the effect, if any, of INTERCEED Barrier on the occurrence of ectopic pregnancies. No teratogenic studies have been performed. Therefore, an avoida conception should be considered during the first complete menstrual cycle after use of INTERCEED E Care should be exercised in applying INTERCEED Barrier to a pelvic organ not to constrict or restric

ADVERSE REACTIONS: To date, no adverse reactions have been reported attributable to the INTERCEED Barrier.

CAUTION: Federal law restricts this device to sale, distribution, or use by or on the order of a phy

References

1. Diamond MP, DeCherney AH. Pathogenesis of adhesion formation/reformation: application to reproductive pelvic surgery. *Microsurgery*. 1987;8:103-107.
2. Bronson RA, Wallach EE. Lysis of periadnexal adhesions for correction of infertility. *Fertil Steril*. 1977;28:613-619.
3. Caspi E, Halpern Y, Bukovsky I. The importance of peritoneal adhesions in tubal reconstructive surgi infertility. *Fertil Steril*. 1979;31:296-300.
4. Gomel V. Salpingo—ovariolysis by laparoscopy in infertility. *Fertil Steril*. 1983;40:607-611.
5. INTERCEED* (TC7) Adhesion Barrier Study Group. *Fertil Steril*. 1989;51:933.

Johnson & Johnson

PATIENT CARE, INC.

New Brunswick, NJ 08901-2023

For product informat call 1-800-526-JJPC.

*Trademark of Johnson & Johnson Patient Care, Inc.

© J.J.P.C.I., 1989 PC 0483 Made in U.S.A.

Comparison of Cytobrush and cervicovaginal lavage sampling methods for the detection of genital human papillomavirus

Gary L. Goldberg, MD,[a] Sten H. Vermund, MD,[b, c] Mark H. Schiffman, MD,[c] Diane B. Ritter, MD,[a] Carol Spitzer, BS, MS,[d] and Robert D. Burk, MD[c, d]

Bronx, New York, and Bethesda, Maryland

The development of an accurate method for the detection and typing of genital human papillomavirus is of substantial clinical importance. This virus has been implicated as an etiologic agent in the development of cervical neoplasia. To detect human papillomavirus infection with maximum sensitivity, cells must be collected and assayed for human papillomavirus deoxyribonucleic acid. We compared two noninvasive methods of sampling exfoliated cervical cells—cervicovaginal lavage and scrape–Cytobrush. Seventy-four patients newly referred to the colposcopy clinic were divided randomly for cell sampling by either cervicovaginal lavage followed by scrape-Cytobrush or, conversely, scrape-Cytobrush followed by cervicovaginal lavage. Restriction analysis and Southern blot hybridization were used to test all the samples thus obtained for human papillomavirus. Overall, test results from 42 patients (56.8%) were positive for human papillomavirus deoxyribonucleic acid. Twenty-six (31.1%) tested positive for human papillomavirus by both sampling methods, and 32 (43.2%) tested negative for human papillomavirus by both methods. One (1.4%) tested positive with scrape-Cytobrush sampling but negative with cervicovaginal lavage, while 15 (20.3%) tested negative with scrape-Cytobrush but positive with cervicovaginal lavage ($p < 0.001$, McNemar's test). These data, combined with previous work from our group, suggest that, of the available methods, cervicovaginal lavage, coupled with human papillomavirus deoxyribonucleic acid hybridization, is the most sensitive noninvasive method for harvesting cells for molecular identification of human papillomavirus in the female lower genital tract. (Am J Obstet Gynecol 1989;161:1669-72.)

Key words: Papillomavirus, cervix diseases, epidemiology

The development of an accurate method for the detection and typing of genital human papillomavirus (HPV) is of substantial clinical importance. Increasing research and mounting evidence have suggested that HPV infection of the cervix is associated with the development of cervical neoplasia.[1, 2] A variety of HPV types is commonly found in cervical cancer tissues, premalignant cervical lesions, and, less commonly, in normal cervical epithelium.[3-8]

At present, HPV cannot be propagated in cell culture or reliably identified by immunologic methods. According to Schneider,[9] only the detection of HPV deoxyribonucleic acid (DNA) gives direct confirmation of the presence or absence of the virus. Various methods of HPV DNA identification have previously been described: dot blot, filter in situ hybridization, Southern blot hybridization, and, recently, polymerase chain reaction.[9-11] Restriction enzyme analysis, coupled with Southern blot hybridization, appears to be the currently preferred method for the detection of HPV DNA.[9]

The clinical sampling technique used to collect the material for DNA analysis is important. A noninvasive test offers the potential to screen a large number of patients. Routine Papanicolaou smear techniques do not always yield enough exfoliated epithelial cells for HPV assessment by DNA hybridization. We have previously compared the scrape-swab technique with cervicovaginal lavage for the collection of exfoliated cells from the female lower genital tract.[12] The cervicovaginal lavage cell collection technique is significantly more sensitive than the scrape-swab technique for assessing HPV infection. The Cytobrush cell collection instrument (Zelsmer) also have recently been shown to be more effective than a cotton-tip swab for obtaining endocervical cells for routine Papanicolaou screening.[13-15] We compared the Cytobrush with the cervicovaginal lavage for sampling and collection of exfoliated cervicovaginal cells.

Methods

Seventy-four patients referred to a City Hospital colposcopy clinic in the Bronx, New York, were enrolled

From the Departments of Obstetrics and Gynecology,[a] Epidemiology and Social Medicine,[b] Pediatrics,[c] and Microbiology and Immunology,[d] Albert Einstein College of Medicine/Montefiore Medical Center, Bronx, and the Environmental Epidemiology Branch,[e] National Cancer Institute, Bethesda.[e]

Supported in part by National Institutes of Health Contract No. 263-MD-706528-2. Dr. Ritter is supported by National Institutes of Health, National Cancer Institute Training Grant No. CA-09173.

Presented at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.

Reprint requests: Dr. Gary L. Goldberg, Department of Obstetrics and Gynecology, Albert Einstein College of Medicine, Room 709, BMHC, Pelham Parkway and Eastchester Road, Bronx, NY 10461.

6/6/15668

CALCUTTA CENTRAL LIBRARY

Table IA. Ordering effects on cell sample recovery by the scrape-Cytobrush method

Order of collection	Cell volume		Total
	0-5 μl	6-50 μl	
Scrape-Cytobrush first*	21 (58%)	15 (42%)	36
Scrape-Cytobrush second	18 (47%)	20 (53%)	38

*$p = 0.5$ by χ^2.

Table IB. Ordering effects on cell sample recovery by the cervicovaginal lavage method

Order of collection	Cell volume			Total
	10-50 μl	51-100 μl	101-500 μl	
Cervical lavage first*	21 (55%)	11 (29%)	6 (16%)	38
Cervical lavage second	16 (44%)	9 (25%)	11 (31%)	36

*$p = 0.3$ by χ^2 with Yates' continuity correction.

Table II. HPV status with respect to sampling technique

	Cervicovaginal lavage, HPV pos.	Cervicovaginal lavage, HPV neg.	Total
Cytobrush, HPV pos.	26	1	27
Cytobrush, HPV neg.	15	32	47
TOTAL	41	33	74

$p < 0.005$ by McNemar's test.

in this study, which was approved by the hospital's institutional review board. All patients were examined by a single gynecologist (G. L. G.). Standard Papanicolaou smears, obtained by use of a cotton-tip swab and then a spatula, were obtained for cytologic study, after which both study methods (scrape-Cytobrush and cervicovaginal lavage) were used to collect cells for HPV testing. Patients were randomly divided into two groups, according to the last digit of their medical record numbers. Thirty-six of the 74 patients had scrape-Cytobrush sampling followed by cervicovaginal lavage, while 38 had cervicovaginal lavage followed by scrape-Cytobrush. Under this method, each woman served as her own control, and ordering effects could be assessed by comparison of the two groups. For the scrape-Cytobrush technique, a Cytobrush was inserted and rotated 360 degrees twice inside the endocervical canal. A wooden cervical spatula was then rotated 360 degrees twice around the external cervical os and squamocolumnar junction. The Cytobrush and the spatula tip were then vigorously shaken to dislodge cells into 10 ml of a 0.9% sodium chloride–50 mmol/L ethylenediaminetetraacetic acid (EDTA) solution in a 50 ml polypropylene tube. A 10 ml syringe with a 2-inch, 18-gauge Teflon extension catheter was used to spray 10 ml of sterile saline solution against the external cervical os and the ectocervix. The fluid that pooled in the posterior vaginal fornix was aspirated into the syringe and transferred to a 50 ml polypropylene tube containing 1.0 ml of 0.5 mol/L EDTA. Specimens collected by both methods were refrigerated at 4° C until processing. Cellular material was quantitated by determining the packed volume of cells after centrifugation at 13,000 rpm for 5 minutes. DNA was then extracted as previously described and resuspended in 30 μl of TE (10 mmol/L Tris buffer, pH 8.;0, and 0.1 mmol/L EDTA).[7]

For the detection of HPV DNA, 10 μl of the unknown sample DNA solution was digested with the *Pst* I restriction enzyme according to conditions specified by the supplier, New England Biolabs. DNA fragments were separated by electrophoresis in 0.8% Agarose slab gels. DNA was visible after staining with ethidium bromide and was photographed under ultraviolet light. Southern blot hybridization was then performed with a mixed probe of HPV-11, HPV-16, and HPV-18 DNA inserts labeled with phosphorus 32.[7] After exposures ranging from 2 to 14 days, the autoradiographs were interpreted by researchers who did not know the origin of the specimens.

To assess the statistical significance of the differences in HPV diagnosis between scrape-Cytobrush and cervicovaginal lavage techniques, we used McNemar's test for correlated proportions.[16] A χ^2 analysis, with the Yates continuity correction where appropriate, was used for testing the significance of differences between cell volumes in samples obtained by cervicovaginal lavage after scrape-Cytobrush, as compared with those obtained by scrape-Cytobrush after cervicovaginal lavage.[16]

Results

More cellular material was obtained with cervicovaginal lavage than with the scrape-Cytobrush method. The median cell volume for scrape-Cytobrush was 5 μl, the mean volume was 11.2 ± 10.4 μl (±SD), and the range was 2 to 50 μl. The median cell volume for cervicovaginal lavage was 62.5 μl, the mean volume was 96.0 ± 96.5 μl (±SD), and the range was 5 to 500 μl. The cervicovaginal lavage method yielded a twelvefold greater median cell volume of recovered sample and provided more cellular material in 66 of the 74 patients (89.2%). The same yield in microliters was noted in 8 of the 74 patients (10.8%). All specimens, irrespective of collection method, contained visible cell pellets.

We were concerned that repeated collection of cervicovaginal cells during a single examination would in-

fluence the cell yield and the possibility of the HPV DNA detection, so we compared the order of testing and the cell sample size. The ordering effects on cell sample recovery by the two collection methods can be seen in Tables IA and IB. There was no statistically significant correlation between test order and amount of cellular material collected by cervicovaginal lavage or scrape-Cytobrush ($p = 0.3$ and 0.5, respectively). A lower overall recovery was obtained with scrape-Cytobrush regardless of the order of the tests.

Twenty-six of the 74 patients' (35.1%) samples collected by both the scrape-Cytobrush and the cervicovaginal lavage techniques revealed HPV DNA (Table II). Thirty-two of the 74 (43.2%) samples collected by both techniques tested negative for HPV DNA. In 15 of the 74 cases (20.2%) the virus was detected in the cervicovaginal lavage sample, but not in the scrape-Cytobrush sample. In one case HPV was detected in the scrape-Cytobrush sample alone. The discordant findings are unlikely to have occurred by chance alone ($p < 0.001$ by McNemar's test).

Southern blot analysis was used to determine the presence and type of HPV DNA, as illustrated in Fig. 1. The virus types detected in the 42 women whose sample tested positive for HPV DNA were consistent between the two techniques. Twenty-four of the 26 patients (92.3%) who tested positive for HPV with both sampling techniques had the same HPV DNA types in the cervicovaginal lavage and scrape-Cytobrush samples. In the 15 samples that were positive with cervicovaginal lavage only, the virus type identified was consistent for cervical infection and for the population being studied (Burk RD, et al., unpublished data). Overall, in this study type 16 was the most prevalent HPV type, a finding consistent with our previous results.[12]

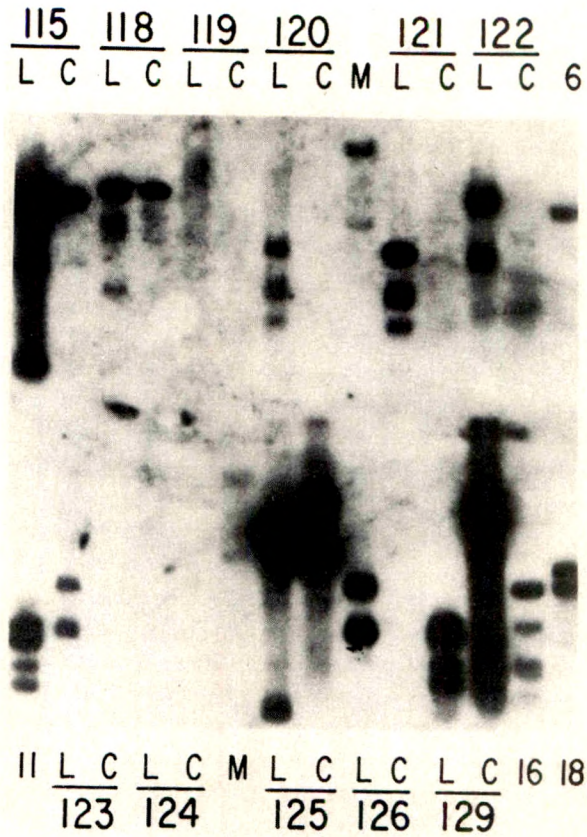


Fig. 1. Detection of HPV DNA by *Pst* I restriction analysis and Southern blot hybridization. DNA samples were isolated from cells collected by either lavage *(L)* or Cytobrush *(C)*, digested with *Pst* I, separated by gel electrophoresis, and transferred to a Nytran filter (Schleicher and Schuell). The filter was hybridized with a mixed probe of HPV-11, HPV-16, and HPV-18 DNA labeled with ^{32}P and washed under stringent conditions (TM-10 C). Patient number is shown above the *solid line*. A total of 10 to 20 pg of gel-isolated linear HPV DNA was mixed with 5 μg of genomic DNA, digested with *Pst* I, and run as hybridization sensitivity control (equivalent to approximately two viral genomes per cell). The lanes containing HPV-6, HPV-11, HPV-16, or HPV-18 DNA are indicated. The *L* and *C* samples from patients *115* and *118* contained the same type of HPV DNA, whereas the *L* sample was positive and the *C* negative in No. *120*, No. *121*, No. *122*, and No. *123*. The DNA samples from the *C* specimen of No. *125* and No. *129* were incompletely digested and the HPV type could not be determined from this blot. Interpretation of HPV types from this blot indicate: patient *115*, HPV-18 and HPV-14; patient *118*, related HPV; patient *120*, HPV-16; patient *121*, HPV-16; patient *122*, HPV-45; patient *123*, HPV-31; patient *125*, HPV-18 and related HPV; patient *126*, HPV-31; patient *129*, HPV-11.

Comment

The definitive role of HPV in cervical neoplasia remains unresolved. Mounting evidence links or associates HPV infection with premalignant and malignant lesions of the cervix.[3-8] Specific HPV DNA types are commonly found in biopsy specimens of cervical carcinoma and cervical intraepithelial neoplasia. Certain HPV types contain genetic information capable of transforming cells in culture, although this oncogenic potential has not been unequivocally demonstrated in vivo.[6] Accurate documentation of the HPV status is of epidemiologic and potential clinical importance.

The Cytobrush is a device specifically designed for sampling the endocervical canal. It has been used to collect clinical samples for testing for chlamydia and other sexually transmitted diseases. Various authors have reported improved endocervical cell collection when they used the Cytobrush instead of a cotton-tip swab for Papanicolaou smears.[13-15] No samples collected by the scrape-Cytobrush technique were labeled "quantity not sufficient for DNA evaluation." Recent data suggest that standard techniques obtain a greater proportion of insufficient cell yields from patients with cervical lesions, compared with samples collected by the

same methods from patients who do not have lesions.[22] This bias is partially mitigated by the use of scrape-Cytobrush and further minimized with the cervicovaginal lavage technique. We were aware that obtaining a Papanicolaou smear before the HPV sampling method might affect the cell collection. Moreover, it could have had more effect on the cell count in samples collected by the scrape-Cytobrush method, in comparison with the cervicovaginal lavage method, because cervicovaginal lavage could have been collecting cells that had already exfoliated into the vaginal fornix. Alternatively, cervicovaginal lavage might have been picking up HPV in vaginal cells, the same types that were infecting the cervix. In practice, many clinicians are not willing to collect cells for HPV determination before the Papanicolaou smear because doing so might compromise the cytologic result. Since HPV tresting is being promoted primarily as an adjunct to the Papanicolaou smear and is usually performed at the same examination, we therefore included a standard Papanicolaou smear before scrape-Cytobrush and cervicovaginal lavage.

The multifocal potential of the squamous epithelium of the lower genital tract for intraepithelial neoplasia and carcinoma is of clinical and epidemiologic significance. Sampling that includes the cervix, endocervix, and vaginal fornices increases the yield of exfoliated cells and the sensitivity of HPV DNA detection while reducing the number of false-negative tests.

We conclude that substitution of the scrape-Cytobrush method for the scrape-swab method improved the cell yield for HPV DNA detection. However, our study reaffirmed that cervicovaginal lavage is the most sensitive collection method for exfoliated cervicovaginal cells for the detection of HPV and should replace the standard or routine scrape techniques currently in use, including the Cytobrush.

We thank Mrs. Audrey Mack for the preparation of the manuscript.

REFERENCES

1. Syrjanen KJ. Papillomavirus infections and cancer. In: Sryjanen KJ, Gissmann L, Koss LG, eds. Papillomaviruses and human disease. Berlin: Springer-Verlag, 1987:467-503.
2. Koss LG. Carcinogenesis in the uterine cervix and human papillomavirus infection. In: Syrjanen KJ, Gissmann L, Koss LG, eds. Papillomaviruses and human disease. Berlin: Springer-Verlag, 1987:257-62.
3. Kadish AS, Burk RD, Fress Y, Calderin Sl, Romney SL. Human papillomaviruses of different types in precancerous lesions of the uterine cervix: histologic, imunocytotechnical and ultrastructural studies. Hum Pathol 1986; 17:384-92.
4. Boshart M, Gissmann L, Ikenberg H, Kleinheinz A, Scheurlen W, zur Hausen H. A new type of papillomavirus DNA, its presence in genital cancer biopsies and in cell lines from cervical cancer. Int J Cancer 1984;3: 1151-7.
5. Ritter DB, Kadish AS, Vermund SH, Romney SL, Villari D, Burk RD. Detection of human papillomavirus dexoyribonucleic acid in exfoliated cervicovaginal cells as a predictor of cervical neoplasia in a high-risk population. Am J Obstet Gynecol 1988;159:1517-25.
6. Reid R, Crum C, Herschman BR, et al. Genital warts and cervical cancer. III. Subclinical papillomavirus infection and cervical neoplasia are linked by a spectrum of continuous morphologic and biologic change. Cancer 1984;53:943-53.
7. Burk RD, Kadish AS, Calderin S, Romney SL. Human papillomavirus infection of the cervix detected by cervicovaginal lavage and molecular hybridization: correlation with biopsy results and Papanicolaou smear. Am J Obstet Gynecol 1986;154:982-9.
8. Crum CP, Ikenberg H, Richart RM, Gissmann L. Human papillomavirus and early cervical neoplasia. N Engl J Med 1984;310:880-3.
9. Schneider A. Methods of identification of human papillomaviruses. In: Syrjanen K, Gissman L, Koss LG, eds. Papillomaviruses and human disease. Berlin: Springer-Verlag, 1987:19-39.
10. Shibata D, Fu YS, Gupta JW, Shah KV, Arnheim N, Martin WJ. Detection of human papillomavirus in normal dysplastic tissue by the polyermase chain reaction. Lab Invest 1988;69:555.
11. Morris BJ, McKinnon KJ, Nightingale BN. Papillomavirus screening of cervical lavages by polymerase chain reaction. Lancet 1988;1:1368.
12. Vermund SH, Schiffman MH, Goldberg GL, Ritter DB, Weltman A, Burk RD. Molecular diagnosis of genital human papillomavirus infection: comparison of two methods used to collect exfoliated cervical cells. Am J Obstet Gynecol 1989;160:304-8.
13. Taylor PT Jr, Andersen WA, Barber SR, Covell JL, Smith EB. The screening papanicolaou smear: contribution of the endocervical brush. Obstet Gynecol 1987;70:734-8.
14. Trimbos JB, Arentz NPW. The efficiency of the Cytobrush versus the cotton swab in the collection of endocervical cells in cervical smears. Acta Cytol 1986;30:261.
15. Boon M, Alonsl-van Kordelaar JJLM, Rietveld-Scheffers PEM. Consequences of the introduction of combined spatula and Cytobrush sampling for cervical cytology: improvements in smear quality and detection rates. Acta Cytol 1986;30:264.
16. Fleiss JL. Statistical methods for rates and proportions. New York: John Wiley, 1981.
17. Breitburd FV. Cell culture systems for the study of papillomaviruses. In: Syrjanen K, Gissman L, Koss LG, eds. Papillomaviruses and human disease. Berlin: Springer-Verlag, 1987:385-8.
18. Ros E, Jiminez AM, Vilapana E, et al. New technique for endocervical sampling with Stormby's brush; preliminary results. Citologia 1983;3:9.
19. Schiffman MH, Kurman Rl, Barnes W, et al. A case-control study of type-specific HPV infection, smoking, and risk of early CIN. In: Sixth international papillomavirus workshop. Washington DC: Georgetown University, 1987:24.

Spontaneously resorbing ectopic pregnancy: Preservation of human chorionic gonadotropin bioactivity despite declining steroid hormone levels

Mark V. Sauer, MD,[a] Robert E. Anderson, MD,[b] Michael Vermesh, MD,[a] Bronte A. Stone, PhD,[c] and Richard J. Paulson, MD[a]

Los Angeles, California

We compared serum hormone profiles of patients with normal intrauterine pregnancies (n = 14), spontaneously resorbing ectopic pregnancies (n = 10), and viable ectopic pregnancies (n = 26). Hormone profiles were evaluated at 5 to 8 weeks' gestational age. Ectopic pregnancies were diagnosed by laparoscopy; intrauterine gestations were confirmed by ultrasonography. Immunoreactive β–human chorionic gonadotropin, progesterone, estradiol, and 17-hydroxyprogesterone were measured by radioimmunoassay. Bioactive human chorionic gonadotropin was measured by a modified mouse Leydig cell bioassay. Diminished steroid production was noted in ectopic pregnancies; levels in serum of patients with resorbing ectopic pregnancies were lower than values expressed in viable ectopic pregnancies ($p < 0.01$). Serum levels of human chorionic gonadotropin bioactivity correlated closely with immunoreactive human chorionic gonadotropin in all three groups ($r = 0.81$, $p < 0.01$). Ratios of bioactive human chorionic gonadotropin to immunoreactive β–human chorionic gonadotropin were similar (0.93 ± 0.26 in resorbing ectopic pregnancies, 1.11 ± 0.16 in viable ectopic pregnancies, and 0.90 ± 0.10 in intrauterine pregnancies). We conclude that although reduced serum levels of steroids noted in ectopic pregnancy suggest an impairment in corpus luteum activity, diminished steroid production was not attributable to lower human chorionic gonadotropin bioactivity. (Am J Obstet Gynecol 1989;161:1673-6.)

Key words: Ectopic pregnancy, progesterone, bioactive human chorionic gonadotropin

Whereas serum levels of human chorionic gonadotropin (hCG) are generally lower in women with ectopic pregnancies than in normal intrauterine gestations, levels of sex steroids are proportionately lower, which facilitates rapid discrimination of normal or abnormal gestations.[1,2] Suggested reasons for diminished serum steroid content include altered hCG bioactivity, perturbed steroidogenesis by the corpus luteum, absence of a still-uncharacterized ovarian stimulator of steroid biosynthesis, and alterations in hCG glycosylation.[3] We previously described the clinical course of women with ectopic pregnancies destined to resolve spontaneously without intervention.[4] In this population, ovarian steroid production was noted to be depressed; progesterone and 17-hydroxyprogesterone levels in serum were <0.8 ng/ml. Because the half-life of steroid hormones in serum is shorter than that of hCG, it is possible that low peripheral levels of steroids that originate from the corpus luteum reflect diminished luteotropic support of hCG (bioactive/immunoreactive ratio) before its clearance from the circulation. This study investigates this latter possibility by examination of the relationship between bioactive hCG and immunoreactive hCG, and the markers of corpus luteum function in women with differing pregnancy outcomes.

From the Departments of Obstetrics and Gynecology, University of Southern California,[a] Cedars-Sinai/University of California Los Angeles,[b] and Hospital of the Good Samaritan.[c]

Presented at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.

Reprint requests: Mark V. Sauer, MD, Women's Hospital, 1240 N. Mission Road, Room L946, Los Angeles, CA 90033.

6/6/15669

Material and methods

Patients. A total of 36 women with laparoscopically visualized ectopic pregnancies were studied. All pregnancies were between 30 and 56 days' gestation (mean ± SE, 42 ± 4 days) at the time of diagnosis, based on recollection of the last menstrual period. Within this population, 10 women were enrolled in a study of nonsurgical treatment as previously described,[5] based on the following criteria: (1) tubal pregnancy <3 cm diameter; (2) tubal serosa intact; (3) no active bleeding present at time of diagnosis; and (4) declining serum β-hCG levels as measured by radioimmunoassay (RIA). All patients in this group went on to resolve their ectopic pregnancies without medical or surgical intervention. The remaining 26 cases were termed *viable ectopic pregnancies* and were treated surgically in standard fashion. A control group comprised 14 women with normal intrauterine pregnancies of similar gestational age. All patients were followed up until

Table I. Hormonal characteristics of all patients

Group	*Progesterone (ng/ml)*	*Estradiol (pg/ml)*	*17-Hydroxyprogesterone (ng/ml)*	*Human chorionic gonadotropin (miu/ml)*	*Ratio Bioactive/immunoreactive hCG*
Resorbing ectopic pregnancy (range)	0.6 ± 0.1 (0.2-0.7)	35 ± 7 (20.1-164.2)	0.6 ± 0.1 (0.2-0.8)	192 ± 132 (10-1050)	0.9 ± 0.26
Ongoing ectopic pregnancy (range)	8.2 ± 1.5* (0.2-42.2)	153 ± 24* (23-601)	2.4 ± 0.5* (0.2-9.2)	3842 ± 1253* (951-6422)	1.1 ± 0.16
Intrauterine pregnancy (range)	28.2 ± 2.8† (12.6-53.9)	606 ± 54† (275-1879)	5.8 ± 0.8† (2.7-13.2)	19873 ± 4724† (6972-30073)	0.9 ± 0.10

Mean ± SEM.

*$p < 0.01$ versus resorbing ectopic pregnancy.

†$p < 0.01$ versus ongoing ectopic pregnancy.

intrauterine cardiac activity was noted by transvaginal ultrasonographic examination. Aliquots taken from sera obtained from patients at the time of pregnancy diagnosis were stored at −20° C until assayed. Blood samples from ectopic gestations were drawn preoperatively.

Radioimmunoassays. Serum β-hCG, estradiol, progesterone, and 17-hydroxyprogesterone were measured in duplicate with commercial RIAs (ICN Biomedical Inc., Carson, Calif.). Validation of these assays has been detailed elsewhere.[6, 7] Intra-assay coefficients of variation were <12% for all hormones studied. Values of immunoreactive β-hCG are reported in terms of the World Health Organization First International Reference Preparation (75/537).

Bioassay of hCG. Serum hCG bioactivity was measured by the modified mouse Leydig cell assay.[8] Briefly, adult mice (strain B6BFL) were killed and the testes were removed. After mechanical dispersion, aliquots (100 μl) of cell suspension that contained approximately 200,000 viable Leydig cells were combined in triplicate with 100 μl of diluted sample or standard hCG (iodination grade, 15,000 IU/mg; Sigma Chemical Company, St. Louis) diluted in culture medium (Dulbecco's Modified Eagle's, GIBCO, New York, N.Y.). After incubation for 3 hours at 34° C, 50 μl aliquots of supernatant were assayed for testosterone content by a direct RIA (Pantex, Santa Monica, Calif.), sensitive to 5 pg testosterone per tube. Average coefficients of variation for testosterone assayed were <10%. Levels of bioactive hCG in individual samples were derived from curves fitted to hCG concentrations in the standards (calculations performed on an IBM-XT based 3001 Quality Control System, Iso-Data Inc., Rolling Meadows, Ill.). Average intra-assay coefficients of variation for the hCG bioassay were <10% and interassay coefficients of variation were <16%.

Statistics. Means of distributions of immunoreactive β-hCG, bioactive hCG, progesterone, estradiol, and 17-hydroxyprogesterone were compared with the nonparametric method of Mann-Whitney. Least squares linear regression correlation coefficients were calculated on log transformed data. Statistical significance was defined as the 5% level for two-tailed data.

Results

Table I lists hormonal values obtained in the three groups of women. Despite similar gestational age, levels of immunoreactive β-hCG and all steroid hormones were significantly lower in ectopic pregnancies than in normal intrauterine gestations. Levels of all hormones in serum of patients whose gestations were destined to resorb spontaneously were significantly lower than corresponding levels of hormone in patients with viable ectopic pregnancies, although some overlap did exist between value ranges.

Overall, measurements of bioactive hCG and immunoreactive β-hCG correlated closely ($r = 0.81$, $p < 0.01$; Fig. 1). As noted in Table I, ratios of bioactive hCG/immunoreactive β-hCG were not dissimilar among groups, and ratios were near unity.

Comment

Heterogeneity has been appreciated in the clinical presentation and course of ectopic pregnancy since Lund first reported on spontaneous resolution in the 1950s.[9] More recently, several authors have reported successful expectant management of selected cases of laparoscopically confirmed ectopic gestations.[10, 11] The concepti are characteristically small in size (<3 cm), ampullary in location, and produce low levels of immunoreactive β-hCG (<1000 mIU/ml). It remains uncertain why some ectopic implantations of trophoblast continue to proliferate, invade the tubal epithelium, and progress to tubal rupture, whereas others remain confined, relatively dormant, and are destined to abort or resorb. Physician intervention may occur any time along this continuum, and there appears to be no easy way to anatomically discriminate between ectopic pregnancies that require treatment and those that would spontaneously resolve.

During the initial course of normal pregnancy the

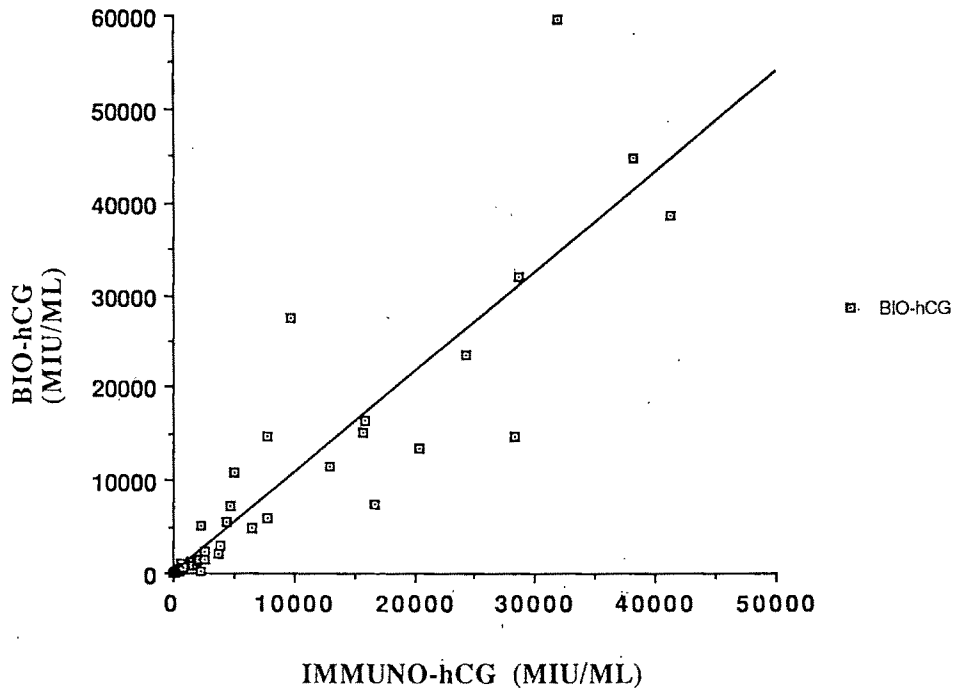


Fig. 1. Correlation of bioactive hCG to immunoreactive β-hCG for all values ascertained ($r = 0.81$, $p < 0.01$).

corpus luteum releases large amounts of sex steroid in response to hCG secreted by the developing trophoblast. However, by 8 weeks' gestation the placenta begins to contribute the majority of steroid hormone, which diminishes further need for corpus luteum support to the developing pregnancy. Although both the placenta and ovary secrete estradiol and progesterone, it is believed that the low levels of steroid hormone seen in abnormal gestations result from aberrant corpus luteum secretion because most ectopic pregnancies are detected before the time of the luteoplacental shift.

Our previous data[1] support suggestions that a persistent downward trend of immunoreactive β-hCG is characteristic of small unruptured ectopic pregnancies destined to resorb. In cases in which the ectopic pregnancy could be visualized, we did not observe tubal rupture during the time that preceded clearance of immunoreactive hormone. Furthermore, as confirmed in this report, we noted a relative dissociation between the clinical course of pregnancy and luteal function. In medically managed patients, a decline in the functional activity of the corpus luteum always preceded the decline in hCG, usually several days in advance of the eventual resolution of the ectopic pregnancy.[2] We attributed this to the diminished synthesis of the steroid hormones, and thought it probable that a reduction in hCG bioactivity could account for this phenomenon. However, as indicated by this report, hCG bioactivity did not differ between the outcome groups.

The ratio of bioactive hCG/immunoreactive hCG has previously been shown to remain constant (near 1:1) throughout normal pregnancy.[12] This also has been demonstrated in an unselected group of ectopic gestations matched to subjects with similar concentrations of immunoreactive hCG.[3] Such findings imply the lower serum steroid levels noted in ectopic gestations are a consequence of abnormal ovarian response to luteotropin (i.e., hCG) rather than diminished hCG activity. We chose not to match subjects by hCG levels because these are very low in resorbing ectopic pregnancy and require controls to be of gestational ages different from the ectopic group. We were more interested in the determination of whether degeneration of the resorbing ectopic pregnancy was associated with reduced bioactivity of its secreted hCG, which could differentiate it endocrinologically from viable proliferating ectopic pregnancies. Our data imply that other factors that modulate luteal function may be altered in or absent from ectopic gestations. Accordingly, peptides such as oxytocin and relaxin have been found in the corpus luteum and may function as paracrine or autocrine modulators of luteal cell steroidogenesis.[13] Alternatively, an improper amount or rate of increase of bioactive hCG may desensitize the corpus luteum to hCG with a subsequent decline in steroid production.

In conclusion, it appears that during the course of spontaneous resorption of ectopic pregnancies immunoreactive hCG levels decline, probably as a result of trophoblastic cell death. Bioactivity of hCG persists during this decline, and even at very low levels parallels

hCG immunoreactivity. This suggests that hCG remains bioactive while being cleared from the circulation during the final stages of secretion by degenerating trophoblast. Despite continuous production of hCG, steroid secretion by the corpus luteum is characteristically low or absent, which indicates prior dissociation from the trophoblastic hCG signal. However, the mechanism of this dissociation remains to be elucidated.

REFERENCES

1. Yeko TR, Gorrill MJ, Hughes LH, Rodi IA, Buster JE, Sauer MV. Timely diagnosis of early ectopic pregnancy using a single blood progesterone measurement. Fertil Steril 1987;48:1048-50.
2. Sauer MV, Vermesh M, Anderson RE, Vijod AG, Stanczyk FZ, Lobo RA. Rapid measurement of urinary pregnanediol glucuronide to diagnose ectopic pregnancy. Am J Obstet Gynecol 1988;159:1531-5.
3. Norman RJ, Buck RH, Kemp MA, Joubert SM. Impaired corpus luteum function in ectopic pregnancy cannot be explained by altered human chorionic gonadotropin. J Clin Endocrinol Metab 1988;66:1166-70.
4. Sauer MV, Gorrill MJ, Rodi IA, Yeko TR, Buster JE. Corpus luteum activity in tubal pregnancy. Obstet Gynecol 1988;71:667-70.
5. Sauer MV, Gorrill MJ, Rodi IA, et al. Nonsurgical management of unruptured ectopic pregnancy: an extended clinical trial. Fertil Steril 1987;48:752-5.
6. Ratcliffe WA, Corrie JET, Dalziel AH, Macpherson JS. Direct I125-radioligand assays for serum progesterone compared with assays involving extraction of serum. Clin Chem 1982;28:1314-8.
7. Jurgen H, Pratt JJ, Woldring MG. Radioimmunoassay of plasma estradiol without extraction and chromatography. J Clin Endocrinol Metab 1975;40:19-25.
8. Stone BA, Tan TT, Koopersmith TB, Quinn K, Marrs RP. Luteotropic activity in serum of women following embryo or gamete transfer in a program of assisted conception. J In Vitro Fert Embryo Transfer 1988;5:275-81.
9. Lund JJ. Early ectopic pregnancy. J Obstet Gynaecol Br Comm 1955;62:70-5.
10. Garcia AJ, Aubert JM, Sama J, Josimovich JB. Expectant management of presumed ectopic pregnancies. Fertil Steril 1987;48:395-400.
11. Fernandez H, Rainhorn JD, Papiernik E, Bellet D, Frydman R. Spontaneous resolution of ectopic pregnancy. Obstet Gynecol 1988;71:171-4.
12. Mandanes AE, Fencl M de M, Tulchinsky D. Comparison of human chorionic gonadotropin measurements by radioimmunoassay and in vitro bioassay. Am J Immunol Microbiol 1985;9:52-7.
13. Khan-Dawood FS, Goldsmith LT, Weiss F, Dawood MY. Human corpus luteum secretion of relaxin, oxytocin, and progesterone. J Clin Endocrinol Metab 1989;68:627-31.

Decreased in vitro production of 6-keto-prostaglandin $F_{1\alpha}$ by uterine arteries from postmenopausal women

Alex Steinleitner, MD,[a] Frank Z. Stanczyk, PhD,[a] Jay H. Levin, MD,[a] Gerritt d'Ablaing III, MD,[b] Marcela A. Vijod, BS,[a] Violette L. Shahbazian, BS,[a] and Rogerio A. Lobo, MD[a]

Los Angeles, California

Cessation of ovarian function is associated with a marked increase in morbidity and mortality secondary to ischemic heart disease. Estrogen replacement has been shown to impart protection against ischemic heart disease. We hypothesized that estrogen may influence vascular production of vasodilators such as prostacyclin. To investigate this relationship we have measured the production of 6-keto-prostaglandin $F_{1\alpha}$ and thromboxane B_2 by superfused uterine arteries from pre- and postmenopausal women. Arterial specimens from healthy normotensive premenopausal ($n = 5$) and postmenopausal women ($n = 5$) were superfused for 5 hours. Production of 6-keto-prostaglandin $F_{1\alpha}$ reached steady state levels by 120 minutes and remained linear for the length of the experiment. Indomethacin (4×10^{-5} mol/L) added at 120 minutes significantly decreased prostanoid production. In subsequent experiments, 17β-estradiol in concentrations of 10, 100, 1000 ng/ml was added to the superfusion media at 120 minutes. Total production of 6-keto-prostaglandin $F_{1\alpha}$ by premenopausal arteries superfused with neat media during the steady state interval (3 hours) was significantly greater than that of postmenopausal specimens (1.25 versus 0.27 ng/mg dry tissue, $p < 0.05$). Thromboxane B_2 levels were undetectable in spent media. However, the addition of 17β-estradiol did not alter production of 6-keto-prostaglandin $F_{1\alpha}$. These data suggest that arterial production of prostacyclin is significantly decreased in uterine arteries from postmenopausal women, but in this in vitro model system estrogens did not affect vascular prostanoid production. (Am J Obstet Gynecol 1989;161:1677-81.)

Key words: Estrogen replacement therapy, prostacyclin, ischemic heart disease, menopause

Loss of ovarian function is known to be associated with a dramatic increase in the incidence of ischemic heart disease,[1] which is the leading cause of death in postmenopausal women.[2] Estrogen replacement therapy is known to ameliorate the risk of ischemic heart disease in these patients, reducing the mortality of myocardial infarction by as much as 50%.[3, 4] Given these data, it has been suggested that protection of postmenopausal women against the development of ischemic heart disease should be the primary indication for the administration of estrogen replacement therapy.[5]

Although beneficial changes in lipids and lipoproteins have been offered as an explanation for the cardioprotection associated with estrogen replacement therapy, emerging evidence suggests that other factors may play an important role in this phenomenon.[6] Estrogens are known to enhance blood flow in many tissues.[7, 8] This may be a result of alterations in the relationship between vasodilatory and vasoconstrictive mediators, resulting in enhanced coronary perfusion and decreased systemic vascular resistance. Prostacyclin (PGI_2) is a potent vasodilator known to play a central role in the regulation of vascular tone. Additionally, the local balance between PGI_2 and thromboxane A_2 (TxA_2) is thought to impact on platelet aggregation and thrombus formation.

We postulated that one consequence of menopause may be reduced vascular production of PGI_2. It is conceivable that alterations in the regulation of PGI_2 metabolism by gonadal steroids might impact significantly on the development and progression of ischemic heart disease. To test this hypothesis we used an in vitro system to measure the production of PGI_2 and TxA_2 metabolites by uterine arteries obtained from pre- and postmenopausal women.

Material and methods

Subjects. Uterine arteries were obtained from pre- and postmenopausal women ($n = 5$ per group) undergoing elective hysterectomy because of benign gy-

From the Departments of Obstetrics and Gynecology, Division of Reproductive Endocrinology[a] and Pathology,[b] University of Southern California School of Medicine.

Supported in part by the 1988-89 American College of Obstetricians and Gynecologists CIBA Fellowship for Research in Endocrinology of Postreproductive Women (A. S.).

Presented at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.

Reprint requests: Rogerio A. Lobo, MD, Women's Hospital, 1240 N. Mission Road, Room 1M2, Los Angeles, CA 90033

6/6/16243

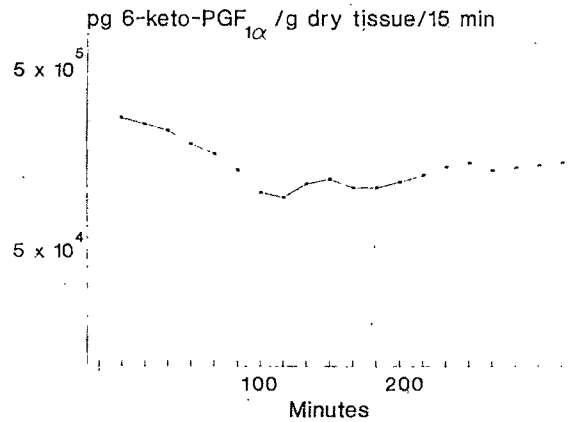


Fig. 1. Typical production curve for in vitro production of 6-keto-$PGF_{1\alpha}$ by uterine artery obtained from premenopausa. woman.

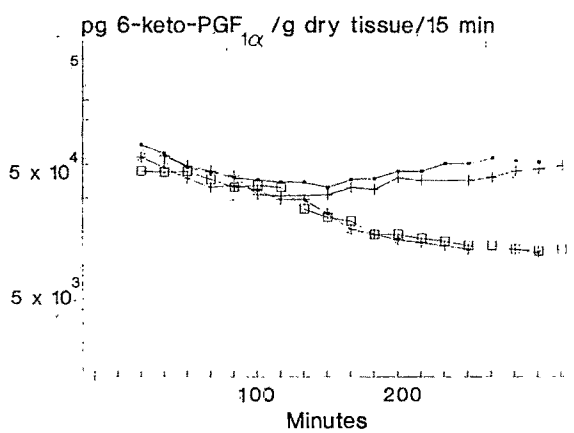


Fig. 2. Inhibition of 6-keto-$PGF_{1\alpha}$ production by uterine arteries from two premenopausal patients after addition of indomethacin (4×10^{-5} mol/L) to superfusate (control, *crosses, dots;* indomethacin, *stars, boxes*).

necologic indications. Mean age of premenopausal women was 36.5 ± 2.2 (±SEM) years, and of postmenopausal women was 62.4 ± 3 years. All subjects were known to be healthy and without evidence of significant medical disorders. Specifically absent were diabetes, hypertension, or ischemic heart disease. Patients had not been exposed to exogenous steroids for at least 6 weeks before surgery.

Superfusion procedure. Specimens of uterine artery were carefully dissected from the surrounding connective tissue immediately on removal of the hysterectomy specimen. All specimens were processed within 45 minutes of uterine artery clamping. Specimens of 20 to 40 mg (wet weight) were opened longitudinally with a scalpel blade. Extreme care was taken to ensure that the intima was not separated from the underlying vessel wall.

An in vitro superfusion system, based on the procedure of Mitchell and Flint,[9] was used to maintain the tissue under near physiologic conditions during these short-term evaluations. Uterine artery specimens were placed between 8.0 μm nylon Millipore filters (type C) in individual polypropylene Swinnex chambers (Millipore Corporation, Bedford, Mass.). Specimens were perfused at a flow rate of 0.2 ml/min with minimal essential medium (Gibco Laboratories, Santa Clara, Calif.) gassed with a mixture of 95% oxygen and 5% carbon dioxide. Temperature was maintained at 37° C with a circulating water bath. An automated fraction collector was used to collect spent media at 15-minute intervals over the course of a 5-hour experiment. Samples were stored at −20° C until assayed.

In the course of preliminary experiments, a steady state of prostanoid production was attained after 2 hours. A typical curve for the production of 6-keto-prostaglandin $F_{1\alpha}$ by a uterine artery taken from a premenopausal woman is depicted in Fig. 1. Substances used to perturb the system were added to the superfusate at 2 hours.

Validation. To determine whether the presence of prostanoids in spent media was a result of de novo synthesis by arterial tissue, two preliminary experiments were performed in which the effect of indomethacin on 6-keto-$PGF_{1\alpha}$ production was assessed. In each experiment indomethacin was added to the media of two specimens at a final concentration of 4×10^{-5} mol/L after 120 minutes. Production of 6-keto-$PGF_{1\alpha}$ in the presence of indomethacin was compared with that of untreated controls.

Effect of 17β-estradiol (E_2) on prostanoid production. Specimens from pre- and postmenopausal arteries ($n = 5$ per group) were subjected to four different conditions for 3 hours beginning at 120 minutes, as follows:

1. Control, superfusion with neat media
2. Superfusion with media + E_2 10 ng/ml
3. Superfusion with media + E_2 100 ng/ml
4. Superfusion with media + E_2 1000 ng/ml.

Radioimmunoassay. The stable metabolites of PGI_2 and TxA_2 (6-keto-$PGF_{1\alpha}$ and TxB_2, respectively) were measured in superfusate by means of direct radioimmunoassays developed in our laboratory. Highly specific antibodies for 6-keto-$PGF_{1\alpha}$ and TxB_2 were provided by Dr. Carlo Patrono, Universita Cattolica del Sacro Cuore, Rome, Italy. Relative cross-reaction to the anti-6-keto-$PGF_{1\alpha}$ serum was <1% for $PGF_{2\alpha}$, 2,3-dinor-6-keto-$PGF_{1\alpha}$, PGE_2, PGD_2, TxB_2, and arachidonic acid; cross-reaction to the anti-TxB_2 serum was 10.5% for 2,3-dinor-TxB_2, and <1% for 11-dehydro-TxB_2, $PGF_{2\alpha}$, PGD_2 PGE_2, 6-keto-$PGF_{1\alpha}$, and arachidonic acid. ^{3}H-labeled 6-keto-$PGF_{1\alpha}$ (specific activity, 157 Ci/mmol) and TxB_2 (specific activity, 180 Ci/mmol) were purchased from Amersham (Arlington Heights, Ill.). Assay sensitivity was 20 pg/ml for both the 6-keto-$PGF_{1\alpha}$ and

TxB_2 radioimmunoassays. Interassay coefficients of variation were 17.4% and 17.5%, respectively. Intraassay coefficients of variation were 7.1% and 7.9%, respectively.

Histologic evaluation. Hematoxylin and eosin sections of vessels used in the superfusion experiments were taken whenever sample mass permitted. Histologic evaluation confirmed in all cases that specimens were, in fact, arteries.

Protein determination. Protein content of pre- and postmenopausal arteries was determined by the bicinchoninic acid method (Pierce Chemicals, Rockford, Ill.). Pre- and postmenopausal arteries were found to be essentially equivalent in their protein content (0.35 to 0.45 mg protein/mg dry weight). Therefore dry weight of desiccated samples was subsequently used as a denominator to calculate prostanoid production by superfused uterine arteries. Results are expressed in terms of total 6-keto-$PGF_{1\alpha}$ production over the steady state portion of the experiment (3 hours) per milligram of dry weight.

Production kinetics. Production kinetics for 6-keto-$PGF_{1\alpha}$ were estimated by the use of linear regression analysis to estimate the slope of the production curve during the steady state portion of the experiment. Results are expressed in picograms of 6-keto-$PGF_{1\alpha}$ per milligram of desiccated tissue per minute.

Statistical analysis. Analysis of variance and nonparametric statistics (Mann-Whitney test) were used as appropriate to evaluate the data. Data are presented as the mean ± SEM.

Results

Validation experiment. Addition of indomethacin to the superfusate of premenopausal specimens resulted in a prompt and significant decline in 6-keto-$PGF_{1\alpha}$ production (Fig. 2).

Basal prostanoid production by pre- and postmenopausal uterine arteries. In vitro 6-keto-$PGF_{1\alpha}$ production by uterine arteries was observed to vary significantly with menopausal status. Integrated steady state 6-keto-$PGF_{1\alpha}$ production by specimens from premenopausal women was nearly fivefold that of postmenopausal specimens (1.25 ± 0.5 versus 0.27 ± 0.2 ng of 6-keto-$PGF_{1\alpha}$/mg of desiccated tissue per 3 hours, respectively; $p < 0.05$, Mann Whitney test, Fig. 3). Production kinetics for 6-keto-$PGF_{1\alpha}$ were also found to differ significantly with menopausal status ($K_{premenopausal} = +0.002$ versus $K_{postmenopausal} = -0.0011$ pg 6-keto-$PGF_{1\alpha}$/mg desiccated tissue per minute).

Steady state concentrations of TxB_2 in spent media were found to be at or below the detectable limits of the assay (<20 pg/ml).

Effect of E_2 on short-term prostanoid production. Addition of E_2 in concentrations that ranged from 10 to 1000 ng/ml to superfusate, beginning 120 minutes into the experiment, did not significantly affect integrated 6-keto-$PGF_{1\alpha}$ production (p = NS, analysis of variance, Fig. 4) or 6-keto-$PGF_{1\alpha}$ production kinetics in pre- or postmenopausal uterine arteries.

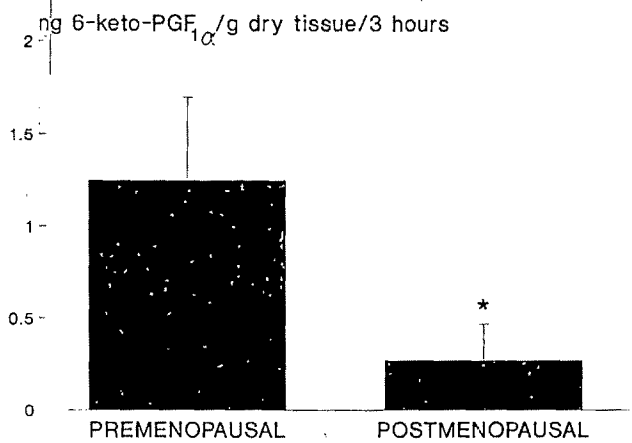


Fig. 3. Total steady state production (3 hours) of 6-keto-$PGF_{1\alpha}$ by uterine arteries from pre- and postmenopausal women. *$p < 0.05$, Mann-Whitney test.

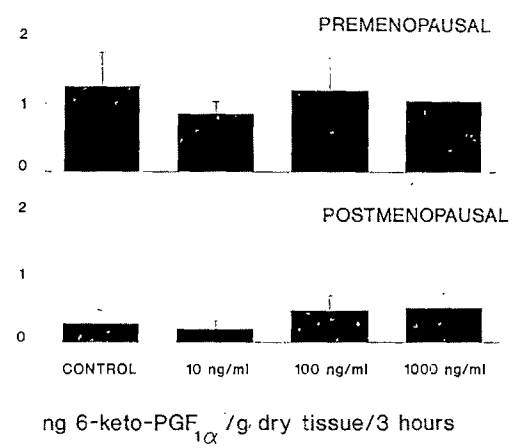


Fig. 4. Effect of 17β-estradiol on total steady state in vitro 6-keto-$PGF_{1\alpha}$ production by uterine arteries from pre- and postmenopausal women.

Comment

The physiologic mechanisms that underly the increased incidence of ischemic heart disease that occurs with menopause and its amelioration by estrogen replacement therapy remain incompletely understood. Alterations in lipoprotein metabolism induced by hypoestrogenism are thought to be the principal effectors of this phenomenon. However, epidemiologic data suggest that additional factors must be sought to explain the observed increase in morbidity.[6] An understanding of the ancillary mechanisms through which changes in estrogen status alter the risk of ischemic heart disease

is essential for the rational design of estrogen replacement strategies.

An important observation in this regard is that estrogens are known to increase blood flow in a variety of vascular beds.[7, 8] The means through which estrogens exert this effect are unknown. Possible mechanisms include a direct vasodilatory effect of estrogen or modulated production of endogenous mediators of vascular tone.

The purpose of this investigation was to determine whether vascular production of PGI_2 differs in pre- and postmenopausal women. PGI_2 is a potent vasodilator and inhibitor of platelet activation and is known to play a central role in vascular homeostasis. The possibility that estrogens modulate vascular PGI_2 metabolism in women is supported by data from a variety of models. Makila and colleagues[10] have shown that perfusion of umbilical vessels by pharmacologic amounts of estrogens increased vascular production of PGI_2. This effect was completely reversed by addition of progesterone to the perfusion media. PGI_2 production by piglet aortic endothelial cells obtained from female (but not male) animals was markedly augmented by incubation with estradiol.[11] Similar results have been obtained with cultured rat aortic smooth muscle cells.[12, 13] In the sheep, vascular PGI_2 production in response to angiotensin II varies dramatically with pregnancy.[14]

The model used in these experiments provides a means to assess the effect of menopausal status and short-term estradiol exposure on prostanoid production. Uterine arteries were used in this model because of their availability in our center. However, we are cognizant that in the selection of uterine arteries as a model we may not be able to extrapolate these findings to PGI_2 production in the heart and central circulation. Nevertheless, studies in the sheep suggest that regulation of PGI_2 production in uterine arteries is similar to that of systemic (omental) vessels in the nonpregnant state.[14] Although differences may exist in the regulation of PGI_2 production in the heart and great vessels (as compared with peripheral vessels), this model provides preliminary data concerning the production of prostanoids by human vessels in vitro.

In these experiments 6-keto-$PGF_{1\alpha}$, the stable metabolite of PGI_2, was actively synthesized by uterine artery specimens from all patients studied. Inhibition of 6-keto-$PGF_{1\alpha}$ production by indomethacin showed the presence of prostanoids in spent media to be a result of de novo synthesis by superfused tissue, as opposed to leaching of adsorbed prostanoid from vascular endothelium or release from disrupted cells. In vitro TxB_2 production during steady state was observed to be at or below the limits of detection in both pre- and postmenopausal patients. These data are consistent with previous observations in sheep[14] and cattle.[15, 16]

Production of 6-keto-$PGF_{1\alpha}$ was found to be significantly depressed in vessels obtained from postmenopausal women. Additionally, production kinetics for 6-keto-$PGF_{1\alpha}$ were found to differ significantly between the two groups. These data suggest that menopausal status has a significant effect on vascular PGI_2 production. This finding may be consistent with a direct effect of long-term estrogen deprivation on the regulation of the vascular PGI_2 biosynthetic pathway. Alternatively, decreased PGI_2 synthesis may be a result of progressive atherosclerotic injury arising as a consequence of prolonged hypoestrogenicity and aging. However, data from Fitzgerald[17] suggest that atherosclerosis may induce a compensatory increase in PGI_2 production, rather than the decrease in production noted in these studies. In addition, the similarities in protein content of uterine arteries from pre- and postmenopausal patients observed in this investigation appear to negate the latter possibility. A third possibility is an indirect effect of altered menopausal lipoprotein profiles on PGI_2 production. In vitro studies[18] suggest that lipoproteins may modulate vascular PGI_2 synthesis. Low-density lipoproteins have generally been shown to exert an inhibitory effect and high-density lipoproteins a stimulatory influence on PGI_2 production. Regardless of the specific mechanism involved, given the importance of PGI_2 as a vasodilator and inhibitor of platelet activation and thrombosis, these data imply that estrogen-related alterations of PGI_2 production may contribute to the increased ischemic heart disease observed in menopausal women. However, we believe that modulation of vascular prostanoid production may account for only a portion of the interaction between estrogens and ischemic heart disease.

Although basal PGI_2 production was found to be significantly decreased in specimens obtained from hypoestrogenic (menopausal) patients, short-term estrogen exposure did not affect vascular PGI_2 production. Uterine vessels are known to possess the ability to respond to appropriate stimuli (e.g., angiotensin II) with increases in PGI_2 production.[14] This response is most consistent with a mediator-receptor-second messenger interaction resulting in an acute allosteric stimulation of prostaglandin synthetase and enzymes contributing to prostaglandin substrate mobilization. The failure of short-term incubation with pharmacologic amounts of estradiol to elicit an increase in vascular production of PGI_2 in these experiments suggests an alternative means of regulation in these patients. Chang and coworkers[13] have shown that estrogen-induced enhancement of PGI_2 synthesis by cultured aortic smooth muscle cells occurred over the course of 3 to 5 days and was a result of an induction of cyclooxygenase and PGI_2 synthetase rather than an acute allosteric phenomenon. Extrapolation of these data to the human

might explain both the disparity in basal PGI_2 production between pre- and postmenopausal women and the failure of short-term estrogen treatment to modulate PGI_2 synthesis.

Although the results of these experiments suggest that menopausal status (and perhaps estrogenicity) modulates vascular PGI_2 tone, it is evident that further studies are required to examine this association. The treatment of a group of postmenopausal women with an extended course of estrogens before hysterectomy would provide a means to examine this question further. Studies designed to specifically assess these factors are in progress.

REFERENCES

1. Gordon T, Castelli WP, Hjortland MC, et al. Menopause and coronary heart disease: the Framingham Study. Ann Intern Med 1978;89:157-61.
2. Johansson S, Vedin A, Wihelmsson C. Myocardial infarction in women. Epidemol Rev 1983;5:67-95.
3. Ross RK, Paganini-Hill A, Mack TM, et al. Menopausal estrogen therapy and protection against death from ischemic heart disease. Lancet 1981;1:858.
4. Bush TL, Barrett-Conner E, Cowan LD, et al. Estrogen use and all-cause mortality. JAMA 1983;249:903-6.
5. Henderson BE, Pike MC, Ross RK, Mack TM, Lobo RA. Reevaluating the role of progestogen therapy after the menopause. Fertil Steril 1988;49(suppl 5):9-15.
6. Bush TL, Barrett-Conner E, Cowan LD, et al. Cardiovascular mortality and noncontraceptive use of estrogen in women: results from the Lipid Research Clinics follow-up study. Circulation 1987;75:1102-9.
7. Rosenfeld CR, Morriss FH Jr, Battaglia FC, Makowski EL, Meschia G. Effect of estradiol-17β on blood flow to reproductive and nonreproductive tissues in pregnant ewes. Am J Obstet Gynecol 1976;124:618-29.
8. Magness RR, Rosenfeld CR. Local and systemic estradiol-17β: effects on uterine and systemic vasodilation. Am J Physiol 1989;256:E536-42.
9. Mitchell MD, Flint APF. Prostaglandin production by intrauterine tissues from periparturient sheep: use of a superfusion technique. J Endocrinol 1978;76:111-21.
10. Makila U-M, Wahlberg L, Viinikka L, Ylikorkala O. Regulation of prostacyclin and thromboxane production by human umbilical vessels: the effect of estradiol and progesterone in a superfusion model. Prostaglandins Leukotrienes Med 1982;8:115-24.
11. Seillan C, Ody C, Russo-Marie F, Duval D. Differential effects of sex steroids on prostaglandin synthesis by male and female cultured piglet endothelial cells. Prostaglandins 1983;26:3-12.
12. Chang W-C, Nakao J, Orimo H, Murota S-I. Stimulation of prostacyclin biosynthetic activity by estradiol in rat aortic smooth muscle cells in culture. Biochem Biophys Acta 1980;619:107-18.
13. Chang W-C, Nakao J, Orimo H, Murota S-I. Stimulation of prostaglandin cyclooxygenase and prostacyclin synthetase activities by estradiol in rat smooth aortic cells. Biochem Biophys Acta 1980;620:472-82.
14. Magness RR, Osei-Boaten K, Mitchell MD, Rosenfeld CR. In vitro prostacyclin production by ovine uterine and systemic arteries: effects of angiotensin II. J Clin Invest 1985;76:2206-12.
15. Terragno MA, McGiff JC, Smigel M, Terragno A. Prostaglandin synthesis by bovine mesenteric arteries and veins. Circ Res 1975;36(suppl I):76-80.
16. Terragno MA, McGiff JC, Smigel M, Terragno A. Prostaglandin synthesis by bovine fetal and maternal vasculature. Prostaglandins 1978;16:847-55.
17. FitzGerald GA, Smith B, Pedersen AK, Brash AR. Increased prostacyclin biosynthesis in patients with severe atherosclerosis and platelet activation. N Engl J Med 1984;310:1065.
18. Beitz J, Forster W. Influence of human low density and high density lipoprotein cholesterol on the in vitro prostaglandin I_2 synthetase activity. Biochem Biophys Acta 1980;620:352-55.

Depressive episodes in premenstrual syndrome

Joseph F. Mortola, MD,* Lyda Girton, PhD, and Samuel S. C. Yen, MD, DSc**
La Jolla, California

Episodic depression, a prominent but poorly defined symptom of premenstrual syndrome, was quantitated in 24-hour cortisol secretory episodes (determined by sampling at 20-minute intervals) as biochemical markers, as well as the Beck Depression Inventory and Profile of Mood States as psychometric measures. Results of 16 patients with premenstrual syndrome were compared with six age-matched women with endogenous depression and 16 control women. On both the Profile of Mood States and Beck Depression Inventory, women with premenstrual syndrome showed a marked worsening of scores ($p < 0.01$) during the luteal phase compared with either their own follicular phase scores or the scores of controls in either cycle phase. However, Beck Depression Inventory scores were threefold higher ($p < 0.005$) in women with depression than in those with luteal phase premenstrual syndrome (33.7 ± 3.6 vs. 11.9 ± 2.5). The Profile of Mood States depression scale was also higher ($p < 0.05$) in women with depression than in those with premenstrual syndrome, while scores on other Profile of Mood States scales were similar. The numbers of cortisol secretory pulses identified by the cluster algorithm were similar (5 to 6 per 24 hours) in all groups, and the times of circadian nadirs as determined by cosinor rhythmometry were comparable. While the mean amplitude and duration of the cortisol pulses were also similar in women with premenstrual syndrome and controls, both were significantly higher ($p < 0.05$) in women with depression. This resulted in markedly enhanced ($p < 0.005$) cortisol secretion during a given secretory episode in women with depression and in higher 24-hour transverse mean cortisol values in women with depression (87.8 ± 5.8 ng/ml) than in either those with premenstrual syndrome (66.7 ± 3.3 ng/ml) or controls (58.9 ± 3.3 ng/ml). These data affirm the clinical impression that depressive episodes occurring selectively in the luteal phase of the cycle in women with premenstrual syndrome are not present in controls and demonstrate, for the first time, that these episodes are distinct from endogenous depression as measured by both cortisol secretory parameters and psychological indices. (Am J Obstet Gynecol 1989;161:1682-7.)

Key words: Premenstrual syndrome, cortisol, depression

Among the most debilitating and commonly reported symptoms premenstrual syndrome (PMS) are depressive episodes.[1-4] As a result of this clinical observation, a possible association between PMS and major affective disorder has been sought with both positive[5-8] and negative[9-11] results. More recently, depressed subtypes of PMS have been described.[12] To date, however, prospective delineation of the similarities and differences between the depressive episodes in endogenous depression and PMS has not been achieved. Moreover, the expanded serum cortisol pulsatile episodes that have been demonstrated to be a sensitive index of endogenous depression[13-15] have not been evaluated in patients with PMS. In the present study well-characterized patients diagnosed as having PMS without major depressive episodes are compared with age-matched women with unipolar endogenous depression and with normally cycling controls by means of well-validated and relaible psychometric instruments. In addition, analysis of pulsatile activities of serum cortisol levels measured at 20-minute intervals around the clock is used as a neuroendocrine marker.

From the Department of Reproductive Medicine, School of Medicine (T-002), and the General Clinical Research Center University of California, San Diego.
This work was supported by National Institutes of Health, National Institute of Child Health and Human Development Center Grant HD-12303 and in part by the Mellon Fuondation and University of California, San Diego General Clinical Research Center, National Institutes of Health—Division of Research Resources Grant RR-00827. This research was conducted in part by the Clayton Foundation for Research.
Presented in part at the Thirty-fifth Annual Meeting of the Society for Gynecologic Investigation, Baltimore, Maryland, March 17-20, 1988.
Reprint requests: Joseph F. Mortola, MD, Department of Reproductive Medicine, T-002, University of California, San Diego, School of Medicine, La Jolla, CA 92093.
**Mellon Foundation Faculty Scholar.*
***Clayton Foundation Investigator.*
6/6/15959

Methods

Subjects. Sixteen women with premenstrual syndrome, 16 controls, and six women with endogenous depression were studied. All women were between 21 and 36 years old and had recorded regular menstrual cycles for the preceding 6 months, which ranged from 26 to 34 days. They had no history of previous medical or psychiatric illness (with the exception of major depression in those included in the endogenous depres-

sion group) and had normal results of physical examination before admission into the study. Women with prior substance abuse or those taking any medications including oral contraceptives and vitamin tablets were excluded. Control subjects who denied any physical or behavioral symptoms associated with the premenstrual phase of the cycle were compensated for their participation in the study. The presence of normal ovarian cyclicity, including normal luteal lengths (≥12 days) was confirmed by daily urine pregnanediol glucuronide levels measured by an immunoassay with monoclonal antibody[16] and normalized by correction for urinary creatinine values.

Women in the endogenous depression group met DSM III criteria[17] for major depression and research diagnostic criteria for major depressive episode, endogenous subtype.[18, 19] None received any medication during the study. However, for ethical reasons their participation was limited to 1 month to allow for appropriate psychopharmacologic intervention. Women included in the PMS group met a set of inclusion criteria that was developed in our research unit. The inclusion criteria for PMS (Table I) was the predictable occurrence of both behavioral and somatic symptoms reproducibly in the late luteal phase of the cycle, as well as the identifiable disruption of interpersonal or socioeconomic functioning during this time. Subjects with any historical evidence of affective disorder were excluded. Thus no women with both endogenous depression and PMS were included in the study. After satisfaction of the initial inclusion criteria for the study all women in the PMS group and controls were prospectively evaluated for a 2-month period before enrollment with the Calendar of Premenstrual Experiences (COPE). This inventory, a four-point Likert scale self-report instrument, further ensured the cyclic occurrence of physical and behavioral symptoms in women with PMS and the absence, by self-report, of these symptoms in controls. All women in the PMS groups showed a twofold increase ($p < 0.001$) in luteal phase scores (defined as the sum of scores on days 20 to 26) over follicular phase scores (defined as the sum of scores on days 3 to 9), while the follicular and luteal phase scores of controls were not statistically different. This study was approved by the Committee on Investigations Involving Human Subjects of the University of California, San Diego (UCSD).

Psychometric measures. On completion of the 2-month screening interval, PMS and control subjects were given the Beck Depression Inventory (BDI)[20] and the Profile of Mood States (POMS)[21] once on day 5 to day 9 and again on day 24 to day 27 of two consecutive menstrual cycles. Depressed women were administered the inventory on one occasion between days 5 and 9 of the menstrual cycle. All subjects were instructed to answer questions on both inventories according to their experience on the particular week the inventory was taken. Raw scores on the BDI were obtained. Scores on the six scales of the POMS, including tension, depression, anger, vigor, fatigue, and confusion, were converted to T scores by the method described in the manual of the POMS.[21]

Table I. Diagnostic criteria for PMS

1. The presence by self-report of at least one of the following somatic *and* affective symptoms during the 5 days before menses in each of the three prior menstrual cycles:

Affective	*Somatic*
Depression	Breast tenderness
Angry outbursts	Abdominal bloating
Irritability	Headache
Anxiety	Swelling of extremities
Confusion	
Social withdrawal	

2. These symptoms are relieved within 4 days of the onset of menses, without recurrence until at least cycle day 13
3. The symptoms are present in the absence of any pharmacologic therapy, hormone ingestion, or drug or alcohol use
4. The symptoms occur reproducibly during two cycles of prospective recording
5. Identifiable dysfunction in social or economic performance by one of the following criteria:
   - Marital or relationship discord confirmed by partner
   - Difficulties in parenting
   - Poor work or school performance, poor attendance, or tardiness
   - Increased social isolation
   - Legal difficulties
   - Suicidal ideation
   - Seeking medical attention for a somatic symptom

Cortisol measurements. Six of the women with PMS had serum cortisol measurements in the midluteal phase of the menstrual cycle (days 6 to 8 after the midcycle luteinizing hormone surge) as determined by rapid urinary assay (Monoclonal Antibodies, Inc.). All six depressed women were studied in the early follicular phase (days 3 to 5 after the onset of menses) so as not to confound results in this group with possible superimposed PMS. Eight controls were also studied in the early follicular phase to avoid potential contamination of the results by subtle PMS manifestations that may occur in normal women during the luteal phase, although evidence indicates that the hypothalamic-pituitary-adrenal axis does not change throughout the course of the normal menstrual cycle.[22] All subjects were admitted to the Clinical Research Center at UCSD for an accommodation night, during which an indwelling catheter was placed into a vein in the nondominant forearm. Light-dark schedules and mealtimes were standardized. Ambulation and daytime napping were

Table II. Mean (±SE) POMS T scores in women with PMS, endogenous depression, and control subjects

	Controls ($n = 16$)		PMS ($n = 16$)		*Endogenous depression: Follicular ($n = 6$)*
	Follicular	*Luteal*	*Follicular*	*Luteal*	
Tension	39.3 ± 1.7	39.2 ± 2.1	41.8 ± 0.8	55.9 ± 1.0*	62.3 ± 3.3*
Depression	41.3 ± 1.1	41.7 ± 2.3	43.2 ± 0.6	54.6 ± 1.1*	67.0 ± 4.7*†
Anger	42.2 ± 1.2	45.6 ± 0.9	45.6 ± 0.9	58.5 ± 1.2*	57.8 ± 4.3*
Vigor	52.1 ± 2.0	51.5 ± 1.6	52.9 ± 0.8	39.2 ± 1.0*	39.1 ± 3.7*
Fatigue	42.4 ± 1.2	40.8 ± 2.2	42.9 ± 0.8	57.3 ± 1.1*	60.6 ± 1.3*
Confusion	35.5 ± 1.0	36.1 ± 1.8	38.5 ± 0.6	49.9 ± 1.2*	56.8 ± 4.4*

*$p < 0.01$, compared with values for follicular or luteal phase controls or follicular phase PMS.
†$p < 0.05$, compared with value for luteal phase PMS.

avoided. Lights were off from 11 PM to 7 AM and sleep was monitored by trained observers. Meals were at 8 AM, 12 noon, and 6 PM. Meals contained approximately 50% of calories as carbohydrate, 35% as fat, and 15% as protein. Blood samples (2 ml) were obtained at 20-minute intervals for 24 hours through the indwelling catheter, which extended into an adjacent room so as not to disturb the subject during sleep hours.

Serum cortisol was measured by radioimmunoassay in each of the unextracted samples by techniques previously described.[23] The interassay coefficient of variation was 6.5% and the sensitivity of the assay was 10 ng/ml. All samples from a given subject were included in the same assay run.

Pulse detection. Cortisol pulses were characterized by using the computerized cluster analysis algorithm.[24] This program searches for significant increases and decreases in a series of hormone values. This analysis affords assessment of attributes of pulse amplitude, duration, and area, as well as frequency. A significant pulse was defined by a cluster configuration of 2 × 2 (two values for the upstroke and downstroke) and a t statistic of 3.34 for both significant increases and decreases and limits the likelihood of false-positive determinations to <5%.[24] A method described by Merriam et al.[25] was used in estimating the within-sample variance at various hormone concentrations. To establish a relationship between the coefficient of variation and its corresponding hormone level, 10 replicates were measured at 10 different doses between 40.3 and 394 ng/ml and a second-degree polynomial regression for standard deviation as a function of hormone concentration was constructed. The resulting coefficients were 0.00014, 0, and 2.12 for the second-degree coefficient, first-degree coefficient, and intercept, respectively. These calculated standard deviations were used in the cluster algorithm. The coefficient of variation at any given hormone concentration was then obtained by division of the calculated standard deviation by the dose. The resulting intraassay coefficients of variation ranged from 2.8% to 6.2% at doses between 40 and 400 ng/ml.

Circadian rhythm. The nadir of the circadian rhythm of cortisol for each subject was determined by single cosinor computation by means of the rhythmometry algorithm described by Nelson et al.[26]

Statistical analysis. The 24-hour transverse mean levels were defined as the arithmetic mean of the 73 determinations obtained for each subject. Differences between groups in 24-hour transverse mean cortisol levels, cortisol pulse parameters, and cortisol circadian rhythm, as well as in psychometric scores (BDI scores and T scores on each of the scales of POMS), were analyzed by one-way analysis of variance followed by Newman-Keuls multiple range tests. The data are presented as mean ± SE.

Results

Psychometric measures. Results of the BDI scores (Fig. 1) were similar in control subjects during both follicular and luteal phases and in subjects with PMS during the follicular phase. In contrast, in the luteal phase, subjects with PMS showed a twofold increase ($p < 0.05$) in the BDI score. Mean luteal phase scores in PMS, however, remained threefold lower ($p < 0.005$) than the scores obtained in women with depression in the follicular phase (11.7 ± 2.5 vs. 33.7 ± 3.6).

Control subjects did not show any differences on any of the Profile of Mood States scales between the follicular and luteal phases (Table II). As with the BDI, follicular phase POMS scores in women with PMS were similar to those of controls on all scales. However, a marked worsening of all mean scores ($p < 0.01$) occurred in women with PMS during the luteal phase to a degree similar to that obtained in depression on tension, anger, confusion, and fatigue, and there was also a decrease in vigor. The greater ($p < 0.05$) depression scale was the only parameter that exhibited departure of PMS from depression in the luteal phase (Table II).

Cortisol 24-hour rhythms. Representative samples of the 24-hour circadian profiles of women with PMS, women with depression, and controls are shown in Fig. 2. In all subjects the circadian variation was maintained. The mean nadir times of the circadian rhythm of cor-

Table III. Transverse mean 24-hour levels and 24-hour pulsatile characteristics of serum cortisol levels in women with PMS, endogenous depression, and control subjects

Subject no.	24 hr mean (ng/ml)	Pulsatile characteristics			
		No./24 hr	Amplitude* (ng/ml)	Duration* (min)	Area* (ng · min/ml × 10^2)
PMS					
PMS 1	61.6	6	56	123	32.6
PMS 2	75.3	6	130	173	71.6
PMS 3	61.6	6	91	200	62.8
PMS 4	71.7	4	78	195	45.1
PMS 5	55.7	8	74	126	36.4
PMS 6	73.9	8	112	123	48.6
Mean ± SE	66.7 ± 3.3	6.3 ± 0.6	90.2 ± 11.0	156.7 ± 15.0	49.6 ± 6.2
Depression					
D1	52.3	4	91	36	103.4
D2	86.3	6	137	200	102.8
D3	98.1	4	151	186	98.0
D4	90.2	6	133	233	134.8
D5	74.0	6	118	153	70.0
D6	125.9	5	161	192	115.4
Mean ± SE	87.8 ± 10.0†	5.2 ± 0.4	132.8 ± 10.5†	215.0 ± 24.5†	103.8 ± 8.7‡
Controls					
C1	55.5	9	72	106	36.7
C2	73.7	5	117	196	94.7
C3	52.2	6	118	153	57.9
C4	53.9	5	85	172	48.9
C5	74.0	5	109	130	51.0
C6	53.0	4	156	168	48.2
C7	62.7	5	84	163	53.7
C8	46.9	6	109	116	59.5
Mean ± SE	58.9 ± 3.3	5.6 ± 0.5	102.5 ± 6.9	169.6 ± 16.6	56.3 ± 6.0

*Mean for a 24-hour period.
†$p < 0.05$ by Newman-Keuls test versus values for PMS or controls.
‡$p < 0.005$ by Newman-Keuls test versus values for PMS or controls.

tisol were similar in all groups and occurred at approximately 9 PM. As illustrated, a loss of the evening quiescent period of cortisol (6 PM to 12 midnight) was noted in women with depression and was accompanied by continued secretory episodes only in this group. Analysis of the 73 sampling times throughout the 24-hour biologic clock revealed that subjects with depression had higher mean cortisol levels ($p < 0.001$) than those of controls at all times, with the exception of a brief period during the early morning hours when values overlapped (Fig. 3), whereas women with PMS showed mean cortisol values that overlapped those of controls throughout the 24-hour circadian clock. This resulted in significantly ($p < 0.05$) higher 24-hour transverse mean levels in depression (87.8 ± 10.0 ng/ml) than in PMS (66.7 ± 3.3 ng/ml) or controls (58.9 ± 3.3 ng/ml), which were similar (Table III).

Analysis of the cortisol pulsatile activity (Table III) among the three groups revealed that the mean number of pulses (pulse frequency) during the 24-hour sampling period were similar (PMS, 6.3 ± 0.6; depression, 5.2 ± 0.4; controls, 5.6 ± 0.5). Compared with controls, subjects with PMS did not differ significantly with respect to either pulse amplitude (PMS, 90.2 ± 11.0 ng/ml; controls, 102.5 ± 6.9 ng/ml) or pulse duration (PMS, 156.7 ± 15.0 minutes; controls, 169.6 ± 16.6 minutes) and these values were lower ($p < 0.05$) than those of women with depression for both amplitude (132.8 ± 10.5 ng/ml) and duration (215.0 ± 24.5 minutes). Thus the combination of increased amplitude and duration led to markedly expanded ($p < 0.005$) cortisol secretory episodes in depressed women (103.8 ± 8.7 × 10^2 ng · min/ml) as compared with those of controls (56.3 ± 6.0 × 10^2 ng · min/ml), which was not evident in PMS (49.6 ± 6.2 × 10^2 ng · min/ml).

Comment

Our subjects with PMS demonstrated depressive episodes that occurred selectively in the luteal phase of the cycle. Characteristics of these episodes included both elevated scores on the BDI and a worsening of all scores on the POMS during the luteal phase. The follicular phase BDI scores in our subjects with PMS were far lower than those found in a previous study using this instrument in PMS.[27] This was due primarily to the

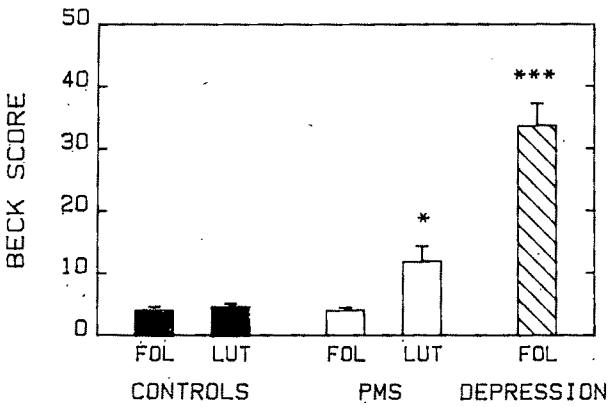


Fig. 1. Mean (±SE) Beck depression scores in controls and subjects with PMS in the follicular and luteal phases and subjects with endogenous depression in the follicular phase. (*Asterisk*, $p < 0.05$; *triple asterisk*, $p < 0.005$.)

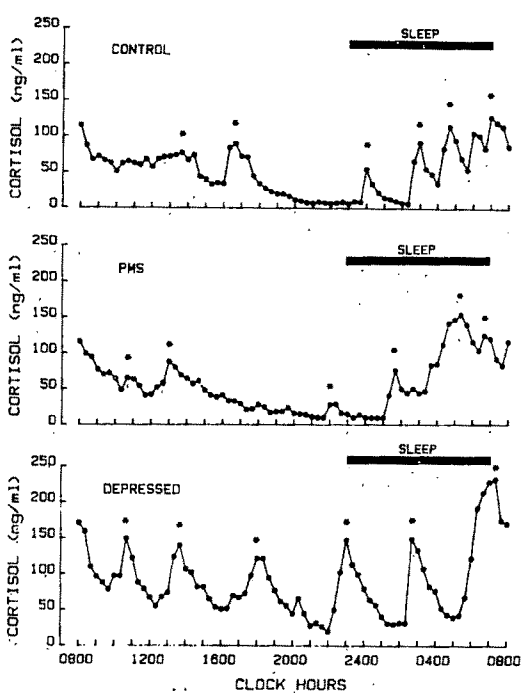


Fig. 2. Representative 24-hour serum cortisol secretory patterns and circadian rhythms in a control subject *(top panel)*, a subject with PMS *(middle panel)*, and a subject with endogenous depression *(bottom panel)*, illustrating larger pulses in the subject with depression. (*Asterisk* denotes pulses.)

specific exclusion of depressed subjects in our study. On the basis of the POMS, the degree of tension, anger, confusion, or fatigue experienced in our subjects with PMS did not differ from the degree of these symptoms seen in major depression, and the loss of vigor was also comparable. These scores in women with PMS, however, returned to control values during the follicular phase of the cycle and reproducibly recurred during the luteal phases of consecutive menstrual cycles. These

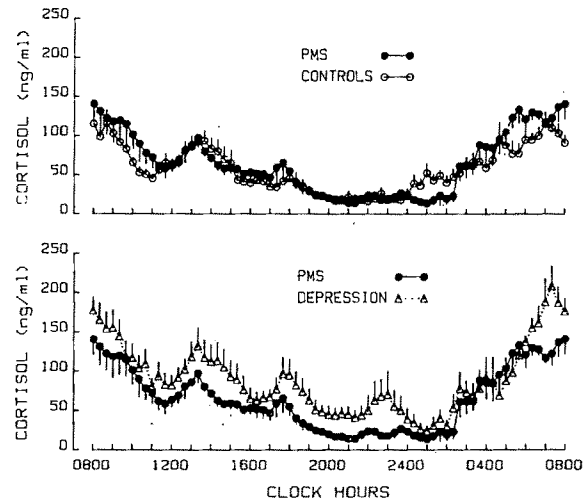


Fig. 3. Comparison of 24-hour mean (±SE) serum cortisol levels in subjects with PMS and controls *(top panel)* and in subjects with PMS and those with endogenous depression *(bottom panel)*.

results are consistent with a previous report using this measure.[3] Several studies have found a high incidence of PMS in women with major affective disorder.[3-8] To date the nature and severity of luteal phase depressive symptoms has not been adequately assessed in women who have PMS with a specifically excluded history of major depression. Our study is the first to compare these scores with those of a population of women with endogenous depression that was simultaneously evaluated. Within the limitations of our psychometric measures, the degree of the symptom of depression itself showed marked differences in the luteal phase between women with PMS and those with major depression. Such differences are explained on the basis of the degrees of self-reproach, loss of interest in life, and sense of helplessness and hopelessness, which are more pronounced in major depression than in our subjects whose lives were significantly disrupted by symptoms of PMS.

In addition to the greater severity of the symptom of depression itself, the cortisol changes that have previously been noted to occur in endogenous depression[13-15] were absent in our subjects with PMS in whom overt depression was specifically excluded. This may be due to differences in the pathophysiology of these disorders or the fact that the hypothalamic-pituitary-adrenal axis changes that occur in depression require longer time to manifest than the relatively brief episodes of affective symptoms in PMS. In either case this study expands on previous findings that abnormalities in the dexamethasone suppression test and elevations of 24-hour urinary cortisol level were not found in PMS.[11] It confirms the results of a small study in which circadian cortisol rhythms in two subjects with PMS

were found to be similar to those of controls.[10] To our knowledge, however, the present study represents the first demonstration that PMS is distinct from endogenous depression on the basis of the differences in both amplitude and duration of serum cortisol pulses, which were used as a biochemical marker, as well as prospective psychometric evaluation.

We are grateful to Gail Laughlin for her assistance in use of the computerized cluster detection algorithm and to Del Alsobrook for her assistance in preparation of the manuscript. We are also indebted to the nurses in the UCSD Clinical Research Center for their invaluable assistance in sample collection.

REFERENCES

1. Reid RL, Yen SSC. Premenstrual syndrome. AM J OBSTET GYNECOL 1981;139:83-103.
2. Rubinow DR, Roy-Byrne P, Hoban MC, Gold PW, Post RM. Prospective assessment of menstrually related mood disorders. Am J Psychiatry 1984;141:684-6.
3. Haskett RF, Abplanalp JM. Premenstrual tension syndrome: diagnostic criteria and selection of research subjects. Psychiatry Res 1983;9:125-38.
4. Steiner M, Haskett RF, Carroll BJ. Premenstrual tension syndrome: the development of research diagnostic criteria and new rating scales. Acta Psychiatr Scand 1980; 62:177-90.
5. Endicott J, Halbreich U, Schacht S, Nee J. Premenstrual changes and affective disorders. Psychom Med 1981; 43:519-29.
6. Kashiwagi T, McClure JH, Wetzel RD. Premenstrual affective syndrome and psychiatric disorder. Dis Nerv Syst 1981;37:116-9.
7. DeJong R, Rubinow DR, Roy-Byrne P, Hoban MC, Grover GN, Post RM. Premenstrual mood disorder and psychiatric illness. Am J Psychiatry 1985;142:1359-61.
8. Schuckit MA, Daly V, Herman G, Hineman S. Premenstrual symptoms and depression in a university population. Dis Nerv Syst 1976;36:516-7.
9. Diamond SB, Rubenstein AA, Dunner DL, Fieve RR. Menstrual problems in women with primary affective illness. Compr Psychiatry 1976;17:541-3.
10. Steiner M, Haskett RF, Carroll BJ, Hays SE, Rubin RT. Circadian hormone secretory profiles in women with severe premenstrual tension. Br J Obstet Gynaecol 1984; 91:466-71.
11. Haskett RF, Steiner M, Carroll BJ. A psychoneuroendocrine study of premenstrual tension syndrome. A model for endogenous depression. J Affective Disord 1984; 6:191-9.
12. Halbreich U, Endicott JE, Nee J. Premenstrual depressive changes. Value of differentiation. Arch Gen Psychiatry 1983;40:535-42.
13. Sachar EO, Hellman L, Roffwarg H, Halpern F, Fusushima D, Gallagher T. Disrupted 24-hour patterns of cortisol secretion in psychotic depression. Arch Gen Psychiatry 1973;28:19-24.
14. Linkowski P, Mendelewicz J, Leclercq R, et al. The 24-hour profile of adrenocorticotropin and cortisol in major depressive illness. J Clin Endocrinol Metab 1985;61:429-38.
15. Mortola JF, Liu JH, Gillin JC, Rasmussen DD, Yen SSC. Pulsatile rhythms of adrenocorticotropin (ACTH) and cortisol in women with endogenous depression: Evidence for increased ACTH pulse frequency. J Clin Endocrinol Metab 1987;65:692-7.
16. Eschar Z, Kim JB, Barnard G, et al. Use of monoclonal antibodies with pregnanediol-3-glucuronide for the development of a solid phase chemoluminescence immunoassay. Steroids 1981;38:889-109.
17. American Psychiatric Association Committee on Nomenclature and Statistics. Diagnostic and statistical manual of mental disorders. 3rd ed. Washington, DC: American Psychiatric Association, 1980.
18. Endicott J, Spitzer RL. A diagnostic interview. The schedule of affective disorders and schizophrenia. Arch Gen Psychiatry 1978;35:837-44.
19. Spitzer RL, Endicott J, Robins E. Research diagnostic criteria. Rationale and reliability. Arch Gen Psychiatry 1978;35:773-82.
20. Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. Arch Gen Psychiatry 1961;4:561-71.
21. McNair DM, Lorr M, Doppleman LF. EITS manual for the Profile of Mood States. San Diego: Educational and Industrial Testing Service, 1971.
22. Liu JH, Rasmussen DD, Rivier J, Vale W, Yen SSC. Pituitary responses to synthetic corticotropin-releasing hormone: absence of modulatory effects by estrogen and progestin. AM J OBSTET GYNECOL 1987;157:1387-91.
23. Judd HL, Parker DC, Rakoff JS, Hopper BR, Yen SSC. Elucidation of the mechanism(s) of the nocturnal rise of testosterone in men. J Clin Endocrinol Metab 1973; 38:134-41.
24. Veldhuis JD, Johnson ML. Cluster analysis: a simple versatile and robust algorithm for endocrine pulse detection. Am J Physiol 1986;250:E486-93.
25. Merriam GR, Kozuch M, Wachter KW. Pulsar user's guide version 1983. 1. Bethesda, Maryland: Developmental Endocrinology Branch, National Institute of Child Health and Human Development, and Berkeley, California: Department of Statistics, University of California, Berkeley, 1983:3.
26. Nelson W, Tong YL, Lee JK, Halberg F. Methods for cosinor rhythmometry. Chromogiologin 1979;6:305-7.
27. Stout AL, Steege J. Psychological assessment of women seeking treatment for premenstrual syndrome. J Psychosom Res 1985;29:621-9.

Peri-implantation phase endometrial estrogen and progesterone receptors: Effect of ovulation induction with clomiphene citrate

Bryan R. Hecht, MD, Firyal S. Khan-Dawood, PhD, and M. Yusoff Dawood, MD
Chicago, Illinois

The effects of clomiphene citrate on endometrial nuclear estradiol receptors and progesterone receptors were examined in 10 normal women during an untreated cycle (control) and during treatment with 50 mg clomiphene citrate and 150 mg clomiphene citrate daily on days 5 through 9. Concentrations and binding constants of the receptors were determined in endometrium obtained 8 to 12 days after midcycle luteinizing hormone surge. Scatchard plots for both estrogen receptors and progesterone receptors were linear, indicating only one type of high-affinity binding sites. In control cycles, estrogen receptor levels (mean ± SEM) were 199.6 ± 23.1 fmol/mg deoxyribonucleic acid, (n = 8) and were not significantly different from either 50 mg clomiphene citrate (180.5 ± 19.1 fmol/mg deoxyribonucleic acid, n = 6) or 150 mg clomiphene citrate (194.3 ± 35.2 fmol/mg deoxyribonucleic acid, n = 4). Similarly, the dissociation constants were unaffected by clomiphene citrate treatment. The concentrations of progesterone receptors in the control cycles (613 ± 31 fmol/mg deoxyribonucleic acid, n = 5) and treatment cycles (50 mg clomiphene citrate – 652.8 ± 121 fmol/mg deoxyribonucleic acid, n = 6; 150 mg clomiphene citrate – 592.6 ± 31 fmol/mg deoxyribonucleic acid, n = 7) were also not significantly different. Clomiphene citrate also did not affect dissociation constants for progesterone receptors. Therefore, ovulation induction with clomiphene citrate apparently did not affect peri-implantation phase endometrial estrogen receptors and progesterone receptors or their respective binding constants. (Am J Obstet Gynecol 1989;161:1688-93.)

Key words: Clomiphene citrate, estrogen receptors, progesterone receptors, implantation, endometrium

In the human uterus, estrogen, followed by progesterone, stimulates endometrial maturation and preparation for blastocyst implantation. These two ovarian hormones exert their effects on the endometrium through their specific hormone receptors, concentrations of which may determine the sensitivity of the endometrium to circulating estrogen or progesterone. Estrogen induces its own receptors and progesterone receptors. Delayed, inadequate, or asynchronous development of the endometrium may arise from inadequate levels of estrogen, progesterone, or their receptors and abnormal receptor activity. Studies of steroid hormone receptors in endometrial tissues of infertile women with luteal phase defect have yielded conflicting results.[1-7] These discrepancies may result from the diverse mechanisms responsible for this disorder and differences in methods and analysis used for steroid hormone receptor populations.

Clomiphene citrate, a nonsteroidal synthetic compound with both estrogenic and antiestrogenic activity,[8] is widely used for ovulation induction. Inasmuch as clomiphene citrate is concentrated in the endometrium, inhibits tissue uptake of estrogen,[8] and has been implicated in delayed endometrial development,[9] a direct adverse effect on the endometrium has been suggested. This effect may contribute to the discrepancy observed between successful ovulation and pregnancy rates when clomiphene citrate is used for ovulation induction and points to possible impaired implantation as a factor. Because clomiphene citrate is known to bind to estrogen receptors and alter their intracellular kinetics[8, 10, 11] it could alter endometrial maturation directly through estrogen receptors or indirectly through progesterone receptors, which are normally induced by estrogen. Previous studies[12-14] on the effect of clomiphene citrate on human endometrial steroid receptors were not designed to address the relationship between the receptors and luteal phase abnormalities or the biochemical

From the Division of Reproductive Endocrinology, Department of Obstetrics and Gynecology, University of Illinois College of Medicine.
Supported in part by an American College of Obstetricians and Gynecologists–Mead Johnson Fellowship for Clinical Research and American College of Obstetricians and Gynecologists District VI, Illinois Section.
Presented at the Thirty-fifth Annual Meeting of the Society for Gynecologic Investigation, Baltimore, Maryland, March 17-20, 1988.
Reprint requests: M. Yusoff Dawood, MD, Department of Obstetrics and Gynecology, University of Illinois College of Medicine, 840 S. Wood St., Chicago, IL 60612.
6/6/16508

basis of implantation. In a study of infertile women treated with clomiphene citrate, only the cytosolic receptor population from secretory endometrium could be studied because of limitations on the quantity of tissue obtained.[15] Because these studies were performed in infertile women, it is not possible to exclude other potential infertility effects on the endometrium. This study was carried out to examine the effect of clomiphene citrate on the concentrations and binding activities of endometrial estrogen and progesterone receptors at the peri-implantation phase. We conducted the present investigation on normal ovulatory women to eliminate other confounding infertility factors on the endometrium. In addition, we determined the effect of high-dose (150 mg) and low-dose (50 mg) clomiphene citrate administration on endometrial steroid hormone receptors in the same subjects.

Material and methods

Subjects. Ten normal fertile female volunteers with regular menstrual cycles were recruited and gave informed consent for the study, which was approved by our Institutional Review Board. All subjects had previously undergone tubal sterilization or were using barrier contraception. The clinical characteristics are summarized in Table I.

Medication and biopsy protocol. Eight subjects were studied for three consecutive menstrual cycles, whereas two were studied for three nonconsecutive cycles. The first cycle was a control cycle during which the subjects did not receive medication. In the second cycle, clomiphene citrate 50 mg was given daily on days 5 through 9 of the cycle; in the third cycle, 150 mg clomiphene citrate was given daily on days 5 through 9.

Basal body temperature (BBT) was recorded by each subject for all cycles studied. First-voided urine samples were collected daily, beginning on cycle day 11 and continuing until a luteinizing hormone (LH) surge was identified or a sustained BBT rise was demonstrated. The urinary LH surge was determined by the Q test ovulation test kit (Clay Adams, Division of Becton Dickinson, Parsippany, N.J.) with a monoclonal LH antibody in an enzyme immunoassay for LH. The day of the LH surge was subsequently confirmed by the measurement of LH in the plasma.

Daily peripheral heparinized venous blood samples were obtained from cycle day 11 until the day of the endometrial tissue biopsy. The plasma was separated and stored at −20° C until assayed. Plasma LH was measured by a specific double antibody radioimmunoassay. Plasma progesterone was measured by a specific radioimmunoassay as previously described,[16] with the use of an antibody raised against progesterone-11-oxime bovine serum albumin conjugate. To reduce interassay variability, all blood samples from the same patient were performed in the same assay for each hormone.

Table I. Clinical characteristics of the 10 subjects studied

Characteristics	*Mean ± SEM*
Age (yr)	30.3 ± 1.1
Weight (kg)	77.3 ± 5.4
Parity	2.4 ± 0.4
Menstrual cycle length (days)	28.5 ± 1.1

Endometrial biopsies were performed 8 to 12 days after the LH surge with a mean of 10.4 to 10.8 days (Table II). The subjects were given ibuprofen 800 mg orally 1 hour before the endometrial biopsy, which was performed with a paracervical block. The uterine cavity was systematically and thoroughly curetted to obtain as much endometrial tissue as possible. Endometrial curettings were immediately rinsed in ice-cold normal saline solution to remove blood and debris, and a small piece was taken for histologic studies. The remaining tissue was snap frozen by immersion in liquid nitrogen and after removal was stored at −70° C until ready for receptor studies (within 2 weeks of collection for most of the samples but in a few cases between 2 and 4 weeks of collection). Preparation of tissue nuclear fraction and determination of endometrial nuclear estradiol and progesterone receptors were as previously described and validated in this laboratory by Khan-Dawood and Dawood.[17, 18]

Preparation of endometrial cell nuclei. The frozen tissue was homogenized at 4° C in eight volumes of Tris-glycerol buffer (10 mmol of Tris-base, 1.5 mmol dithiothreitol, and 30% vol/vol of glycerol at a pH of 7.4) with a Tissumizer homogenizer (Tekmar, Cincinnati, Ohio) at a setting of 50. Three 10-second bursts with 30-second cooling intervals were used for homogenization. The homogenate was centrifuged at 800 *g* for 10 minutes at 4° C. The 800 *g* pellet was washed three times with 20 volumes of Tris-glycerol buffer and resuspended in the same volume of buffer for measurement of nuclear receptors. The nuclear deoxyribonucleic acid (DNA) concentration was determined by the method of Burton.[19] Approximately 400 μg of tissue was needed for a seven-point saturation assay to enable satisfactory Scatchard analysis of hormone receptor binding.

Measurement of nuclear receptors. Concentrations of nuclear progesterone and estradiol receptors were determined by exchange assays with the use of [^{3}H] promegestone (R5020) and [^{3}H] moxestrol (R2858), respectively (New England Nuclear Research Products, Boston, Mass.). Aliquots of 500 μl nuclear suspension were incubated with 100 μl of tritiated moxestrol (specific activity, 87 Ci/mmol) or promegestone

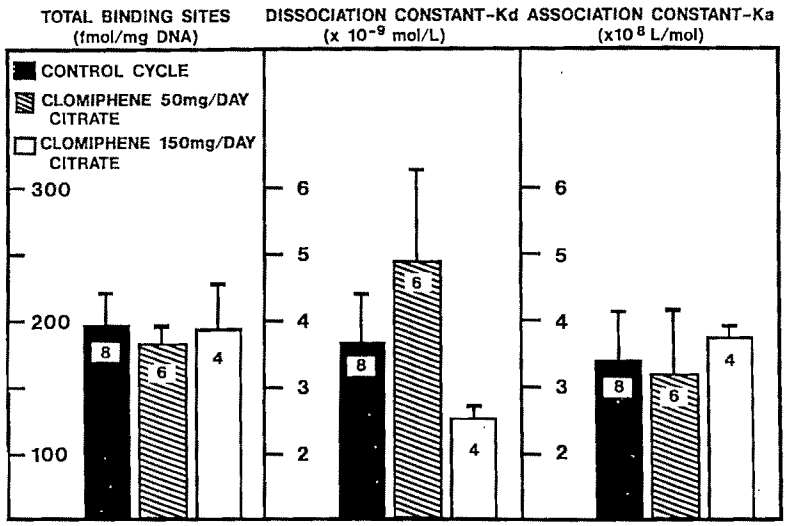


Fig. 1. Mean ± SEM nuclear estrogen receptors, K_d and K_a in peri-implantation phase (luteal phase) endometrium. Treatment with clomiphene citrate in doses of 50 mg/day and 150 mg/day from days 5 through 9 of the cycle had no effect on nuclear estrogen receptor concentrations or binding affinities. Numbers within the *bar graphs* represent number of subjects whose endometrial tissues were studied.

Table II. Characteristics of cycles before and during treatment with clomiphene citrate (mean ± SEM)

	Cycles		
		Clomiphene citrate	
Characteristics	*Control*	*50 mg*	*150 mg*
Cycle length (days)	28.5 ± 1.1	28.2 ± 0.6	29.0 ± 0.4
LH surge (cycle day)	16.4 ± 1.2	16.5 ± 0.5	17.1 ± 0.7
LH surge–biopsy interval (days)	10.4 ± 0.2	10.7 ± 0.4	10.8 ± 0.1
Progesterone on biopsy day (ng/ml)	9.4 ± 1.6	10.5 ± 1.6	*16.5 ± 2.6

*$p < 0.05$.

(specific activity, 87 Ci/mmol) in concentrations that ranged from 2.5 to 20 nmol. A parallel set of tubes were incubated with 100-fold excess of nontritiated ligand (moxestrol or promegestone) to measure nonspecific binding. For the estradiol receptor assay, the mixtures were incubated at 37° C for 30 minutes, and for the progesterone receptor determination the incubation was carried out at 4° C for 16 hours. The binding reaction was terminated by the addition of 1 ml of ice-cold Tris-glycerol buffer and centrifuged at 800 *g*. The pellet was washed three times in the same buffer. One ml of 100% ethanol was used to elute the bound nuclear receptors from the washed pellet. The ethanolic supernatant was added to 10 ml of high-efficiency scintillation fluid (Biocount, Research Products International Corp, Mount Prospect, Ill.) and radioactivity was counted in a liquid scintillation counter with an efficiency of 58% for tritium.

Data analysis. A Scatchard plot was constructed for analysis of the total number of binding sites and the dissociation constant (K_d) and association constant (K_a). Specific binding is expressed as femtomoles of hormone bound per milligram of DNA. Data are expressed as mean ± SEM. Means between different treatment cycles were compared by one-way analysis of variance before the paired or nonpaired Student *t* test between two different treatment regimens. A *p* value >0.05 was not considered significant.

Results

Clinical findings. All menstrual cycles in control and treatment cycles were ovulatory as determined by identification of an LH surge, biphasic BBT, elevated progesterone concentrations, or the presence of secretory endometrium on biopsy. The cycle length, day of the LH surge, and plasma progesterone concentrations are indicated in Table II. Only the plasma progesterone levels on the day of the biopsy during the 150 mg clomiphene cycles were significantly different from control values.

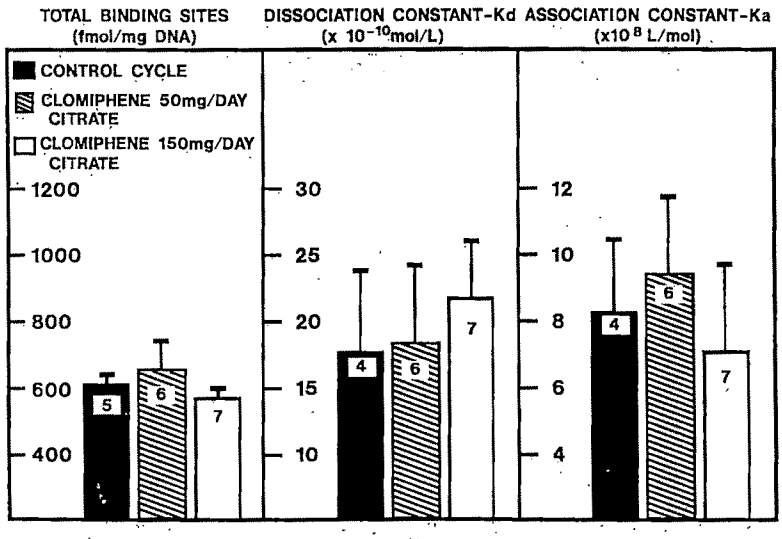


Fig. 2. Mean ± SEM nuclear progesterone receptors, K_d, and K_a in peri-implantation phase (luteal phase) endometrium. Clomiphene citrate treatment (50 mg and 150 mg daily from days 5 through 9 of the cycle) had no significant effect on nuclear progesterone receptor concentrations or binding affinities.

Receptor concentrations and binding affinities. The mean ± SEM concentrations, K_d and K_a of endometrial nuclear estradiol receptors in control cycles and clomiphene citrate–treated cycles are shown in Fig. 1. In control cycles, estrogen receptors levels were 199.6 ± 23.1 fmol/mg DNA (n = 8) and were not significantly different from either treatment with 50 mg clomiphene citrate (180.5 ± 19.1 fmol/mg DNA, n = 6) or 150 mg clomiphene citrate (194.3 ± 35.2 fmol/mg DNA, n = 4). The K_d and K_a were also unaffected by treatment with clomiphene citrate.

Similarly, there was no significant change in the concentrations, K_d, and K_a of nuclear progesterone receptors with 50 mg and 150 mg clomiphene citrate as compared with untreated control cycles (Fig. 2). The concentrations of progesterone receptors were 613 ± 31 fmol/mg DNA in control cycles, 652.8 ± 121 fmol/mg DNA with 50 mg clomiphene citrate, and 592.6 ± 31 fmol/mg DNA with 150 mg clomiphene citrate. Fig. 3 shows representative Scatchard plots for endometrial nuclear estradiol receptors in control, 50 mg, and 150 mg clomiphene citrate–treated cycles from one subject. Representative Scatchard plots for nuclear progesterone receptors in control and clomiphene citrate–treated cycles from one patient are shown in Fig. 4.

Comment

Because of the disparity between ovulation rates and viable pregnancy rates in women undergoing ovulation induction with clomiphene citrate,[16] we examined the estrogen and progesterone nuclear receptors of endometrial tissues for inappropriate changes around the time of implantation during the luteal phase. Given in doses of 50 mg and 150 mg daily from days 5 through 9 of the cycle, clomiphene citrate did not produce a demonstrable effect on either the concentrations or the binding affinities of both estrogen and progesterone nuclear receptors in luteal phase peri-implantation endometrium. The use of rapid LH determinations in urine and plasma enabled us to obtain endometrium of similar luteal ages for all cycles studied. To eliminate any potential endometrial factors unrelated to clomiphene citrate use (which may be present in infertile women) and to obtain adequate endometrial tissues for dependable Scatchard analysis of the receptors studied, only fertile normal ovulatory women not desirous of pregnancy were studied. In addition, each subject acted as her own control by having her own untreated ovulatory cycle studied.

Previous limited studies[12, 15] have examined the effect of clomiphene citrate on endometrial steroid receptors but differed in either the type of subjects, the cycle time when medication was given, or the analysis and type of receptors measured. Clomiphene citrate given to ovulating women during the luteal phase depressed both cytosolic and nuclear estrogen receptors but only cytosolic progesterone receptors.[21] However, there is no clinical indication to give clomiphene citrate in the luteal phase. Both total and cytosolic estrogen and progesterone receptors were reported to be significantly reduced but the corresponding nuclear receptors were significantly increased in endometrium obtained at oocyte retrieval after follicle recruitment with clomiphene, human menopausal gonadotropin, and human chorionic gonadotropin for in vitro fertilization.[13] With the measurement of only luteal phase endometrial cy-

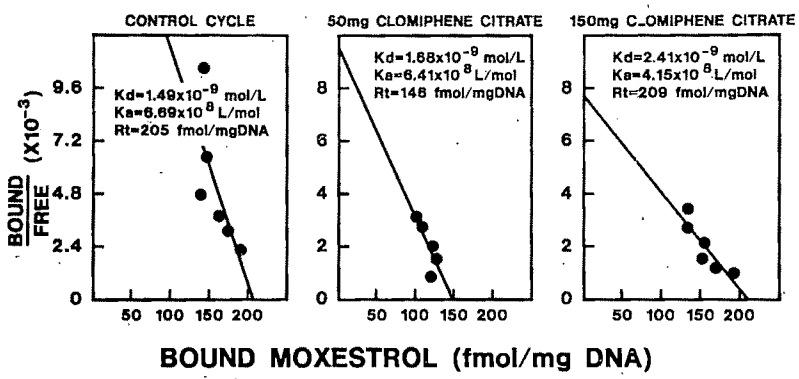


Fig. 3. Representative Scatchard analysis of luteal phase (peri-implantation) human endometrial nuclear estrogen receptor binding activity in one patient during a control cycle and in cycles treated with 50 mg/day and 150 mg/day of clomiphene citrate from days 5 through 9.

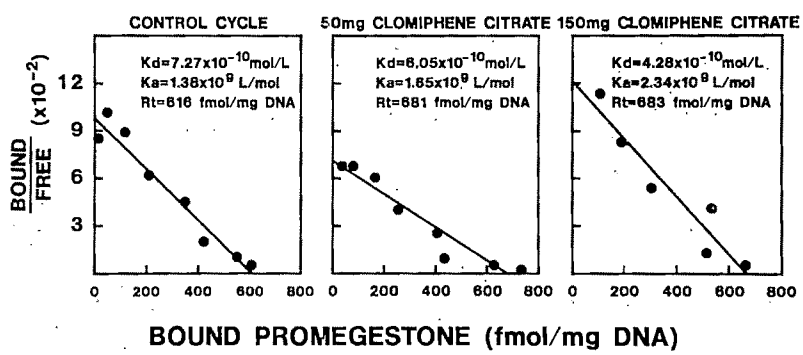


Fig. 4. Representative Scatchard analysis of luteal phase (peri-implantation) human endometrial nuclear progesterone receptor binding activity in one patient during a control cycle and in cycles treated with 50 mg/day and 150 mg/day of clomiphene citrate from days 5 through 9.

tosol estrogen and progesterone receptors in infertile women given clomiphene to induce ovulation, Aksel et al.[15] found decreased levels of these receptors without any correlation with luteal phase defects. In postmenopausal women, clomiphene given to antagonize the proliferative effects of estrogen replacement induced a significant decrease in cytosolic estrogen and progesterone receptors.[14] Whereas these studies are interesting, current concepts about the localization of steroid hormone receptors being in the nucleus[20] and not in the cytosol render interpretation of the findings difficult at best, if not impossible, when the nuclear receptors are not measured. It is precisely for this reason that we undertook to measure receptors in the nuclear material.

It is noteworthy that tamoxifen, another triphenylethylene derivative with mixed agonist-antagonist properties, given during the follicular phase for up to 21 days did not cause any significant change in late luteal phase endometrial estrogen receptor and progesterone receptor concentrations.[21] Whereas we found no appreciable effect of clomiphene on peri-implantation phase endometrial steroid receptor concentrations and binding affinities, it does not exclude possible effects on mechannisms at the postreceptor translational level. The results of our study lend biochemical support to the currently evolving challenge that clomiphene may actually have no adverse effect on the endometrium[22, 23] and that the discrepancy between successful ovulation and pregnancy rates disappears when life-table analysis of pregnancy and other infertility factors are carefully considered.[24] That the antiestrogenic effects of clomiphene may have attained unearned significance is further compounded by the difficult and controversial diagnosis of luteal phase defect,[25] a condition often overdiagnosed. Furthermore, the currently marketed formulations of clomiphene consist of a mixture of enclomiphene and zuclomiphene isomers, each of which has a different ratio of agonist-antagonist properties so that the ratio of estrogenic and antiestrogenic effect may vary from preparation to preparation.

In conclusion, it is reassuring from a clinical standpoint that clomiphene citrate in customarily used doses of 50 mg and 150 mg from day 5 through 9 of the cycle does not produce any significant change in the con-

centrations and binding affinities of peri-implantation phase endometrial estrogen and progesterone receptors.

We thank Serono Laboratories, Inc., Randolph, Mass., for providing the clomiphene citrate (Serophene) and Clay Adams Division of Becton Dickinson and Company, Parsippany, N. J., for providing the Q test ovulation test kits used in this study.

REFERENCES

1. Keller DW, Wiest WG, Askin FB, Johnson LW, Strickler RC. Pseudocorpus luteum insufficiency: a local defect of progesterone action on endometrial stroma. J Clin Endocrinol Metab 1979;48:127-32.
2. Jacobs MH, Balasch J, Gonzalez-Merlo JM, et al. Endometrial cytosolic and nuclear progesterone receptors in the luteal phase defect. J Clin Endocrinol Metab 1987; 64:472-5.
3. Laatikainen T, Anderson B, Karkkainen J, Wahlstrom T. Progestin receptor levels in endometrium with delayed or incomplete secretory changes. Obstet Gynecol 1983; 62:592-5.
4. Gravanis A, Zorn J-R, Tanguy G, Nessman C, Cedard L, Robel P. The "dysharmonic luteal phase" syndrome: endometrial progesterone receptor and estradiol dehydrogenase. Fertil Steril 1984;42:730-6.
5. Spirtos NJ, Yurewicz EC, Moghissi KS, Magyar DM, Sundareson AS, Bottoms SF. Pseudocorpus luteum insufficiency: a study of cytosol progesterone receptors in human endometrium. Obstet Gynecol 1985;65:535-40.
6. McRae MA, Blasco L, Lyttle CR. Serum hormones and their receptors in women with normal and inadequate corpus luteum function. Fertil Steril 1984;42:58-63.
7. Saracoglu OF, Aksel S, Yeoman RR, Wiebe RH. Endometrial estradiol and progesterone receptors in patients with luteal phase defects and endometriosis. Fertil Steril 1985;43:851-5.
8. Clark JH, Markaverich BM. The agonistic-antagonistic properties of clomiphene: a review. Pharmacol Ther 1982;15:467-519.
9. Cook CL, Schroeder JA, Yussman MA, Sanfilippo JS. Induction of luteal phase defect with clomiphene citrate. Am J Obstet Gynecol 1984;149:613-6.
10. Bowman SP, Leake A, Morris ID. Time-related effects of enclomiphene upon central and peripheral oestrogen target tissues and cytoplasmic receptors. J Endocrinol 1981;89:117-28.
11. Adashi EY, Hsueh AJW, Yen SSC. Alterations induced by clomiphene in the concentrations of oestrogen receptors in the uterus, pituitary gland and hypothalamus of female rats. J Endocrinol 1980;87:383-92.
12. Fritz MA, Westfahl PK, Graham RL. The effect of luteal phase estrogen antagonism on endometrial development and luteal function in women. J Clin Endocrinol Metab 1987;65:1006-13.
13. Ronnberg L, Isotalo H, Kauppila A, Martikainen H, Vihko R. Clomiphene-induced changes in endometrial receptor kinetics on the day of ovum collection after ovarian stimulation: a study on cytosol and nuclear estrogen and progestin receptors and 17β-hydroxysteroid dehydrogenase. Ann NY Acad Sci 1985;442:408-15.
14. Kokko E, Janne O, Kauppila A, Vihko R. Cyclic clomiphene citrate treatment lowers cytosol estrogen and progestin receptor concentrations in the endometrium of postmenpausal women on estrogen replacement therapy. J Clin Endocrinol Metab 1981;52:345-9.
15. Aksel S, Saracoglu OF, Yeoman RR, Wiebe RH. Effects of clomiphene citrate on cytosolic estradiol and progesterone receptor concentrations in secretory endometrium. Am J Obstet Gynecol 1986;155:1219-23.
16. Dawood MY. Circulating maternal serum progesterone in high-risk pregnancies. Am J Obstet Gynecol 1976; 125:832-40.
17. Khan-Dawood FS, Dawood MY. Implantation of the rabbit blastocyst: sequential changes in estradiol and progesterone and their receptors. Am J Obstet Gynecol 1984;150:450-5.
18. Khan-Dawood FS, Dawood MY. Estrogen and progesterone receptor and hormone levels in human myometrium and placenta in term pregnancy. Am J Obstet Gynecol 1984;150:501-5.
19. Burton K. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem J 1956;62:315-23.
20. Gorski J, Welshons WV, Sakai D, et al. Evolution of a model of estrogen action. Recent Prog Horm Res 1986; 42:297-329.
21. Kokko E, Janne O, Kauppila A, Vihko R. Effects of tamoxifen, medroxyprogesterone acetate, and their combination on human endometrial estrogen and progestin receptor concentrations, 17β-hydroxysteroid dehydrogenase activity and serum hormone concentrations. Am J Obstet Gynecol 1982;143:382-8.
22. Thatcher SS, Donachie KM, Glasier A, Hillier SG, Baird DT. The effects of clomiphene citrate on the histology of human endometrium in regularly cycling women undergoing in vitro fertilization. Fertil Steril 1988;49:296.
23. Sundstrom P, Nilsson O, Liedholm P. Scanning electron microscopy of human preimplantation endometrium in normal and clomiphene/human chorionic gonadotropin–stimulated cycles. Fertil Steril 1983;40: 642.
24. Hammond MG, Halme JK, Talbert LM. Factors affecting the pregnancy rate in clomiphene citrate induction of ovulation. Obstet Gynecol 1983;62:196-202.
25. Li T-C, Rogers AW, Lenton EA, Dockery P, Cooke I. A comparison between two methods of chronological dating of human endometrial biopsies during the luteal phase, and their correlation with histologic dating. Fertil Steril 1987;48:928-32.

Aromatase in human fetal tissues

Kevin J. Doody, MD, and Bruce R. Carr, MD
Dallas, Texas

The placenta has been shown to be the major source of estrogen production during pregnancy. This investigation was undertaken to compare the content and activity of aromatase in the placenta and various other human fetal tissues. Tissues were obtained from first- and second-trimester human abortuses. The amount of aromatase P-450 (aromatase cytochrome P-450) in tissue homogenates was determined after sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting by use of a polyclonal antibody directed against aromatase cytochrome P-450. The activity of aromatase in microsomal preparations was assayed by determining the rate of incorporation of tritium from 1-[^{3}H]androstenedione into [^{3}H]water. The greatest amount of aromatase cytochrome P-450 (55 kd) was detected in placenta and lesser amounts were detected in other tissues. Aromatase activity also was highest in placental microsome fractions (368 ± 62.4 pmol/mg/hr [mean ± SE], n = 9). A significant amount of aromatase activity was also detected in fetal liver (19 ± 4.8 pmol/mg/hr, n = 7). Much less activity was found in brain (2.8 ± 1.0 pmol/mg/hr, n = 6) and intestine (2.7 ± 1.3 pmol/mg/hr, n = 7). Minimal activity was noted in adrenal (n = 5), spleen (n = 4), stomach (n = 4), and muscle (n = 5) (1.2 to 1.5 pmol/mg/hr). Activity in kidney (n = 7), heart (n = 4), and lung (n = 4) was extremely low (<0.8 pmol/mg/hr). In conclusion, the placenta is a major site of conversion of C19 steroid precursors to estrogens because of the amount of enzyme and the high rate of activity of aromatase compared with those of other fetal tissues. However, considering the size and rate of aromatase activity in other fetal tissues such as liver, brain, and intestine, these tissues also may contribute to the total estrogen production in the fetal-placental unit. (Am J Obstet Gynecol 1989;161:1694-7.)

Key words: Aromatase, placenta, aromatase cytochrome P-450

The enzyme aromatase P-450 (aromatase cytochrome P-450) catalyzes the formation of C18 estrogens from C19 steroid precursors. During pregnancy the placenta is considered to be the principal site of estrogen synthesis. However, aromatase activity is known to be present in several other tissues. The ability of the fetal liver to convert C19 steroids to estrogens is well documented.[1-5] The metabolism of androstenedione to estrogens by the human fetal brain also has been previously demonstrated.[1, 6-8] Additionally, the fetal adrenal, kidney, lung, and thymus appear to be capable of converting androstenedione to estrone.[1-4] The methods used by previous investigators include radioisotope infusion of previable fetuses[9] or single- and double-isotope incubations followed by thin-layer chromatography and crystallization to homogeneity with the use of homogenates or slices of fetal tissues.[1-8]

The physiologic role of the large quantity of estrogens synthesized by the fetal-placental unit is not completely understood. It has been proposed that estrogen may regulate or fine-tune the events leading to parturition since pregnancies are often prolonged when estrogen levels in maternal blood and urine are low, as in placental sulfatase deficiency, or when associated with anencephaly.[10, 11] Estrogens also may play a role in fetal organ maturation and development. This investigation was undertaken as an attempt to further define the mechanisms of fetal estrogen production. Our primary goal was to quantitate the activity of aromatase and the content of aromatase cytochrome P-450 in the placenta and various other human fetal tissues.

From The Cecil H. and Ida Green Center for Reproductive Biology Sciences and Department of Obstetrics and Gynecology, The University of Texas Southwestern Medical Center at Dallas.
Supported by National Institutes of Health grant HD-17814.
Presented in part at the Thirty-fifth Annual Meeting of the Society for Gynecologic Investigation, Baltimore, Maryland, March 17-20, 1988.
Reprint requests: Bruce R. Carr, MD, Department of Obstetrics and Gynecology, The University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Blvd., Dallas, TX 75235.
6/6/16236

Material and methods

Source and preparation of tissue. Tissues were obtained from fetuses of 12 to 18 weeks' gestation at induced abortion. Tissues were obtained in accordance with the Donors Anatomical Gift Act of the State of Texas after consent in writing from the woman who was to undergo the abortion. Consent form and protocol were approved by the Human Research Review Committee of the University of Texas Southwestern Medical Center at Dallas. Fetal tissues were transported on ice and homogenates and microsomal fractions were prepared as rapidly as possible (usually <1 hour).

Preparation of homogenates. A homogenizing buffer containing 0.25 mol/L sucrose, 10 mmol/L tris(hydroxymethyl)aminomethane hydrochloride

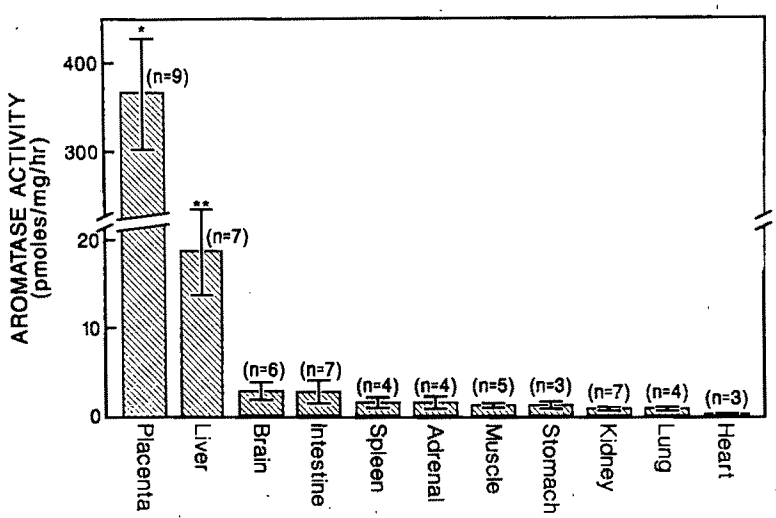


Fig. 1. Aromatase activity in various human fetal tissues. Aromatase activity was determined by the measurement of the formation of [^{3}H]water from 1[^{3}H]androstenedione as described in Material and methods. Each *bar* represents the mean ± SEM of the results obtained. *n*, Number of fetuses used for each determination. Data are expressed as picomoles per milligram of microsomal protein per hour of aromatase activity; *asterisk*, $p < 0.01$ where placental activity differed significantly from other tissues; *double asterisk*, $p < 0.05$ where liver activity differed significantly from other fetal tissues.

(Tris-HCl), 1 mmol/L ethylenediaminetetraacetic acid (EDTA), and 1 mmol/L dithiothreitol was used. The protease inhibitors phenylmethylsulfonyl and fluoride (1 mmol/L), pepstatin (10 μg/ml), leupeptin (10 μg/ml), and antipain (10 μg/ml) were added to the homogenization buffer to prevent degradation. Tissues were homogenized in the above buffer at pH 7.6 with a Teflon-glass homogenizer. Samples were then centrifuged at 100 *g* for 10 minutes at 4° C to remove cellular debris.

Preparation of microsomal fractions. Homogenates were centrifuged at 20,000 *g* for 20 minutes at 4° C. The supernatant fraction was centrifuged at 100,000 *g* for 1 hour at 4° C. The microsomal fractions were stored in liquid nitrogen until assayed for aromatase activity.

Sodium dodecyl sulfate and polyacrylamide gel electrophoresis and immunoblotting of aromatase cytochrome P-450. One-dimensional electrophoresis was conducted with a 3% polyacrylamide stacking gel and a 10% polyacrylamide running gel. Samples were applied to the gel in buffer containing 0.2% sodium dodecyl sulfate, 50 mmol/L Tris-HCl, 10% glycerol, 2 mmol/L EDTA, 2 mmol/L β-mercaptoethanol, and 0.05% bromophenol blue. Electrophoresis was carried out at 15 mA at 4° C for 16 hours in an electrode buffer containing Tris base (50 mmol/L), glycine (383 mmol/L), sodium dodecyl sulfate (0.1%), and EDTA (2 mmol/L). Proteins were transferred from gels onto nitrocellulose paper in a Trans-Blot cell apparatus (Bio-Rad Laboratories, Richmond, Calif.). A second electrophoresis was carried out at 170 mA for 16 hours at 4° C with an electrode buffer of Tris base (20 mmol/L), glycine (50 mmol/L), and methanol (20%). After transfer, the nitrocellulose was preincubated in phosphate-buffered saline solution–Tween (0.5%) for 30 minutes at room temperature. Incubation with the primary antibody (polyclonal anti–aromatase cytochrome P-450) was carried out for 1 hour. Fresh phosphate-buffered saline solution–Tween containing the second antibody [^{125}I]goat antirabbit immunoglobulin G (New England Nuclear, Boston; 500 μCi) was added for 2 hours. In other experiments [^{125}I]protein A (New England Nuclear, 1×10^6 cpm/ml) was used (data not shown). The nitrocellulose was washed three times with water and four times with phosphate-buffered saline solution–Tween. After an additional rinse with water, the nitrocellulose was blotted dry, wrapped in plastic, and exposed to Kodak Omat-X film for 48 hours with the use of enhancing screens. Polyclonal rabbit anti–aromatase cytochrome P-450 was generously provided by Drs. Carol Mendelson and Evan Simpson, Departments of Biochemistry and Obstetrics and Gynecology, University of Texas Southwestern Medical School, Dallas.[12-15]

Assay of aromatase activity. 1β[^{3}H]Androstenedione (28 Ci/mmol) was purchased from New England Nuclear. Aliquots of microsomal fractions were assayed for protein content by the method of Lowry et al.[16] Microsomal fractions (50 to 100 μg protein per sample) were incubated in buffer containing 50 mmol/L monofasic potassium phosphate and 3 mmol/L magnesium chloride at a pH of 7.4., A β-nicotinamide adenine dinucleotide phosphate, reduced form, generating sys-

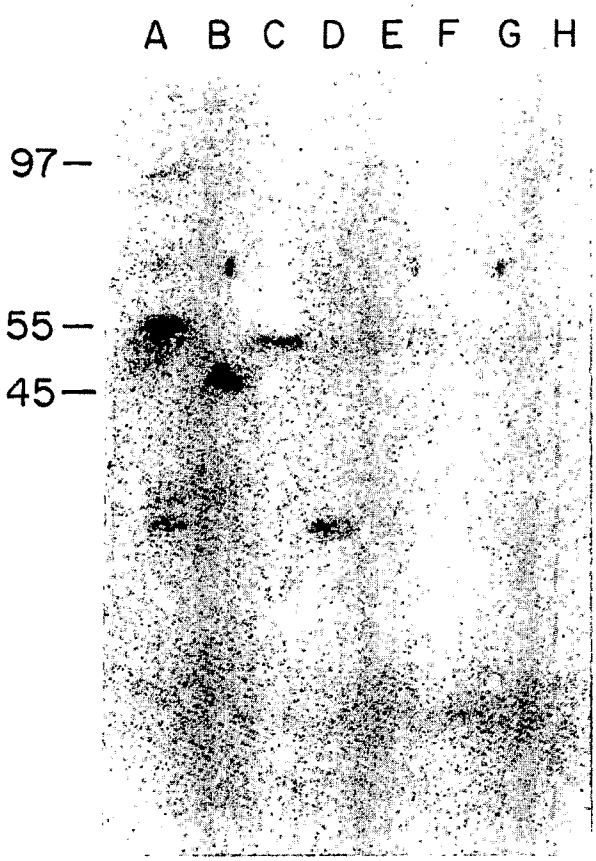


Fig. 2. Sodium dodecyl sulfate and immunoblotting of aromatase cytochrome P-450 of various fetal tissues. Lane *A*, Placenta; *B*, liver; *C*, brain; *D*, intestine; *E*, lung; *F*, kidney; *G*, muscle; *H*, skin; 200 μg of protein was subjected to electrophoresis in polyacrylamide gels containing 0.1% sodium dodecyl sulfate. The proteins were transferred electrophoretically to nitrocellulose paper and incubated with polyclonal antiaromatase antibody and then incubated with [^{125}I]goat antirabbit immunoglobulin (10^6 cpm/ml) as discussed in Material and methods. Molecular weight standards were in kilodaltons as shown.

tem of 1 mmol/L β-nicotinamide adenine dinucleotide phosphate, reduced form, 10 mmol/L glucose-6-phosphate, and 1.2 U/ml glucose-6-phosphate dehydrogenase was used. 1β[^{3}H]Androstenedione was added to a final concentration of 150 nmol/L (final volume 200 μl). Aromatase activity was assayed by determining the incorporation of tritium from 1β[^{3}H]androstenedione into [^{3}H]water as previously described.[17, 18]

Materials. All reagents, unless otherwise specified, were purchased from Sigma Chemical Co., St. Louis.

Statistical analysis. One-way analysis of variance and Student–Newman-Keuls test were used.

Results

The specific activity of aromatase was determined in individual microsome preparations of the various tissues obtained from each fetus (Fig. 1). The rate of aromatase activity is expressed as picomoles of 1β[^{3}H]androstenedione metabolized to estrogen per milligram of protein per hour. The level of aromatase activity did not appear to vary significantly with gestational age in any of the tissues studied (data not shown). Placental microsome fractions exhibited the greatest level of aromatase activity (368 ± 62.4 pmol/mg/hr, mean ± SE, $n = 9$). The liver also demonstrated a significant ability to aromatize androstenedione (19 ± 4.8 pmol/mg/hr, $n = 7$).

A lower level of activity of aromatase was found in the other fetal tissues. Activity in the brain microsome preparations was 2.8 ± 1.0 pmol/mg/hr ($n = 6$). Similar aromatase activity was seen in intestine (2.7 ± 1.3 pmol/mg/hr, $n = 6$). Aromatase activity in the adrenal and spleen microsomes was 1.5 ± 0.7 pmol/mg/hr ($n = 6$) and 1.5 ± 0.6 pmol/mg/hr ($n = 5$), respectively. Muscle aromatase activity was 1.2 ± 0.3 pmol/mg/hr ($n = 7$). Some activity was also measured in the stomach (1.2 ± 0.4 pmol/mg/hr, $n = 3$). Minimal aromatase activity was observed in kidney (0.78 ± 0.2 pmol/mg/hr, $n = 7$), lung (0.75 ± 0.3 pmol/mg/hr, $n = 5$), and heart (0.19 ± 1 pmol/mg/hr, $n = 4$).

Immunoblot analysis of tissue homogenates demonstrated a large quantity of immunoreactive aromatase cytochrome P-450 (55 kd) in the placenta (Fig. 2). A smaller (45 kd) band was noted to be consistently present in liver homogenates. In addition, a 52 kd immunoreactive protein was found to be present in brain tissue.

Comment

In this study we demonstrated that the placenta is the major site of aromatase activity and contains the greatest amount of aromatase cytochrome P-450 of all fetal tissues; thus it is confirmed as the primary location of conversion of C19 steroid precursors to estrogens. Furthermore, it also appears probable that the fetal liver (in view of its size, blood flow, and aromatase activity) also contributes substantially to the total estrogen production of the fetal-placental unit.

All of the other fetal tissues studied had significantly lower levels of aromatase activity, and it is unlikely that they contribute significantly to overall estrogen production. However, it is possible that estrogens produced in these tissues act locally to induce metabolic effects. The significance of estrogens produced in these other sites is a subject for further investigation.

From our study only placental tissue demonstrated immunoreactive protein for aromatase cytochrome P-450 at 55 kd. The significance of the smaller immunoreactive proteins noted in brain and liver tissue is unclear. Posttranslational modification of the enzyme

may account for the presence of the smaller immunoreactive protein. Protein inhibitors were used to minimize the possibility that these findings were artifactual in origin. The consistency of the finding of an immunoreactive band at 45 kd in the fetal liver (which had the highest aromatase activity of any nonplacental tissue), together with the lack of this finding in the many other tissues tested, is evidence that tends to support the possibility of posttranslational modification. Control immunoblots that used only the second antibody did not demonstrate these smaller bands, thus excluding the possibility of nonspecific binding of this antibody. The possibility that the 45 kd band results from nonspecific binding of the polyclonal antibody cannot be excluded altogether. Currently, absolute identification of this band is not possible. Development of other antibodies against aromatase in the future may help to clarify this issue.

Previous investigators have reported similar qualitative results for aromatase activity by use of isotope incubations with homogenates from fetal tissues obtained from a relatively small number of fetuses. George and Wilson[19] reported that total estrogen formation in tissue slices incubated with testosterone and androstenedione was greatest in fetal ovaries. However, we were unable to obtain enough fetal ovaries to prepare a microsomal fraction in this study.

This investigation is believed to be the first to report use of the [^{3}H]water assay to measure aromatase activity in fetal tissues, as well as to measure the amount of immunoreactive aromatase cytochrome P-450. In summary, the placenta exhibits significant amounts of aromatase cytochrome P-450 and a high rate of aromatase activity. Significant activity is also found in the fetal liver when compared with other fetal tissues.

We thank Sandy Nance for editorial assistance and Nora Cline for expert technical assistance.

REFERENCES

1. Schindler AE. Steroid metabolism of fetal tissues. Am J Obstet Gynecol 1975;123:265.
2. Schindler AE, Hayashi K. Steroid metabolism of foetal tissues. V. Comparative studies in a 18 trisomic foetus. Endocrinol Exp (Bratisl) 1977;11:133.
3. Yamamoto T, Sakai C, Okada H. Aromatization of androstenedione to estrogen by human fetal tissues in vitro. Asia Oceania J Obstet Gynaecol 1984;10:241.
4. Wu CH, Flickinger GL, Archer DF, Touchstone JC. Estrogen formation in vitro by fetal liver, fetal adrenal gland, and placenta of early human pregnancy. Am J Obstet Gynecol 1970;107:313.
5. Yamamoto T, Sakai C, Yamaki J, et al. Estrogen biosynthesis in human liver—a comparison of aromatase activity for C-19 steroids in fetal liver, adult liver and hepatoma tissues of human subjects. Endocrinol Jpn 1984;31:277.
6. Naftolin F, Ryan KJ, Petro Z. Aromatization of androstenedione by the diencephalon. J Clin Endocrinol Metab 1971;33:368.
7. Naftolin F, Ryan KJ, Petro Z. Aromatization of androstenedione by limbic system tissue from human foetuses. J Endocrinol 1971;51:795.
8. Ryan KJ, Naftolin F, Reddy V, Flores F, Petro Z. Estrogen formation in the brain. Am J Obstet Gynecol 1972; 114:454.
9. Mancuso S, Dell'Acqua S, Eriksson G, Wiqvist N, Diczfalusy E. Aromatization of androstenedione and testosterone by the human fetus. Steroids 1965;5:183.
10. Carr BR, Simpson ER. Lipoprotein utilization and cholesterol synthesis by the human fetal adrenal gland. Endocr Rev 1981;2:306.
11. Bradshaw KD, Carr BR. Placental sulfatase deficiency: maternal and fetal expression of steroid sulfatase deficiency and X-linked ichthyosis. Obstet Gynecol Surv 1986;41:401.
12. Mendelson CR, Wright EE, Evans CT, Porter JC, Simpson ER. Preparation and characterization of polyclonal and monoclonal antibodies against human aromatase cytochrome P450 and their use in its purification. Arch Biochem Biophys 1985;243:480.
13. Evans CT, Corbin CJ, Saunders CT, Merrill JC, Simpson ER, Mendelson CR. Regulation of estrogen biosynthesis in human adipose stromal cells. J Biol Chem 1987; 262:6914.
14. Fournet N, MacLusky NJ, Leranth CZ, et al. Immunological localization of aromatase cytochrome P-450 and estradiol dehydrogenase in the syncytiotrophoblast of the human placenta. J Clin Endocrinol Metab 1987;65:757.
15. Steinkampf MP, Mendelson CR, Simpson ER. Regulation by FSH of the synthesis of aromatase cytochrome P-450 in human granulosa cells. Mol Endocrinol 1987;1:465.
16. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265.
17. Lanoux MJ, Cleland WH, Mendelson CR, Carr BR, Simpson ER. Factors affecting the conversion of androstenedione to estrogens by human fetal hepatocytes in monolayer culture. Endocrinology 1985;117:361.
18. Ackerman GE, Smith ME, Mendelson CR, MacDonald PC, Simpson ER. Aromatization of androstenedione by human adipose tissue stromal cells in monolayer culture. J Clin Endocrinol Metab 1981;53:412.
19. George FW, Wilson JD. Conversion of androgen to estrogen by the human fetal ovary. J Clin Endocrinol Metab 1978;47:550.

Comparison of in vivo bioavailability of progestational agents into the rat uterus and liver and the effect of plasma protein binding

Bradley Van Voorhis, MD, Dennis W. Matt, PhD, Maria L. Tedeschi, and Kenneth A. Steingold, MD

Richmond, Virginia

The in vivo bioavailabilities of three clinically available progestational agents were evaluated with a previously described rat model. With this model a single-injection, double-isotope technique was performed to calculate and compare the unidirectional extractions of progesterone, 19-nortestosterone, and medroxyprogesterone acetate into the rat uterus and liver. To assess the role of plasma protein binding in the extraction of these agents, steroids were presented in four different vehicles: (1) Ringer's solution, 0.1% bovine serum albumin; (2) Ringer's solution, 4% bovine serum albumin; (3) Ringer's solution, 0.8 mg/ml, α_1-acid glycoprotein; (4) human pregnancy serum. The results revealed that liver extraction always exceeded uterine extraction for each progestin, regardless of the vehicle, and that liver extraction was approximately 100% for all steroids. With regard to the uterus, when binding proteins were not present in the injectate, the extraction of the three steroids was similar; the uterine vasculature was relatively permeable to these progestins, and bovine serum albumin and α_1-acid glycoprotein had no major effect on the uptake into the uterus. However, when human pregnancy serum was in the injectate, the extractions of progesterone and 19-nortestosterone into the rat uterus were significantly diminished. In contrast, pregnancy serum had no effect on the uptake of medroxyprogesterone acetate into the uterus, with the extraction equal to that of Ringer's solution alone. This suggests that, regardless of potential binding proteins, medroxyprogesterone acetate displays greater bioavailability than that of the other progestogens studied. (Am J Obstet Gynecol 1989;161:1698-703.)

Key words: Progestin bioavailability, plasma protein binding, albumin, α_1-acid glycoprotein, progesterone

The uterine and hepatic bioavailability of various progestational agents is an important variable in the determination of the optimal progestogen. Whereas the uterus is an important target organ for progestational activity, the liver assumes a key role as a site of metabolism of progestins and a possible site of adverse reactions that result from the first-pass effect of oral administration of certain progestins.[1]

Previous in vitro studies have shown that medroxyprogesterone acetate is poorly bound to known steroid binding proteins and may be more bioavailable than other progestins.[2] Therefore this study was designed to evaluate and compare the in vivo bioavailability of three progestins into the rat uterus and liver, and to assess whether plasma or plasma proteins affect the bioavailability of these hormones.

The progestins studied were progesterone, medroxyprogesterone acetate, and 19-nortestosterone, chosen as a test steroid to represent the potential derivatives of 19-nortestosterone currently available. The hepatic and uterine bioavailabilities were assessed with a previously described in vivo rat model.[3] With this model, the influxes of the progestins from the microcirculation into the rat uterus and liver were evaluated. In addition, the effects of two plasma proteins, albumin and α_1-acid glycoprotein (AAG), and of the potential steroid binding proteins found in human pregnancy serum on the influx of these steroids were examined.

Previous studies have similarly used this model to evaluate available estrogens used for replacement therapy.[4] Differential effects in the uterine and hepatic vasculature relating to differences in membrane permeability and plasma protein binding of these individual agents were described. This study expands these data to include progestational agents.

Material and methods

Radiolabeled progesterone, medroxyprogesterone acetate, and butanol were obtained from New England Nuclear Corp. (Boston, Mass.); the 19-nortestosterone isotope was obtained from Amersham Laboratories

From the Department of Obstetrics and Gynecology, Medical College of Virginia.

Presented in part at the Thirty-fifth Annual Meeting of the Society for Gynecologic Investigation, Baltimore, Maryland, March 17-20, 1988.

Reprint requests: Kenneth A. Steingold, MD, Box 34, MCV Station, Richmond, VA 23298.

6/6/15953

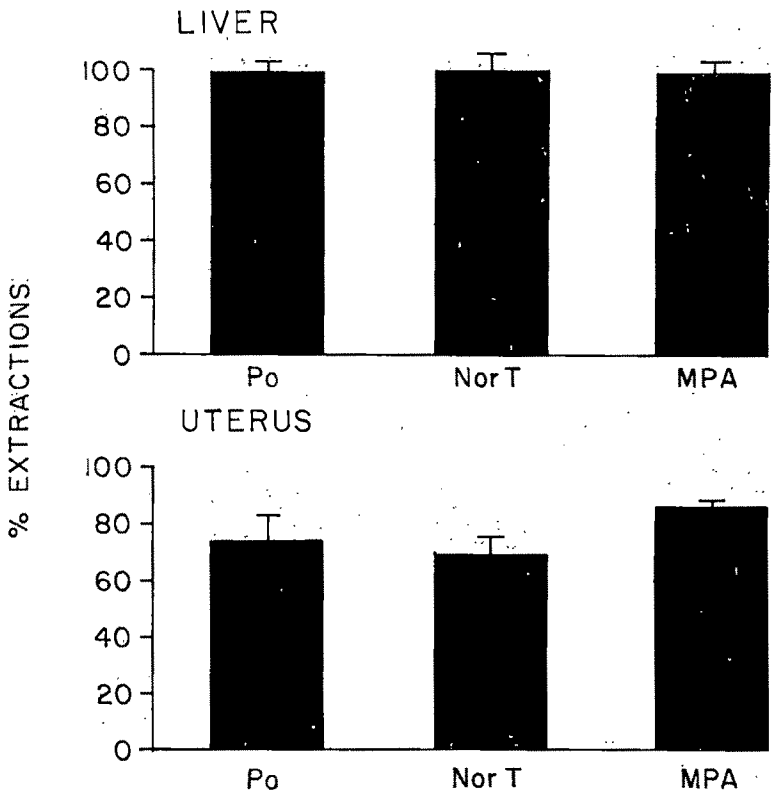


Fig. 1. Mean percent extraction (±SEM) of progesterone *(Po)*, 19-nortestosterone *(NOR-T)*, and medroxyprogesterone acetate *(MPA)* with Ringer's solution (0.1% BSA) as injection vehicle; liver *(upper panel)* and uterine *(lower panel)* extractions are shown.

(Buckinghamshire, England). The specific activities were as follows: [1, 2, 6, 7 - ^{3}H (N)]progesterone, 106.8 Ci/mmol; 6α - [1, 2, - ^{3}H(N)]medroxyprogesterone acetate, 50.0 Ci/mmol; 19-[6, 7 (N) - ^{3}H]nortestosterone, 19 Ci/mmol; n - [1 - ^{14}C]butanol, 0.9 mCi/mmol. The radiolabeled steroids were separated from the solvent by evaporation, and buffered Ringer's solution that contained 0.1% bovine serum albumin (BSA) was added to the steroids. The BSA and AAG were purchased from Sigma Chemical Co. (St. Louis, Mo.). Human serum obtained from volunteers during the third trimester of pregnancy was pooled and frozen at −20° C. The unidirectional extractions of test substances by the uterus and liver were measured by a previously described single bolus injection, double-isotope technique.[5, 6] Female Long-Evans rats, 100 days old, were ovariectomized 7 to 14 days before the injection studies. After ketamine (235 mg/kg) was administered intramuscularly to induce anesthesia, the right common iliac artery and left femoral artery were surgically exposed and clamped for studies that involved the uterus. The test solution was administered by a 250 μl rapid bolus injection into the lower abdominal aorta. The left uterine horn was resected 15 seconds after the injection. The portal vein was cannulated and a similar bolus injection was used immediately after ligation of the hepatic artery for studies that involved the liver. The right major lobe of the liver was removed 18 seconds after the injection. These time intervals were determined in previous studies to be sufficiently long to allow the injectate to exit the vasculature in question but sufficiently short to prevent reentry of the test substances into the circulation after they have passed out of the intravascular space.[5, 6]

The injectate contained either tritiated progesterone, 19-nortestosterone or medroxyprogesterone acetate. Butanol labeled with carbon 14 was present in each injectate as the freely diffusable reference, with the ratio of the tritiated steroid to [^{14}C]butanol between 10 and 30. The different injection vehicles evaluated were (1) Ringer's solution with 0.1% BSA; (2) Ringer's solution with 4% BSA; (3) Ringer's solution with AAG at the human physiologic concentration of 0.8 mg/ml; and (4) third-trimester human pregnancy serum. When serum was used as the injection vehicle, the concentration of labeled test substances was deliberately set to be less than the concentration of previously determined unbound serum globulin binding sites. The concentration of test steroids in serum was 6 nmol/L, and in the

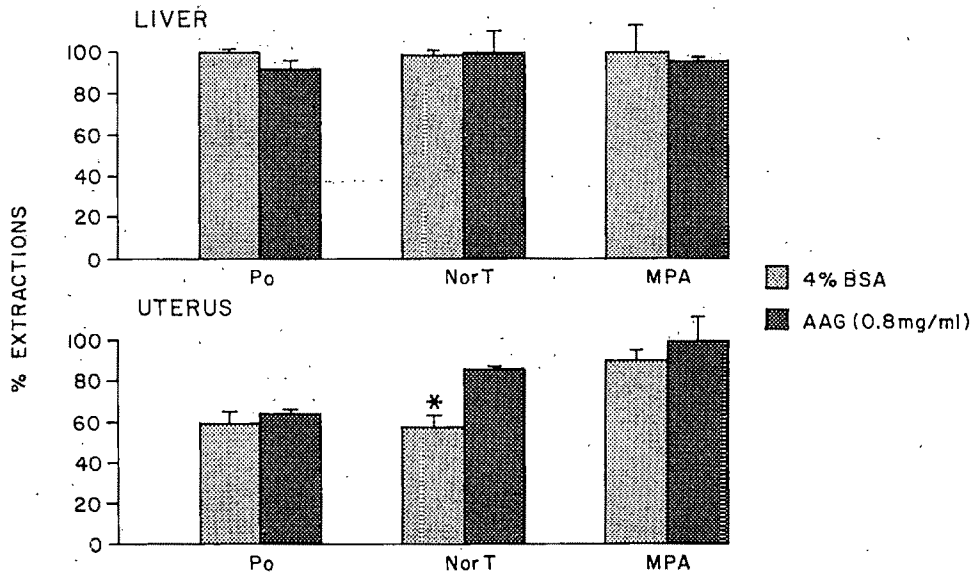


Fig. 2. Mean percent extraction (±SEM) of progesterone *(Po)*, 19-nortestosterone *(NOR-T)*, and medroxyprogesterone acetate *(MPA)* with 4% BSA in Ringer's solution compared with AAG (0.8 mg/ml) in Ringer's solution as injection vehicles; liver *(upper panel)* and uterine *(lower panel)* extraction are shown. $*p < 0.005$.

Ringer's solution it was 24 nmol/L. In both organs studied, three to six injections were performed with each of the progestogens in each of the injection vehicles.

The resected tissues were then solubilized in 2.0 ml Soluene-350 (Packard Instrument Co., Downers Grove, Ill.). The tissue and the injection solution samples were counted by double-isotope liquid scintillation measurements for disintegrations per minute (DPM) with quench correction. The liver or uterine uptake was computed as follows:

$$\text{Uptake} = \frac{(^3H/^{14}C)\ \text{DPM in tissue}}{(^3H/^{14}C)\ \text{DPM in injectate}} \times 100$$

The results are depicted as the unidirectional extraction for each progestin. The organ uptake can be converted to extraction if the extraction of butanol is known. It is assumed that there is 100% extraction of butanol into the uterus and liver, but butanol effluxes these organs at a rate on the basis of blood flow. It has previously been shown that the extraction of butanol in the uterus at 15 seconds is 77%,[5] and in the liver at 18 seconds is 84%.[6] Therefore all uptake determinations were converted to unidirectional extraction as follows: uterine extraction = uterine uptake × 0.77; liver extraction = liver uptake × 0.84.

All results are reported as mean ± SEM extraction of test steroid. Statistical analysis was performed with the Student *t* test.

Results

The tissue unidirectional extractions of the tritiated progestogens by the uterus and liver with Ringer's solution (0.1% BSA) as the injection vehicle are shown in Fig. 1. There was 100% extraction of progesterone, 19-nortestosterone, and medroxyprogesterone acetate by the liver. For the uterus, somewhat lower values were noted for each of the steroids studied, with extractions of 74 ± 8% for progesterone, 69 ± 7% for 19-nortestosterone, and 86 ± 2% for medroxyprogesterone acetate. Because essentially no binding proteins were present in the injectate, the data show that all test substances are freely diffusible into the liver, and are relatively freely diffusible into the uterus.

The effect of the potential serum binding proteins (albumin and AAG) on progestogen extraction can be seen in Fig. 2. With regard to the liver, there was no inhibition of extraction of any of the test steroids by these two binding proteins, with levels ranging from 94% to 121%. In the uterus there was no difference in the percent of progesterone extraction when 4% BSA was compared to AAG. However, the values noted here are slightly, but not significantly, decreased as compared to the 74% extraction in Ringer's solution illustrated in Fig. 1. When 4% BSA was used as the injection vehicle for 19-nortestosterone, the percent extraction was significantly lower than the extraction with AAG ($p < 0.005$), but not different from Ringer's solution. However, for medroxyprogesterone acetate there was no inhibition of extraction with either BSA or AAG. In general, all three progestins did display relatively high extraction rates into the uterus despite the presence of these binding proteins, inasmuch as the extraction of all three steroids was ≥60%.

Fig. 3 shows the extraction of the progestins when

human pregnancy serum was the injection vehicle. Again, with regard to the liver, all three progestins were freely diffusible despite the presence of potential binding proteins in pregnancy serum. In the uterus, the extractions of progesterone and 19-nortestosterone were significantly diminished, as compared with their extraction in Ringer's solution ($p < 0.02$), with the percent extraction of progesterone, 34 ± 8%, and 19-nortestosterone, 26 ± 4%. In contrast, medroxyprogesterone acetate displayed marked bioavailability into the rat uterus with a percent extraction of 84 ± 8%, despite pregnancy serum in the injectate. Therefore in the presence of binding proteins and serum globulins that are normally found in human serum, the apparent bioavailability of progesterone and 19-nortestosterone into the uterus was significantly diminished, as compared with that of medroxyprogesterone acetate ($p < 0.005$).

Comment

Progestins are commonly used in clinical practice to prevent the effects of unopposed estrogen on the endometrium. A variety of progestins are available to the clinician including natural progesterone and progesterone derivatives. Oral administration of progesterone in a micronized form is available and has displayed adequate oral absorption with decreased metabolism.[7] The 19-nortestosterone derivatives, such as norethindrone acetate, norgestrel, and norethynodrel are some of the active progestational agents found in oral contraceptives. These compounds may also be administered as single agents, both in the progestin-only contraceptives and for estrogen opposition in the anovulatory or postmenopausal female. Medroxyprogesterone acetate is also widely used for these purposes as single-agent therapy. For these reasons, progesterone, 19-nortestosterone, and medroxyprogesterone acetate were evaluated in this study.

In choosing among these progestational agents, clinical parameters including efficacy and safety need to be considered. One of the key concerns with regard to safety is the potential effect of the various progestins on hepatic metabolism. For example, the effects of progestins on hepatic lipoprotein production are major factors in the comparison of various agents.[8-10] Therefore in this study the uterus and liver were chosen as specific organs to evaluate extraction of these three steroids.

To determine whether these clinically observed differences in hepatic effects could be a result of differing bioavailability of the progestins to the liver, we first looked at hepatic uptake of the progestins. No difference was observed, as all progestins displayed 100% extraction into the liver despite the presence of pregnancy serum. One interesting observation was that hepatic uptake always exceeded uterine uptake, regardless of the progestin or injection vehicle studied. This finding may result from differences in anatomical architecture and in capillary transit time. The capillary transit time of an organ is the time required for a substance to pass from the afferent to the efferent vessels through the capillaries. The capillary transit time in the liver is between 5 and 10 seconds, whereas the transit time for the uterus is not known but is believed to be between 1 and 3 seconds.[11] Thus circulating hormones have a longer time to pass from the circulation into the liver than into the uterus. In addition, the liver vasculature has wide sinusoidal pores that allow equilibration of plasma proteins and steroids between intravascular and interstitial spaces.[12] This allows for a larger surface area for steroid influx in the liver, as compared with the uterus. In a previous study of clinically available estrogens,[4] similar findings were shown for the liver with enhanced bioavailability of these estrogenic compounds, as compared with uterus and brain.

The second portion of this study evaluated the unidirectional extraction of these progestogens by the rat uterus. Availability to the uterus is an important prerequisite for the protective progestin action on the endometrium. The effects of membrane permeability and plasma protein binding can be separated in this model by altering injection vehicles. It was observed that in the absence of binding proteins (Ringer's solution), all three progestins were relatively permeable to the rat uterus. Furthermore, the presence of physiologic concentrations of the binding proteins, albumin and AAG, had little effect on uptake of any of the progestins into the uterus. Corresponding values for extraction into the uterus were always less than those found for the liver.

Human pregnancy serum was also studied as an injection vehicle. This particular physiologic state was chosen for its known high concentrations of the binding globulins—sex hormone binding globulin and transcortin.[13, 14] In pregnancy serum, the extractions of progesterone and 19-nortestosterone into the uterus were significantly diminished, as compared with Ringer's solution alone, albumin, and AAG. Therefore proteins in pregnancy serum exhibited binding to both progesterone and 19-nortestosterone effectively to prevent the in vivo uptake into the rat uterus. In marked contrast, medroxyprogesterone acetate remained freely permeable into the rat uterus, despite the presence of human pregnancy serum in the injectate. Regardless of potential binding proteins that are found in significant concentrations in pregnancy serum, medroxyprogesterone acetate exited the uterine vasculature at a level similar to that found when Ringer's solution alone (0.1% BSA) was used as the injectate. The extraction of medroxyprogesterone acetate was signifi-

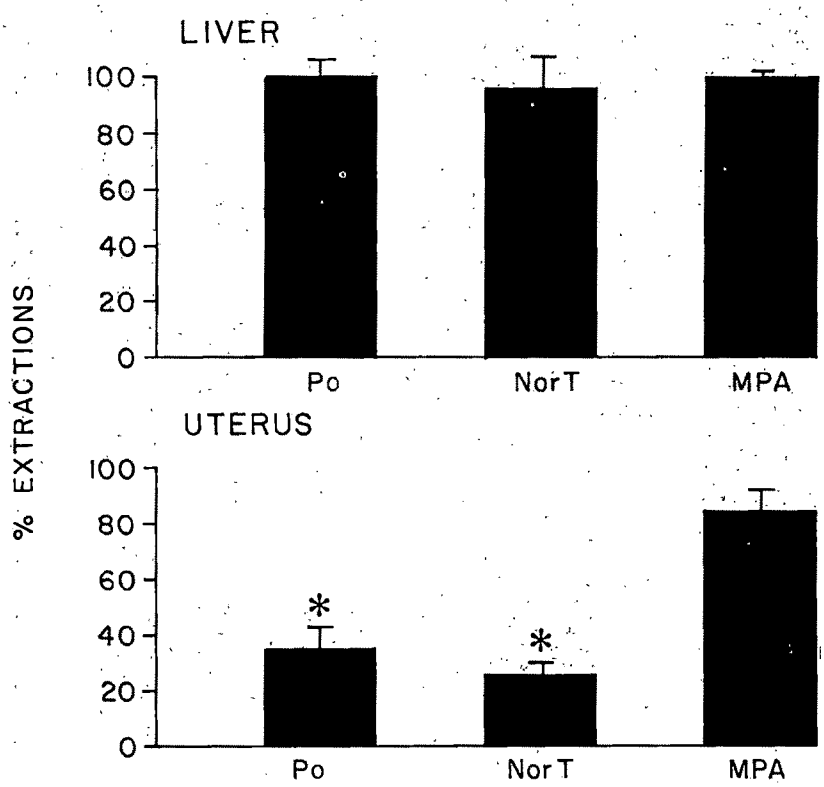


Fig. 3. Mean percent extraction (±SEM) of progesterone *(Po)*, 19-nortestosterone *(NOR-T)*, and medroxyprogesterone acetate *(MPA)* with human pregnancy serum as injection vehicle; liver *(upper panel)* and uterine *(lower panel)* extractions are shown. $*p < 0.005$.

cantly greater than that found for progesterone or 19-nortestosterone in the presence of pregnancy serum.

In vitro studies support our in vivo data with regard to the lack of effect of serum globulins on the bioavailability of medroxyprogesterone acetate. Results from competitive binding analysis and polyacrylamide gel electrophoresis suggest that medroxyprogesterone acetate does not demonstrate significant binding to sex hormone binding globulin or corticosteroid binding globulin.[2] In contrast, progesterone is known to bind with corticosteroid binding globulin, whereas 19-nortestosterone and its derivatives bind with varying affinity to sex hormone binding globulin.[15-18] There are also differences in circulating plasma levels of progestins achieved after clinical administration with decreased levels of medroxyprogesterone acetate, as compared with 19-nortestosterone derivatives.[19, 20] This is believed to be a result of differences in compartmentalization of the drugs, in addition to decreased affinity for plasma proteins, medroxyprogesterone acetate exhibits extravascular storage. These differing properties may lead to the greater uterine in vivo bioavailability of medroxyprogesterone acetate seen in our study.

However, in vitro studies also suggest binding of progesterone and 19-nortestosterone to albumin; progesterone also has affinity for AAG.[17, 19] In this study albumin and AAG did not inhibit uptake of these compounds into the uterus, although the BSA values for 19-nortestosterone were lower than those for AAG. These compounds were still relatively free for transport into this target organ, despite these proteins, which is compatible with previous in vivo studies.[20]

In summary, using an in vivo model, hepatic and uterine extraction of these three progestins with and without potential binding proteins were evaluated. Because of characteristics of the hepatic vasculature, these agents were essentially 100% available to the liver despite the presence of potential binding proteins. This effect is believed to be an inherent property of the architecture of hepatic vasculature. In contrast, uterine extraction was always lower than that found for hepatic tissue at each corresponding point. Progesterone and 19-nortestosterone behaved relatively similarly and uptake from the uterine vasculature was diminished in the presence of binding proteins found in human pregnancy serum. Medroxyprogesterone acetate displayed a marked difference in that extraction from the vasculature of the uterus was greater at all corresponding points, and the presence of potential binding in pregnancy serum resulted in no diminution of uterine bioavailability. This study emphasizes the effect of binding globulins on progestogen bioavailability, an effect that

varied among the progestational agents tested and may influence hormone actions.

REFERENCES

1. Bradley DD, Wingerd J, Petitti DB, Krauss RM, Ramcharan S. Serum high-density-lipoprotein cholesterol in women using oral contraceptives, estrogens and progestins. N Engl J Med 1978;299:17-20.
2. Akpoviroro JA, Mangalam M, Jenkins N, Fotherby K. Binding of the contraceptive steroids medroxyprogesterone acetate and ethynyloestradiol in blood of various species. J Steroid Biochem 1981;14:493-8.
3. Verheugen C, Pardridge WM, Judd HL, Chaudhuri G. Differential permeability of uterine and liver vascular beds to estrogens and estrogen conjugates. J Clin Endocrinol Metab 1984;59:1128-32.
4. Steingold KA, Cefalu W, Pardridge W, Judd HL, Chaudhuri G. Enhanced hepatic extraction of estrogens used for replacement therapy. J Clin Endocrinol Metab 1986; 62:761-6.
5. Laufer LR, Gambone JC, Chaudhuri G, Pardridge WM, Judd HL. The effect of membrane permeability and binding by human serum proteins on sex steroid influx into the uterus. J Clin Endocrinol Metab 1983;56:1282-7.
6. Pardridge WM, Mietus LJ. Transport of protein-bound steroid hormones into liver in vivo. Am J Physiol 1979; 237:E367-72.
7. Maxson WE, Hargrove JT. Bioavailability of oral micronized progesterone. Fertil Steril 1985;44:622-6.
8. Wahl P, Walden C, Knopp R, et al. Effect of estrogen/progestin potency on lipid/lipoprotein cholesterol. N Engl J Med 1983;308:862-7.
9. Ottosson UB. Oral progesterone and estrogen/progestogen therapy. Acta Obstet Gynecol Scand 1984; 127 (suppl):7-37.
10. Hirvonen E, Malkonen M, Manninen V. Effect of different progestogens on lipoproteins during postmenopausal replacement therapy. N Engl J Med 1981;304:560-3.
11. Goresky CA, Rose CP. Blood-tissue exchange in liver and heart; the influence of heterogeneity of capillary transit times. Fed Proc 1977;26:2629-34.
12. Baker KL, Bradley SE. Binding of sulfobromphthalein (BSP) sodium by plasma albumin. J Clin Invest 1966; 45:281-6.
13. Vermeulen A, Verdonk L, Van der Straeten M, Orie N. Capacity of the testosterone-binding globulin in human plasma and influence of specific binding of T on its metabolic clearance rate. J Clin Endocrinol Metab 1969; 29:1470-80.
14. Rosenthal HE, Slaunwhite WR Jr, Sandberg AA. Transcortin: a corticosteroid-binding protein of plasma. X. Cortisol and progesterone interplay and unbound levels of these steroids in pregnancy. J Clin Endocrinol Metab 1969;29:352-67.
15. Rosner W. the binding of steroid hormones in human serum. In: Jamieson GA, Greenwalt TJ, eds. Trace components of plasma: isolation and clinical significance. New York: Alan R Liss, Inc. 1976:377-95.
16. Burton RM, Westphal U. Steroid hormone-binding proteins and blood plasma. Metabolism 1972;21:253-76.
17. Ballard PL. Delivery and transport of glucocorticoids to target cells. In: Baster JD, Rousseau GG, eds. Glucocorticoid hormone action. New York: Springer-Verlag, 1979: 25.
18. Pugeat MM, Dunn JM, Nisula BC. Transport of steroid hormones: interaction of 70 drugs with testosterone-binding globulin and corticosteroid-binding globulin in human plasma. J Clin Endocrinol Metab 1981;53:69-75.
19. Victor A, Johansson EDB. Pharmacokinetic observations on medroxyprogesterone acetate administered orally and intravaginally. Contraception 1976;14:319-29.
20. Slaunwhite WR Jr, Sandberg AA. Disposition of radioactive 17-hydroxyprogesterone, 6α-methyl-17α-acetoxyprogesterone and 6α-methyl prednisolone in human subjects. J Clin Endocrinol Metab 1961;21:753-64.
21. Ganguly M, Carnighan RH, Westphal U. Steroid-protein interactions. XIV. Interaction between human α1-acid glycoprotein and progesterone. Biochemistry 1967;6: 2803-14.
22. Pardridge WK. Transport of protein-bound hormones into tissues in vivo. Endocr Rev 1981;2:103-23.

Androgen sulfate and glucuronide conjugates in nonhirsute and hirsute women with polycystic ovarian syndrome

Robert K. Matteri, MD, Frank Z. Stanczyk, PhD, Elisabet E. Gentzschein, BA, Clara Delgado, BA, and Rogerio A. Lobo, MD

Los Angeles, California

Peripheral androgen action largely determines the occurrence of hirsutism in women. Although serum 5α-androstane-3α,17β-diol (3α-diol) glucuronide signifies skin 5α-reductase activity and has been used as a marker of hirsutism and peripheral androgen metabolism, other C_{19} androgen conjugates have recently been measured and may also be useful markers of hirsutism in women. In addition to normal controls we studied both hirsute and nonhirsute patients with polycystic ovarian syndrome who had similar levels of circulating androgen precursors. In these three groups we measured various C_{19} sulfates and glucuronides including serum 3α-diol glucuronide. Serum androgen precursors were elevated, but were equal in the hirsute and nonhirsute patients. Serum androsterone sulfate and glucuronide, and 3α-diol sulfate and glucuronide clearly differentiated the hirsute from the nonhirsute group. Among the conjugates, androsterone glucuronide was most reflective of the difference between the two groups (100.3 ± 28.0 versus 42.9 ± 4.0 ng/ml, $p < 0.05$). In hirsute compared with nonhirsute patients with polycystic ovarian syndrome, serum 3α-diol glucuronide was increased by the smallest amount (32%), followed by androsterone sulfate (38%), 3α-diol sulfate (59%), and androsterone glucuronide with the largest increase (134%). Serum androsterone glucuronide and 3α-diol glucuronide both correlated with androstenedione and dehydroepiandrosterone sulfate in hirsute women but not in nonhirsute women. These data suggest that besides serum 3α-diol glucuronide, other C_{19} sulfate and glucuronide conjugates may reflect peripheral androgen action. (AM J OBSTET GYNECOL 1989;161:1704-9.)

Key words: Hirsutism, polycystic ovarian syndrome, 5α-reductase, androsterone sulfate, androsterone glucuronide, 5α-androstane-3α,17β-diol sulfate, 5α-androstane-3α,17β-diol glucuronide

Circulating androgens, which reflect ovarian and adrenal production, such as total or free testosterone and dehydroepiandrosterone (DHEA) sulfate may not always explain hirsutism in women.[1] Hirsutism is largely determined by skin 5α-reductase activity, but serum dihydrotestosterone unreliably reflects this peripheral androgen action.[2, 3] A dihydrotestosterone metabolite, 5α-androstane-3α,17β-diol glucuronide (3α-diol glucuronide), has been shown to be a good indicator of peripheral androgen action and reflects 5α-reductase activity.[4-9] In our preliminary work we found that other C_{19} conjugates such as androsterone sulfate and glucuronide as well as 3α-diol sulfate are not only different in men and women but also reflect hirsutism in women.[10] Also androsterone glucuronide was the most elevated conjugate in hirsute women, which implies an important role for the androsterone pathway in androgen metabolism of hirsute women. Increased production of androsterone was also seen in our previous studies on androgen metabolism in the skin of hirsute patients.[10]

Many women with polycystic ovarian syndrome who have elevated levels of serum androgens are not hirsute.[11] Further evidence that serum 3α-diol glucuronide reflects 5α-reductase activity was suggested in our studies of nonhirsute women with polycystic ovarian syndrome who, despite elevated levels of circulating androgens, have normal levels of serum 3α-diol glucuronide.[3] Whether other androgen conjugates reflect 5α-reductase activity directly or through changes in ovarian and adrenal precursors is unknown. Therefore the purpose of our study was to investigate the usefulness of androgen sulfates and glucuronides in patients with polycystic ovarian syndrome who were hirsute or nonhirsute. Besides the determination of how these conjugates reflect 5α-reductase activity, we specifically compared their measurements with a well-characterized androgen marker, serum 3α-diol glucuronide.

Material and methods

Subjects. We studied 20 patients referred to our medical center because of oligomenorrhea, anovula-

From the Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, University of Southern California School of Medicine.

Presented in part at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.

Reprint requests: Rogerio A. Lobo, MD, Department of Obstetrics and Gynecology, Women's Hospital, Room 1M2, 1240 N. Mission Road, Los Angeles, CA 90033.

6/6/15966

Table I. The mean (±SE) age, weight, height, and Ferriman-Gallwey score in normal women, nonhirsute women with polycystic ovary syndrome, and hirsute women

	Age (yr)	*Weight (kg)*	*Height (cm)*	*Ferriman-Gallwey score*
Normal women (n = 8)	31.3 ± 2.3	67.2 ± 5.8	162.6 ± 1.3	5.2 ± 1.4
Nonhirsute women with polycystic ovarian syndrome (n = 10)	27.8 ± 1.3	71.9 ± 2.7	158.2 ± 1.3	7.2 ± 1.6
Hirsute women (n = 10)	29.5 ± 2.5	70.1 ± 5.1	163.9 ± 6.4	18.3 ± 3.2*

*For Ferriman-Gallwey score, the difference between hirsute versus normal or nonhirsute women was significant at $p < 0.001$.

tion, or hirsutism. All patients met the criteria for polycystic ovarian syndrome including inappropriate gonadotropin secretion (luteinizing hormone/follicle-stimulating hormone ratio >2:1), perimenarchial onset of oligomenorrhea, and evidence of androgen excess, either having elevated serum testosterone or DHEA sulfate levels, or hirsutism.[12] The patients were divided into two groups of 10 patients solely on the basis of the presence or absence of hirsutism as reflected by an elevated Ferriman-Gallwey score.[13] Besides the gynecologic endocrine abnormalities, patients had no other endocrine or systemic medical diseases (normal thyroid function studies, normal glucose screening, normal prolactin, and no evidence of congenital adrenal hyperplasia). Patients were compared with a control group of eight normally ovulating women in the early follicular phase, who were matched for age, weight, and height. Venous blood (10 ml) was drawn between 8 and 10 AM in each subject.

Reagents. 9,11-^{3}H-androsterone (specific activity, 53.3 Ci/mmol), 1α,2α-^{3}H-testosterone (specific activity, 55.2 Ci/mmol), and 7-^{3}H-DHEA sulfate (specific activity, 16.3 Ci/mmol) were obtained from NEN Research Corp. (Boston, Mass.). 1α,2α-^{3}H-5α-androstane-3α,17β-diol (specific activity, 52.8 Ci/mmol) was obtained from Amersham (Clearbrook, Ill.). ^{3}H-3α-diol glucuronide was a gift from Nichols Institute (San Juan Capistrano, Calif.). All ^{3}H-labeled steroids were purified by Celite column chromatography before use.

Assays. DHEA sulfate and androstenedione were measured directly by radioimmunoassay with an iodine 125–DHEA sulfate kit (Diagnostic Products Corporation, Los Angeles, Calif.) and an ^{125}I-androstenedione kit (ICN Biomedicals Inc., Carson, Calif.), respectively. Serum testosterone, androsterone, and 3α-diol were measured by radioimmunoassay after extraction and separation by Celite column chromatography.[14] The non-sex hormone-binding globulin-bound testosterone was measured by ammonium sulfate precipitation of sex hormone-binding globulin as previously described.[15] The glucuronide and sulfate conjugates of 3α-diol and androsterone were measured either after β-glucuronidase hydrolysis of the glucuronides or solvolysis of the sulfates. Briefly, after removal of unconjugated steroids from serum by extraction with diethyl ether, the glucuronide conjugates were hydrolyzed at 37° C by addition of β-glucuronidase (type VIII, Sigma Chemical Co., St. Louis, Mo.) to the serum, which was first adjusted to pH 6.8. After 16 hours of hydrolysis, unconjugated hormones were extracted with diethyl ether, and 3α-diol and androsterone were separated by Celite column chromatography, and the specific fractions were measured by radioimmunoassay. The method for hydrolysis of sulfates also involved previous extraction of unconjugated steroids with diethyl ether, and preceded addition of sodium chloride to the serum and extraction with ethyl acetate. Acid solvolysis was performed at 37° C for 2 hours after adjustment of the pH of the ethyl acetate to

Table II. Correlations between serum androsterone glucuronide and androstanediol glucuronide (3α-diol glucuronide) and androgen precursors* with linear regression analysis

	r Value	*p Value*
Hirsute women		
Androsterone glucuronide versus DHEA sulfate	0.880	<0.004
3α-diol glucuronide versus DHEA sulfate	0.672	<0.10
Androsterone glucuronide versus androstenedione	0.597	<0.12
3α-diol glucuronide versus androstenedione	0.846	<0.02
Androsterone glucuronide versus testosterone	0.142	<0.74
3α-diol glucuronide versus testosterone	0.030	<0.94
Nonhirsute women		
Androsterone glucuronide versus DHEA sulfate	0.786	<0.06
3α-diol glucuronide versus DHEA sulfate	0.709	<0.11
Androsterone glucuronide versus androstenedione	0.231	<0.77
3α-diol glucuronide versus androstenedione	0.870	<0.13
Androsterone glucuronide versus testosterone	0.822	<0.04
3α-diol glucuronide versus testosterone	0.763	<0.08

*Androstenedione and testosterone.

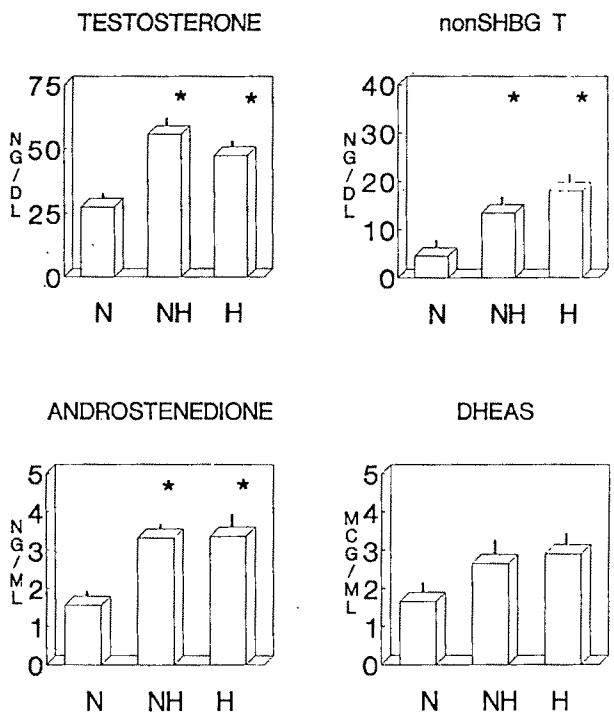


Fig. 1. Mean (± SE) of serum testosterone, androstenedione, and DHEA sulfate in normal women (*N*, $n = 8$), nonhirsute women with polycystic ovarian syndrome (*NH*, $n = 10$), and hirsute women (*H*, $n = 10$). *Significance of difference of *NH* and *H* compared with normal women was $p < 0.01$.

l with a modification of the solvolysis procedure of Aso.[16] After neutralizing the serum with sodium carbonate, the unconjugated steroids were extracted with diethyl ether, separated by Celite column chromatography, and measured by radioimmunoassay. ^{3}H-androsterone and ^{3}H-3α-diol were added to the serum immediately after hydrolysis of the sulfates and glucuronides to allow for monitoring of procedural losses. It was estimated that the percentages of hydrolysis of the sulfates and glucuronides were 67 and 100, respectively.

Analysis of data. The sulfate conjugate values were corrected to reflect 100% solvolysis. All statistics were performed with the Statgraphics statistical package (Statistical Graphics Corporation, Rockville, Md.). The Mann-Whitney U test was used to compare values between different sample groups. Linear regression analysis was used to obtain correlations between groups.

Results

The heights, weights, and ages of normal women who served as controls, nonhirsute women with polycystic ovarian syndrome, and hirsute women are shown in Table I. There was no significant difference between groups for any category except in the Ferriman-Gallwey score ($p < 0.001$). The Ferriman-Gallwey score in hirsute women (18.3 ± 3.2) was significantly greater than that of both normal and nonhirsute women, (5.2 ± 1.4 and 7.2 ± 1.6, respectively; $p < 0.001$). There was no significant difference between the normal and nonhirsute groups.

When the common serum markers of ovarian and adrenal androgens were compared between patients and controls, total testosterone, non-sex hormone-binding globulin-bound testosterone, and androstenedione were significantly greater ($p < 0.001$) compared with controls in both nonhirsute and hirsute patients (Fig. 1). Serum DHEA sulfate was higher in nonhirsute and hirsute patients, as compared with the control group, but this difference was not significant. None of these serum androgens were significantly different between nonhirsute and hirsute patients. Also, there were no significant differences among the three groups with respect to serum 3α-diol or androsterone levels (Fig. 2).

Levels of the sulfate conjugates in all groups were higher than the corresponding glucuronide levels. The sulfates were 9.3 times higher in the case of 3α-diol, and 22.1 times higher for androsterone. In the normal and nonhirsute groups, the conjugates of 3α-diol and androsterone were similar. However, hirsute women had values significantly greater than those of nonhirsute women, not only for serum 3α-diol glucuronide (5.5 ± 0.8 versus 4.2 ± 0.5 ng/ml), but also for 3α-diol sulfate (53.5 ± 9.6 versus 31.7 ± 6.3 ng/ml), and serum androsterone glucuronide (100.3 ± 28.0 versus 42.9 ± 4.1 ng/ml) ($p < 0.05$, Fig. 2). When the conjugate levels were compared between nonhirsute and hirsute women, serum 3α-diol glucuronide was increased by the smallest amount (32%), followed by androsterone sulfate (38%), 3α-diol sulfate (69%), and androsterone glucuronide, which exhibited the largest increase (134%).

The hirsute and nonhirsute groups showed different types of correlations between the conjugates and precursor steroids (Table II). In hirsute, but not in nonhirsute women, serum androsterone glucuronide correlated with DHEA sulfate ($r = 0.880$, $p < 0.004$) and 3α-diol glucuronide with androstenedione ($r = 0.846$, $p < 0.02$). For androsterone glucuronide and 3α-diol glucuronide there was no correlation with testosterone in hirsute women, but there was a correlation between androsterone glucuronide and testosterone in nonhirsute women. In addition, serum 3α-diol sulfate correlated with androsterone sulfate ($r = 0.923$, $p < 0.03$), but 3α-diol glucuronide did not correlate with androsterone glucuronide ($r = 0.708$, $p < 0.12$).

Because androstenedione has been shown to be the most important precursor of peripherally derived androgens, we compared ratios of the C_{19} conjugates to

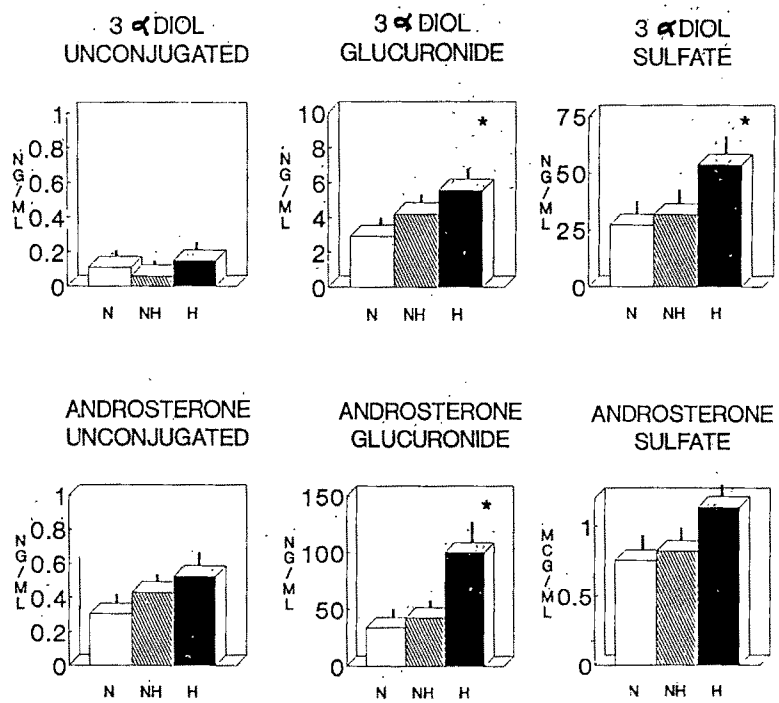


Fig. 2. Mean (± SE) of serum unconjugated 3α-diol and androsterone as well as their glucuronide and sulfate conjugates in normal women *(N)*, nonhirsute women with polycystic ovarian syndrome *(NH)*, and hirsute women *(H)*. *Significance of difference of *H* women compared with either *N* or *NH* women was $p < 0.05$.

Table III. The mean (±SE) of the ratios of serum androgen conjugates to androstenedione in nonhirsute women with polycystic ovarian syndrome and hirsute women

	Androsterone glucuronide/ androstenedione	*Androsterone sulfate/ androstenedione*	*3α-diol glucuronide/ androstenedione*	*3α-diol sulfate/ androstenedione*
Nonhirsute women with polycystic ovarian syndrome	13.1 ± 1.2	244.3 ± 41.9	1.29 ± 0.15	9.8 ± 2.1
Hirsute women	26.2 ± 4.7*	438.0 ± 163.3	2.03 ± 0.40	20.2 ± 4.1

*Between nonhirsute and hirsute women, only androsterone glucuronide/androstenedione is significant at $p < 0.02$.

serum androstenedione between nonhirsute and hirsute women (Table III). Changes in the ratio of androsterone glucuronide/androstenedione most clearly reflected differences between nonhirsute and hirsute women; androsterone glucuronide/androstenedione was 98% higher in hirsute women than in nonhirsute women. For 3α-diol glucuronide, 3α-diol glucuronide/androstenedione was only 57% higher in hirsute women.

Comment

Our finding of elevated C_{19} conjugate levels in hirsute patients confirms, at least for androsterone sulfate, previous data by others with the use of different methodology. Elevations in androsterone sulfate levels in hirsute women confirms the earlier work of Mauvais-Jarvis[17] for urinary androsterone sulfate and Conrad et al.[18] for plasma androsterone sulfate. Our data are novel in that we have measured in serum several androgen conjugates simultaneously and compared these measurements to one another and to precursor steroid levels.

In this study we compared serum 3α-diol and androsterone sulfate and glucuronide levels in hirsute patients who have equivalent circulating levels of non-sex hormone-binding globulin-bound and total testosterone, DHEA sulfate, and androstenedione. Our finding of an increase in these conjugates in only the hirsute group suggests that these androgens, like 3α-diol glucuronide, reflect peripheral androgen action. In contrast with differences seen in previous work,[3] neither unconjugated serum 3α-diol nor androsterone was different among the three groups. Because dihydrotestosterone was not found to be elevated in our previous

work and is not considered to be reflective of the hirsute state, we did not pursue measurements of dihydrotestosterone conjugates.

For all groups, the sulfate conjugates (3α-diol sulfate and androsterone sulfate) were approximately tenfold to twentyfold higher than the glucuronide conjugates (3α-diol glucuronide and androsterone glucuronide). Similarly, androsterone conjugates (androsterone glucuronide and androsterone sulfate) were approximately tenfold higher than their corresponding 3α-diol conjugates. In this study, we confirmed that serum 3α-diol glucuronide clearly differentiates between the nonhirsute and hirsute patients. However, the other androgen conjugates better differentiated the hirsute and nonhirsute groups. Androsterone glucuronide showed the largest increase and was found to best reflect the hirsute state.

In our previous in vitro studies in genital skin, we showed that androstenedione is the most important substrate for 5α-reductase activity and is an important precursor of 3α-diol glucuronide in women.[19] Only in hirsute patients did 3α-diol glucuronide correlate with androstenedione. When we compared androgen conjugates to possible precursor steroids with serum androstenedione as a model to further examine possible substrate use, we found that the conjugate that increased the most in hirsute women was androsterone glucuronide. The ratios of the conjugates to their principal substrate (androstenedione), presumably reflecting 5α-reductase activity, clearly demonstrated a difference between nonhirsute and hirsute women. In this regard, androsterone glucuronide was more reflective than 3α-diol glucuronide.

Our study does not address whether some of these conjugates, such as androsterone glucuronide, are produced directly by the skin, as has been shown for 3α-diol glucuronide. However, the differences in conjugate levels between two groups of very similar women with the exception of hirsutism implies that these other androgen conjugates also reflect peripheral androgen action. The sulfate conjugates of androsterone and 3α-diol were highly correlated, implying a possible androsterone sulfate to 3α-diol sulfate conversion. There was no correlation between the glucuronide conjugates of androsterone and 3α-diol, which supports Rittmaster's finding with the use of isomers of 3α-diol glucuronide, and suggests that there is no significant interconversion between 3α-diol glucuronide and androsterone glucuronide.[20] The fact that androsterone glucuronide is the conjugate most elevated in hirsute women, and that the androsterone glucuronide/androstenedione ratio was most reflective of the hirsute state, gives further support to an alteration in the pathway of peripheral androgen metabolism in hirsutism. The elevation of androsterone conjugates implies perhaps that androsterone glucuronide and androsterone sulfate production by means of androstenedione and androsterone in hirsute women is at least as important, if not more important than the production of 3α-diol glucuronide and 3α-diol sulfate by means of androstenedione and dihydrotestosterone.

In conclusion, we have found that several C_{19} sulfate and glucuronide conjugates reflect peripheral androgen action in hirsute patients, and that androsterone glucuronide may be more valuble than 3α-diol glucuronide in this regard, particularly when androstenedione is elevated. There appears to be a close correlation between these androgen conjugates and both serum androstenedione and DHEA sulfate. Further examination of the interconversions between androsterone sulfate and 3α-diol sulfate, as well as efforts to determine the source of these androgen sulfate and glucuronide conjugates, will allow us to further understand the altered androgen metabolism of hirsute patients.

REFERENCES

1. Abraham G, Chakmakjian Z. Plasma steroids in hirsutism. Obstet Gynecol 1974;44:171.
2. Meikle A, Stringham J, Wilson D, Dolman L. Plasma 5α-reduced androgens in men and hirsute women: role of adrenals and gonads. J Clin Endocrinol Metab 1979; 48:969-75.
3. Lobo RA, Goebelsmann U, Horton R. Evidence for the importance of peripheral tissue events in the development of hirsutism in polycystic ovary syndrome. J Clin Endocrinol Metab 1983;57:393-7.
4. Deslypere JP, Sayed A, Punjami Y, Verdonck L, Vermeulen A. Plasma 5α-androstane-3α-17β-diol and urinary 5α-androstane-3α-17β-diol glucuronide, parameters of peripheral androgen action: a comparative study. J Clin Endocrinol Metab 1982;54:386-91.
5. Samojlik E, Kirschner MA, Silber D, Schneider G, Ertel NH. Elevated production and metabolic clearance rates of androgens in morbidly obese women. J Clin Endocrinol Metab 1984;59:949-54.
6. Gompel A, Wright F, Kuttenn F, Mauvais-Jarvis P. Contribution of plasma androstenedione to 5α-androstanediol glucuronide in women with idiopathic hirsutism. J Clin Endocrinol Metab 1986;62:441-4.
7. Morimoto I, Edmiston A, Hawks D, Horton R. Studies on the origin of androstanediol and androstanediol glucuronide in young and elderly men. J Clin Endocrinol Metab 1981;52:772-8.
8. Horton R, Hawks D, Lobo R. 3α,17β-Androstanediol glucuronide in plasma: a marker of androgen action in idiopathic hirsutism. J Clin Invest 1982;69:1203-6.
9. Lobo RA, Paul WL, Gentzschein E, et al. Production of 3α-androstanediol glucuronide in human genital skin. J Clin Endocrinol Metab 1987;65:711-4.
10. Serafini P, Ablan F, Lobo RA. 5α-Reductase activity in the genital skin of hirsute women. J Clin Endocrinol Metab 1985;60:349-55.
11. Lobo RA, Granger LR, Paul WL, Goebelsmann U, Mishell DR Jr. Psychological stress and increases in urinary norepinephrine metabolites, platelet serotonin, and adrenal androgens in women with polycystic ovary syndrome. Am J Obstet Gynecol 1983;145:496.
12. Goldzieher JW. Polycystic ovarian disease. Fertil Steril 1981;35:371-94.
13. Ferriman D, Gallwey JD. Clinical assessment of body hair

growth in women. J Clin Endocrinol Metab 1961; 21:1440-7.
14. Serafini P, Lobo RA. Increased 5α-reductase activity in idiopathic hirsutism. Fertil Steril 1985;43:74-8.
15. Stumpf PG, Nakamura RM, Mishell DR Jr. Changes in physiologically free circulating estradiol and testosterone during exposure to levonorgestrel. J Clin Endocrinol Metab 1981;52:138-43.
16. Aso T, Aedo A-R, Cekan SZ. Simultaneous determination of the sulphates of dehydroepiandrosterone and pregnenolone in plasma by radioimmunoassay following a rapid solvolysis. J Steroid Biochem 1977;3:1105-8.
17. Mauvais-Jarvis P, Baulieu EE. Urinary 17-ketosteroid conjugates in hirsutism. J Clin Invest 1962;41:1690-8.
18. Conrad SH, Lindberg MC, Herrmann WL. Significance of plasma dehydroisoandrosterone and androsterone sulfates in the diagnosis of virilizing disorders. Am J Obstet Gynecol 1965;91:449-56.
19. Silva PD, Gentzschein EEK, Lobo RA. Androstenedione may be a more important precursor of tissue dihydrotestosterone than testosterone in women. Fertil Steril 1987; 48:419-22.
20. Rittmaster RS, Thompson DL, Listwak S, Loriaux DL. Androstanediol glucuronide isomers in normal men and women and in men infused with labeled dihydrotestosterone. J Clin Endocrinol Metab 1988;66:212-6.

In vivo administration of allopurinol affects ovulation and early embryonic development in rabbits

Toyohiko Miyazaki, MD, PhD, Tsung-Cheng Kuo, MD, PhD, Arunasalam M. Dharmarajan, PhD, Susan J. Atlas, PhD, and Edward E. Wallach, MD

Baltimore, Maryland

The potential role of the hypoxanthine–xanthine oxidase system in human chorionic gonadotropin–induced ovulation, nuclear maturation, and preimplantation development was investigated in the rabbit by use of the xanthine oxidase inhibitor allopurinol. Allopurinol (50 mg/kg) or vehicle was injected, followed by human chorionic gonadotropin (100 IU). Administration of inhibitor or vehicle was repeated 3 hours later. Ovaries and ovulated ova were inspected 12 or 24 hours after human chorionic gonadotropin administration for follicle rupture, cumulus dispersal, and nuclear maturation. Ova were inseminated in vitro and assessed for development to the blastocyst stage, up to 96 hours after insemination. Allopurinol significantly reduced ovulatory efficiency, defined as the percent of large follicles that ovulate, and decreased the percent of ova with cumulus dispersal. Allopurinol significantly inhibited morula and blastocyst formation. These data suggest that the xanthine oxidase system may play a role in ovulation and early embryonic development. (Am J Obstet Gynecol 1989;161:1709-14.)

Key words: Allopurinol, ovulation, early embryonic development, oocyte maturation, free radicals

We have recently reported that oxygen free radicals play a role in ovulation in the in vitro perfused rabbit ovary (Miyazaki T, et al. The role of oxygen free radicals in ovulation in the in vitro perfused rabbit ovary [Abstract 007]. Presented at the Forty-fourth Annual Meeting of the American Fertility Society, Atlanta, Ga., October 10-13, 1988). The (hypo)xanthine-xanthine oxidase system is one of the physiologic mechanisms of free radical generation. This system generates superoxide anion radicals during the oxidation of hypoxanthine and xanthine to uric acid. Although the source of oxygen free radicals involved in the process of ovulation is not known, hypoxanthine has been found in the follicular fluid of several species and is reported to inhibit oocyte nuclear maturation.[1-3] Xanthine oxidase has been found in endothelial cells of many organs,[4, 5] and the metabolism of hypoxanthine by cumulus-oocyte complexes[3] suggests the presence of this superoxide-generating enzyme system within the follicle.

Allopurinol inhibits the enzyme xanthine oxidase, which results in maintenance of the substrates hypo-

From the Department of Gynecology and Obstetrics, The Johns Hopkins University School of Medicine.
Supported by grant HD 19430 from the National Institutes of Health, a SmithKline Beckman Fellowship (T. M.), and the Lalor and Mitchell and Lillian Duberstein Foundations.
Presented in part at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.
Reprint requests: Edward E. Wallach, MD, The Johns Hopkins Medical Institutions, 600 N. Wolfe St., Houck 264, Baltimore, MD 21205.
6/6/15961

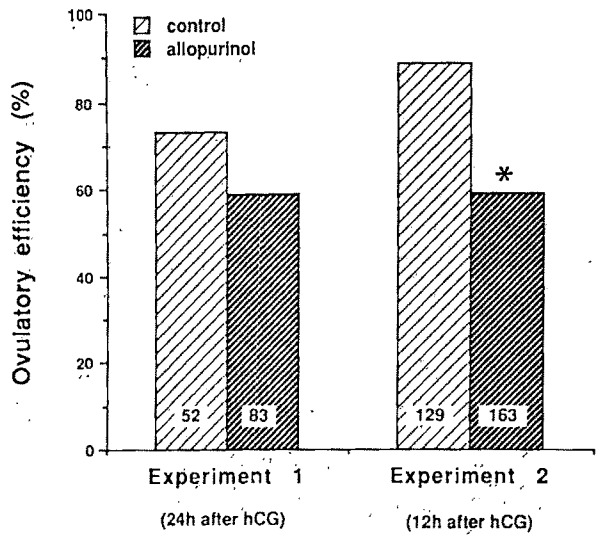


Fig. 1. Ovulatory efficiency—percentage of large follicles (≥1.5 mm) releasing ova. *Numbers* indicate total large follicles. *Asterisk*, $p < 0.001$, compared with control.

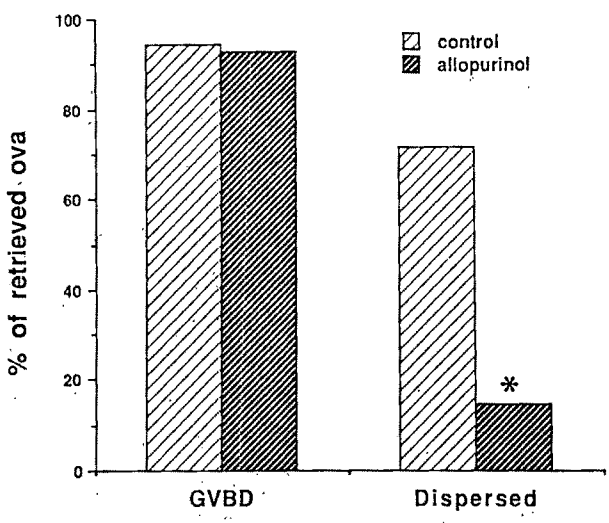


Fig. 2. Percentage of retrieved ova with germinal vesicle breakdown *(GVBD)* and with dispersed cumulus (less than one layer of adherent cumulus cells). *Asterisk*, $p < 0.001$, compared with control.

xanthine and xanthine and in inhibition of superoxide anion radical generation.[5,6]

The objective of this study was to determine, through the use of allopurinol, whether the hypoxanthine-xanthine oxidase system plays a role in ovulation and oocyte maturation in the rabbit. In order to address this question, we assessed the effects of administering allopurinol, together with an ovulation-inducing dose of human chorionic gonadotropin (hCG), on follicle rupture, nuclear maturation, cumulus dispersal, and early embryonic development.

Material and methods

Sexually mature New Zealand White female ($n = 32$) and male ($n = 6$) rabbits were used in all experiments. Female rabbits weighing 3.5 to 4.5 kg were isolated for a minimum of 3 weeks before the experimental procedure. Epididymal spermatozoa were collected from male rabbits of proved fertility. Animals were caged individually and fed water and a diet of Purina Rabbit Chow (Purina Mill, Richmond, Ind.) as desired under conditions of controlled light and temperature. All studies were performed in accordance with the *Guidelines for the Use of Experimental Animals at the Johns Hopkins University*. Female rabbits (treated and control) were given hCG (100 IU; Organon Inc., West Orange, N.J.) to induce ovulation via a marginal ear vein. The treated group also received allopurinol (50 mg/kg; Sigma Chemical Co., St. Louis) simultaneously via a marginal ear vein, while the control group received an equal volume of vehicle alone. The allopurinol solution was prepared according to the methods of Baker et al.[7] A second dose of allopurinol (50 mg/kg) or vehicle was administered 3 hours later. This dose of allopurinol was selected on the basis of its effectiveness in inhibiting free radical generation via xanthine oxidase in vivo[7,8] and was within the effective dose response range observed in vitro.[9]

Experiment 1. Eleven female rabbits (control, $n = 4$; allopurinol, $n = 7$) were put to death with an intravenous overdose of pentobarbital sodium 24 hours after administration of hCG and the initial dose of allopurinol or vehicle, and the ovary and oviduct were removed. Ovaries were examined for number of unruptured follicles and corpora lutea, and ovulated ova were recovered from the oviduct by injection of Brackett's defined medium[10] into the oviduct adjacent to the tubouterine junction. Ova were assessed for both the stage of nuclear maturation and the degree of attachment of cumulus cells by phase-contrast microscopy. The ovum maturity was expressed as the percentage of ova achieving germinal vesicle breakdown. Cumulus dispersal was expressed as the percentage of retrieved ova with less than one layer of cumulus cells. Ovulatory efficiency, defined as the percent of large follicles (≥1.5 mm) that proceed to rupture, also was calculated.

Experiment 2. Twenty-one female rabbits (control, $n = 11$, allopurinol, $n = 10$) were used. Animals were anesthetized with 32 mg/kg pentobarbital sodium, and ovaries and oviducts were removed 12 hours after administration of hCG and the initial dose of allopurinol or vehicle. Ovaries were examined for number of unruptured follicles and corpora lutea, and ovulatory efficiency was calculated as described in experiment 1. Cumulus-oocyte complexes were retrieved by flushing oviducts with Brackett's medium and were then fertilized in vitro.

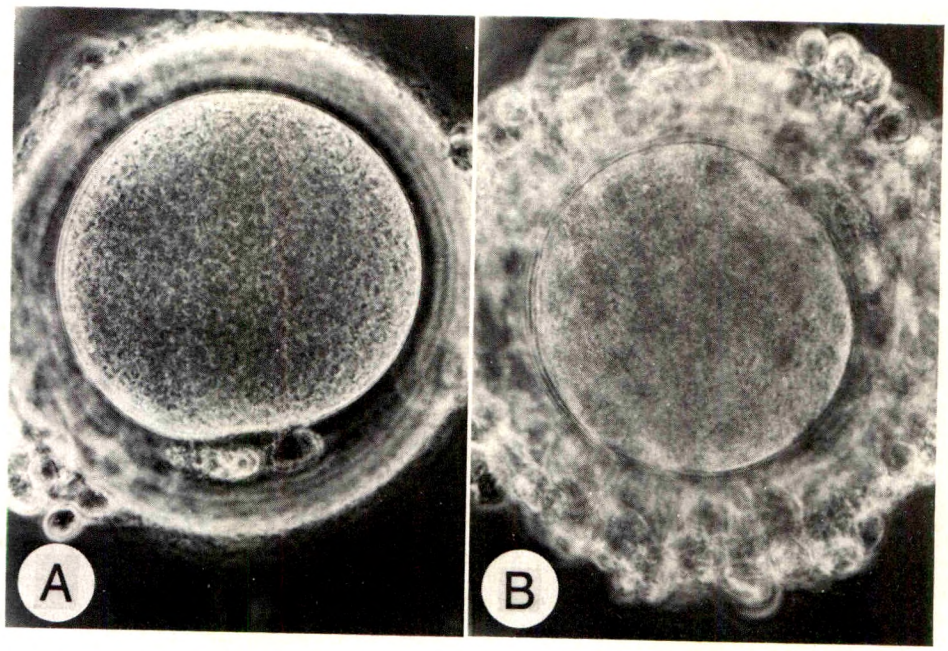


Fig. 3. Ovulated ova retrieved from oviducts 24 hours after hCG administration. **A,** Control. Cumulus cells dispersed (less than one layer). (Original magnification ×500.) **B,** Treatment with allopurinol. Ovum surrounded by cumulus cells. (Original magnification ×500.)

In vitro fertilization. The male rabbit was anesthetized with pentobarbital sodium, the scrotum was incised, and the epididymis with attached vas deferens was removed from the testis. Epididymal spermatozoa were collected as previously described.[11] Washed sperm were resuspended in 1.0 ml of Brackett's medium to yield 1 to 2 × 10^6 spermatozoa per milliliter and preincubated for 10 hours with 5% carbon dioxide in air at 37° C and 100% humidity. Only sperm suspensions with good motility were used for insemination.

Retrieved cumulus-surrounded oocytes were washed three times in Brackett's medium and inseminated with preincubated sperm. Twelve hours later inseminated ova were transferred to Ham's F10 medium (Gibco Laboratories, Grand Island, N.Y.) with 20% heat-inactivated fetal calf serum (Gibco Laboratories). The inseminated ova were examined by phase-contrast microscopy daily for 4 days after insemination, and their developmental stage was assessed. Cleavage rate, defined as the percentage of inseminated ova containing two or more cells, was determined 36 hours after insemination. Sixty hours after insemination, morula formation rate, defined as the percentage of inseminated ova that had proceeded to morula or blastocyst stage, was determined. Ninety-six hours after insemination, morula formation rate and blastocyst formation rate, defined as the percentage of inseminatd ova that had proceeded to blastocyst stage, were determined. Degeneration rate, defined as the percentage of inseminated ova that showed evidence of degeneration (vacuolation, cytolysis, necrosis, changes in the shape of early embryo, and fragmentation), was calculated at each time point.

Statistical analysis. The χ^2 method was used to assess ovulatory efficiency, percent of ova with dispersed cumulus, percent germinal vesicle breakdown, percent degeneration, and developmental stage. A probability of <0.05 was considered to be significant.

Results

Experiment 1. Twenty-four hours after hCG administration, 38 ruptured follicles were observed in 8 control ovaries and 49 ruptured follicles were seen in 14 allopurinol-treated ovaries. Ovulatory efficiency was lower in the treated group (59.0%) than in control (73.1%), but this difference was not statistically significant (Fig. 1). Thirty-five ovulated ova were recovered from controls and 41 from the treated group. No significant difference was noted in percentage of ova achieving germinal vesicle breakdown (control, 94.3%; allopurinol, 92.7%; Fig. 2). Allopurinol significantly inhibited cumulus dispersal (Fig. 3, *A* and *B*) and reduced the percentage of cumulus dispersal from 71.4% to 14.6% ($p < 0.001$; Fig. 2).

Experiment 2. Twelve hours after hCG administration, 114 ruptured follicles were recorded in 22 control ovaries and 96 ruptured follicles in 20 allopurinol-treated ovaries. Allopurinol significantly reduced ovulatory efficiency ($p < 0.001$) from 88.4% to 58.9% (Fig. 1). Totals of 68 and 61 ovulated ova were recovered from control and treated groups, respectively, and used for in vitro fertilization. At this early stage both control

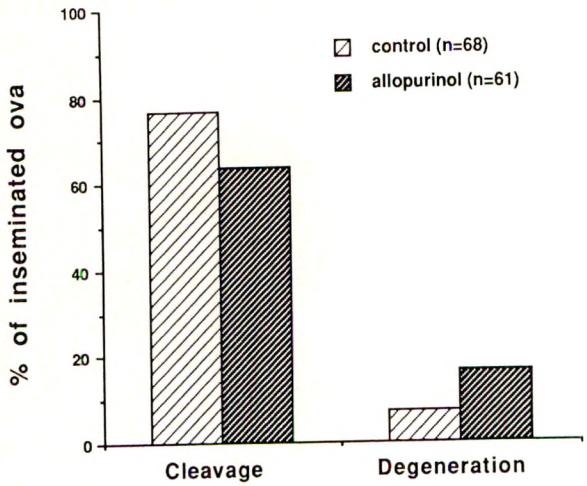


Fig. 4. Cleavage rate (percentage of inseminated ova containing two or more cells) and degeneration rate of ova observed 36 hours after insemination.

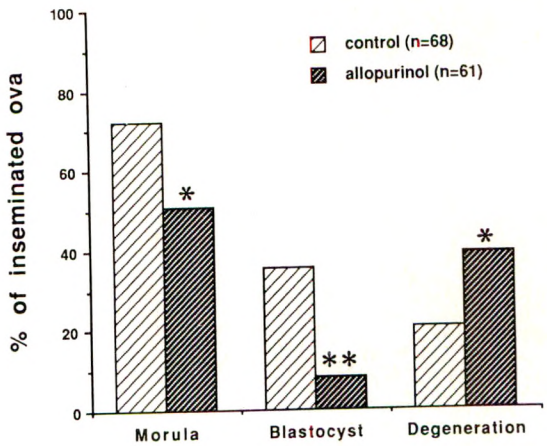


Fig. 6. Morula and blastocyst formation rate (percentage of inseminated ova proceeding to blastocyst stage) and degeneration rate observed 96 hours after insemination. *Asterisk*, $p < 0.025$, compared with control. *Double asterisks*, $p < 0.005$, compared with control.

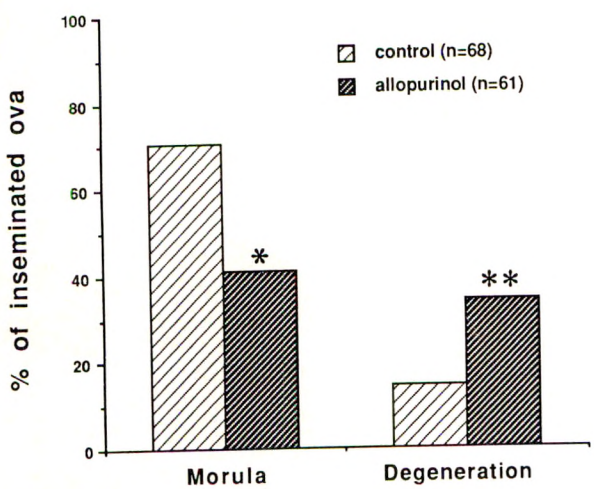


Fig. 5. Morula formation rate (percentage of inseminated ova proceeding to morula or blastocyst stage) and degeneration rate observed 60 hours after insemination. *Asterisk*, $p < 0.05$, compared with control. *Double asterisks*, $p < 0.01$, compared with control.

and treated ova were still surrounded by cumulus cells. Thirty-six hours after insemination, cleavage rate was lower in the treated group (63.9%) than in the control group (76.5%) and degeneration rate was higher (treated, 16.4%; control, 7.4%), but these differences were not statistically significant (Fig. 4). Morula formation rate 60 hours after insemination was significantly reduced ($p = 0.005$) by treatment with allopurinol (41.0%) when compared with the controls (70.6%; Fig. 5). Allopurinol significantly increased ($p < 0.01$) degeneration rate 60 hours after insemination from 14.7% to 34.4% (Fig. 5). Ninety-six hours after insemination, allopurinol significantly reduced morula formation rate from 72.1% to 50.8% ($p < 0.025$) and blastocyst formation rate from 35.3% to 8.2% ($p < 0.005$; Fig. 6). Furthermore, degeneration rate was significantly increased ($p < 0.025$) by treatment with allopurinol (39.3%) as compared with the control group (20.6%; Fig. 6).

Comment

Many factors are involved in ovulation, oocyte maturation, and early embryonic development. Recently, we demonstrated that oxygen free radicals play a role in the process of ovulation in the in vitro perfused rabbit ovary. Since this ovarian perfusion technique is performed with synthetic medium in the absence of blood cells, circulating neutrophils could not have been the source of free radical generation during in vitro ovulation. The possible sources of free radical generation include mitochondrial, endoplasmic reticular, and nuclear membrane electron transport systems. Oxidant enzymes such as cytochrome P_{450}, xanthine oxidase, and lipoxygenase, and the nicotinamide adenine dinucleotide phosphate, reduced form, oxidase of phagocytic cells also are involved in the production of oxygen free radicals.[12, 13] High concentrations of hypoxanthine, a substrate of xanthine oxidase, have been found in both pig and mouse follicular fluid,[1, 2] and endothelial cells are a rich source of xanthine oxidase.[4, 5] The apical follicular region contains evidence of ischemia in hCG-induced ovulation in the rabbit,[14] and hypoxanthine is produced via breakdown of adenosine triphosphate during ischemia.[15] In addition, hypoxanthine is reported to be metabolized within cumulus-oocyte complexes.[3] These observations support the possibility that the superoxide-generating hypoxanthine–xanthine

oxidase system could play a role in ovulation and oocyte maturation.

Allopurinol (4-hydroxypyrazolo[3,4-*d*]pyrimidine) prevents generation of oxygen radicals and maintains the concentration of hypoxanthine by inhibiting xanthine oxidase. In these experiments allopurinol inhibited ovulation, cumulus dispersal, and early embryonic development in rabbits. These effects may have been due either to a decrease in free radical generation or to a direct toxic effect on the ovary. Oxygen free radicals have been found to exert a direct effect on cell membrane, connective tissue, and hyaluronic acid degradation.[12, 16] Hyaluronic acid is a major component of the intercellular matrix that connects cumulus cells to one another and to the oocyte. Inhibition of cumulus dispersal by allopurinol in this study suggests that free radicals generated during follicle rupture degrade hyaluronic acid and result in cumulus dispersal. Allopurinol most likely acts to prevent generation of the superoxide anion radical by inhibiting xanthine oxidase. Since both allopurinol and oxypurinol (4,6-dihydroxypyrazolo[3,4-*d*]pyrimidine), a major metabolite of allopurinol and xanthine oxidase inhibitor,[6] are also free radical scavengers,[9, 17] allopurinol's action on the ovary may be via inhibition of xanthine oxidase and/or scavenging free radicals.

The oocyte and cumulus cells are metabolically coupled through gap junctions[18, 19] that are thought to be involved in the control of oocyte maturation.[20] Meiotic resumption in the oocyte has been thought to be related to either a quantitative or a qualitative change in the signal from cumulus cells to the oocyte[20-22] or to termination of gap junctions, thus interrupting the transfer of substances that inhibit oocyte maturation.[23, 24] That allopurinol treatment did not affect nuclear maturation in ovulated ova in this experiment, despite the maintenance of cumulus-oocyte contacts, supports the hypothesis that a change in the cumulus cell-oocyte signal, rather than termination of gap junctions, is responsible for resumption of oocyte maturation.

Hypoxanthine is known to be a potent inhibitor of oocyte nuclear maturation.[1-3] If a hypoxanthine–xanthine oxidase system exists within the ovary, allopurinol treatment, which maintains the concentration of hypoxanthine, would be expected to inhibit oocyte nuclear maturation. However, allopurinol did not affect the percentage of ovulated ova achieving germinal vesicle breakdown 24 hours after hCG administration. Since nuclear maturation occurs once oocytes are removed from their follicles,[25, 26] in these experiments allopurinol may have exerted its inhibitory influence on oocytes while still contained within the follicles. However, once follicle rupture occurred, approximately 12 hours after hCG administration, the released oocyte would have been liberated from this inhibitory influence. It has been reported that gonadotropins can overcome the inhibitory influence of hypoxanthine on oocyte nuclear maturation.[27]

Although allopurinol did not significantly affect cleavage and degeneration rates, it was associated with a significant reduction of morula and blastocyst formation and increased degeneration of fertilized ova, when assessed 60 and 96 hours after insemination, respectively. These results suggest that allopurinol affects cytoplasmic maturation of oocytes either through a direct cytotoxic effect or as a result of maintaining high concentrations of hypoxanthine. Our data are consistent with previous findings that hypoxanthine inhibits early embryonic development in the mouse.[28]

In conclusion, allopurinol affects hCG-induced ovulation and suppresses cytoplasmic maturation of ovulated ova in this rabbit model. These data suggest that the (hypo)xanthine–xanthine oxidase system may play a role in the periovulatory interval.

We wish to thank Ms. Fran Karas for her assistance in preparation of the manuscript.

REFERENCES

1. Downs SM, Coleman DL, Ward-Bailey PF, Eppig JJ. Hypoxanthine is the principal inhibitor of murine oocyte maturation in a low molecular weight fraction of porcine follicular fluid. Proc Natl Acad Sci USA 1985;82:454-8.
2. Eppig JJ, Ward-Bailey PF, Coleman DL. Hypoxanthine and adenosine in murine ovarian follicular fluid: concentrations and activity in maintaining oocyte meiotic arrest. Biol Reprod 1985;33:1041-9.
3. Downs SM, Coleman DL, Eppig JJ. Maintenance of murine oocyte meiotic arrest: uptake and metabolism of hypoxanthine and adenosine by cumulus cell-enclosed and denuded oocytes. Dev Biol 1986;117:174-83.
4. Jarasch E-D, Bruder G, Heid WH. Significance of xanthine oxidase in capillary endothelial cells. Acta Physiol Scand 1986;548(suppl):39-46.
5. Ratych RE, Chuknyiska RS, Bulkley GB. The primary localization of free radical generation after anoxia/reoxygenation in isolated endothelial cells. Surgery 1987; 102:122-31.
6. Spector T. Oxypurinol as an inhibitor of xanthine oxidase-catalyzed production of superoxide radical. Biochem Pharmacol 1988;37:349-52.
7. Baker GL, Corry RJ, Autor AP. Oxygen free radical induced damage in kidneys subjected to warm ischemia and reperfusion. Ann Surg 1985;202:628-41.
8. Parks DA, Granger DN. Ischemia-induced vascular changes:role of xanthine oxidase and hydroxyl radicals. Am J Physiol 1983;245:G285-9.
9. Moorhouse PC, Grootveld M, Halliwell B, Quinlan JG, Gutteridge JMC. Allopurinol and oxypurinol are hydroxyl radical scavengers. FEBS Lett 1987;213:23-8.
10. Brackett BG, William W. Fertilization of rabbit ova in a defined medium. Fertil Steril 1968;19:144-55.
11. Hosoi Y, Niwa K, Hatanaka S, Iritani A. Fertilization in vitro of rabbit eggs by epididymal serpmatozoa capacitated in a chemically defined medium. Biol Reprod 1981;24:637-42.
12. Weiss SJ. Oxygen, ischemia and inflammation. Acta Physiol Scand 1986;548(suppl):9-37.
13. Cross CE, Halliwell B, Borish ET, et al. Oxygen radicals and human disease. Ann Intern Med 1987;107:526-45.
14. Kanzaki H, Okamura H, Okuda Y, Takenaka A, Morimoto K, Nishimura T. Scanning electron microscopic

study of rabbit ovarian follicle microvasculature using resin injection-corrosion casts. J Anat 1982;134:697-704.
15. Granger DN, Rutili G, McCord JM. Superoxide radicals in feline intestinal ischemia. Gastroenterology 1981;81: 22-9.
16. Del Maestro RF, Thaw HH, Bjork J, Planker M, Arfors K-E. Free radicals as mediators of tissue injury. Acta Physiol Scand 1980;492(suppl):43-57.
17. Peterson DA, Kelly B, Gerrard JM. Allopurinol can act as an electron transfer agent. Is this relevant during reperfusion injury? Biochem Biophys Res Commun 1986; 137:76-9.
18. Amsterdam A, Josephs R, Lieberman ME, Lindner HR. Organization of intramembrane particles in freeze-cleaved gap junctions of rat Graafian follicles: optical-diffraction analysis. J Cel Sci 1976;21:93-105.
19. Gilula NB, Epstein ML, Beers WH. Cell-to-cell communications and ovulation. J Cell Biol 1978;78:58-75.
20. Moor RM, Cran DG. Intercellular coupling in mammalian oocytes. In: Johnson MH, ed. Development in mammals. Amsterdam: Elsevier/North-Holland Biomedical Press. 1980:3.
21. Eppig JJ. The relationship between cumulus cell-oocyte coupling, oocyte meiotic maturation, and cumulus expansion. Dev Biol 1982;89:269-72.
22. Racowsky C, Satterlie RA. Metabolic, fluorescent dye and electrical coupling between hamster oocytes and cumulus cells during meiotic maturation in vivo and in vitro. Dev Biol 1985;108:191-202.
23. Dekel N, Beers WH. Rat oocyte maturation in vitro: relief of cyclic AMP inhibition by gonadotropins. Proc Natl Acad Sci USA 1978;75:4369-73.
24. Dekel N, Beers WH. Development of the rat oocyte in vitro: inhibition and induction of maturation in the presence or absence of the cumulus oophorus. Dev Biol 1980;75:247-54.
25. Scheutz AW. Role of hormones in oocyte maturation. Biol Reprod 1974;10;150-78.
26. Pincus G, Enzmann EV. The comparative behavior of mammalian eggs in vivo and in vitro. I. The activation of ovarian eggs. J Exp Med 1935;62:665-75.
27. Eppig JJ, Downs SM. The effect of hypoxanthine on mouse oocyte growth and developmemt in vitro: maintenance of meiotic arrest and gonadotropin-induced oocyte maturation. Dev Biol 1987;119:313-21.
28. Loutradis D, John D, Kiessling AA. Hypoxanthine causes a 2-cell block in random-bred mouse embryos. Biol Reprod 1987;37:311-6.

Effects of platelet activating factor on mouse oocyte fertilization in vitro

Brijinder S. Minhas, PhD,[a] Raj Kumar, PhD,[b] Deborah D. Ricker, MS,[a] William E. Roudebush, PhD,[a] Melvin G. Dodson, MD, PhD,[a] and Stephen J. Fortunato, MD[a]

Johnson City, Tennessee, and San Antonio, Texas

Platelet activating factor is rapidly gaining acceptance as a potent mediator in many reproductive processes. This study presents data that indicate a direct role of platelet activating factor in fertilization. Plate et activating factor was shown to significantly increase ($p < 0.001$) the fertilization rate of mouse oocytes in vitro. Furthermore, CV3988, an inhibitor of platelet activating factor, was noted to significantly decrease in vitro fertilization rates at 10^{-5} and 10^{-4} mol/L concentrations. (Am J Obstet Gynecol 1989;161:1714-7.)

Key words: Platelet activating factor, in vitro fertilization

Platelet activating factor (PAF) [1-0-alkyl-2-acetyl-*sn*-glyceryo-3-phosphocholine (AGEPC)] is a potent phospholipid with a diverse spectrum of biologic activities.[1]

From the Department of Obstetrics and Gynecology, Quillen-Dishner College of Medicine, East Tennessee State University,[a] and the Department of Biochemistry, University of Texas Health Science Center.[b]

Presented in part at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.

Reprint requests: Brijinder S. Minhas, PhD, Department of Obstetrics and Gynecology, Quillen-Dishner College of Medicine, East Tennessee State University, P.O. Box 19570A, Johnson City, TN 37614.

6/6/15670

Recently, Abisogun et al.[2] noted that ovulation was inhibited in human chorionic gonadotropin (hCG)–primed rats with platelet activating factor receptor antagonist BN 52021. This inhibition of ovulation was overcome by the administration of additional platelet activating factor.[2] Our laboratory reported the presence of platelet activating factor–like activity in human spermatozoa.[3] Platelet activating factor was also noted to increase the motility of human spermatozoa up to 53%.[4] Human and mouse preimplantation embryos have been reported to produce and secrete platelet activating factor into the culture medium in vitro.[5, 6] Embryo-derived platelet activating factor is associated with a mild thrombocytopenia during the first week of preg-

Table I. CV3988 inhibition of mouse in vitro fertilization

Sperm treatment	*Oocytes inseminated*	*Fertilization (second polar body)*	*Cleavage (two-cell formation)*
Control	150	135 (90.0%)	126 (84.0%)
10^{-7} mol/L CV3988	100	88 (88.0%)	78 (78.0%)
10^{-6} mol/L CV3988	100	90 (90.0%)	80 (80.0%)
10^{-5} mol/L CV3988	80	56 (70.0%)*	52 (65.0%)*
10^{-4} mol/L CV3988	85	10 (11.7%)†	7 (8.2%)†

*Significantly different from control ($p < 0.01$).
†Significantly different from control ($p < 0.001$).

nancy in mice.[7] Thrombocytopenia has also been observed in women who receive fertilized human embryos after in vitro fertilization–embryo transfer.[8] There is also evidence for the role of platelet activating factor in early pregnancy in humans, mice, rats, and rabbits.[9-12] This study reports the involvement of platelet activating factor in mouse oocyte fertilization in vitro.

Material and methods

Superovulation and oocyte recovery. Six- to 10-week-old virgin $B_6D_2F_1/J$ mice were maintained on a cyclic 14-hour light/10-hour-dark schedule and were superovulated with intraperitoneal injections of 5 IU pregnant mare's serum gonadotropin followed by 10 IU hCG 48 hours later. Mice were killed by cervical dislocation 14 to 16 hours after hCG administration and ovulated oocytes were recovered from excised oviducts by tearing the dilated ampulla with a 30-gauge needle.

Sperm capacitation and treatment with platelet activating factor inhibitor CV3988 or platelet activating factor. Spermatozoa were collected from the cauda epididymides of proven fertile male mice and allowed to capacitate for 90 minutes at 37° C in a 5% carbon dioxide atmosphere in Ham's F-10 medium supplemented with 30 mg/ml bovine serum albumin. After 90 minutes 1×10^5 spermatozoa were transferred to 1 ml of preequilibrated Ham's F-10 medium (3 mg/ml bovine serum albumin) containing varying concentrations (1×10^{-7} through 1×10^{-4} mol/L) of CV3988 [*rac*-3-(*N*-*n*-octadecylcarbomyloxy)-2-methoxypropyl-2-thiazolyoethyl phosphate], a structural analog and inhibitor of platelet activating factor, or 1×10^{-7} mol/L platelet activating factor and further incubated for 30 minutes before the addition of oocytes.

In vitro fertilization with CV3988-treated sperm. Oocyte-cumulus masses were randomly distributed to Falcon center well tissue culture dishes (Becton Dickinson, Lincoln Park, N.J.) that each contained CV3988-treated sperm. Six hours after insemination oocytes were examined for second polar body extrusion and pronuclei formation. Fertilized ova were transferred to 100 λ microdrops of fresh growth medium (Ham's F-10, 3 mg/ml bovine serum albumin) overlaid by equilibrated mineral oil. Eighteen hours later the fertilized oocytes were scored for two-cell formation. To evaluate for the possibility of parthenogenetic activation of oocytes caused by CV3988, oocytes ($n = 50$) were cultured for 6 hours in the presence of varying concentrations (10^{-7} through 10^{-4} mol/L) of the inhibitor CV3988 in the absence of spermatozoa.

In vitro fertilization with platelet activating factor–treated sperm. Oocyte-cumulus masses were transferred to platelet activating factor–treated spermatozoa or untreated (control) spermatozoa and coincubated for 5 minutes. After coincubation, the oocytes were treated with 300 U/ml of hyaluronidase for 2 to 5 minutes to remove remaining cumulus cells. The oocytes were washed three times with fresh culture medium before culture in 100 λ microdrops (Ham's F-10, 3 mg/ml bovine serum albumin) under equilibrated mineral oil. Six hours later oocytes were assessed for second polar body extrusion and pronuclear formation. Eighteen hours later the fertilized oocytes were scored for two-cell formation. To evaluate for the possibility of parthenogenetic activation of oocytes caused by platelet activating factor, oocytes ($n = 50$) were exposed to 1×10^{-7} mol/L platelet activating factor for 5 minutes in the absence of spermatozoa. Data were subjected to χ^2 analyses.

Results

In the experiment to evaluate the effects of CV3988 on in vitro fertilization of mouse oocytes, 90% fertilization (second polar body extrusion) and 84% two-cell formation rate were observed for control oocytes. In the presence of 10^{-7} and 10^{-6} mol/L CV3988 fertilization rates (88%, 90%) and two-cell formation rates (78%, 80%) were similar to controls. At higher concentrations of the inhibitor (10^{-5} mol/L and 10^{-4} mol/L) a statistically significant reduction in fertilization rates (70%, $p < 0.01$; 12%, $p < 0.001$) and two-cell formation (65%, $p < 0.01$; 8%, $p < 0.001$) were noted (Table I). CV3988 did not parthenogenetically activate oocytes at experimental concentrations.

At the 10^{-4} mol/L concentration of CV3988 10% to

Table II. Platelet activating factor enhancement of mouse in vitro fertilization

Sperm treatment	*Oocytes inseminated**	*Fertilization (second polar body)*	*Cleavage (two-cell formation)*
Control	114	33 (28.9%)	30 (26.3%)
Platelet activating factor (1×10^{-7} mol/L)	159	85 (54.1%)†	80 (50.3%)†

*5-minute coincubation period.
†$p < 0.001$.

15% of the sperm were noted to be agglutinated with a mild reduction in motility.

Oocytes exposed to sperm cells for only 5 minutes resulted in a 29% fertilization rate with 26% of the oocytes developing to the two-cell stage. In the presence of platelet activating factor, 54% of the oocytes were fertilized ($p < 0.001$) with 50% developing to the two-cell stage ($p < 0.001$, Table II). Almost twice the number of spermatozoa were noted to be bound to the zona pellucida in the platelet activating factor group when compared with the non-PAF-treated control group. A 5-minute exposure of oocytes to platelet activating factor in the absence of sperm did not result in parthenogenetic activation of control oocytes.

Comment

The mouse in vitro fertilization system is highly efficient with fertilization rates of 85% to 90%. The two approaches used in the past to demonstrate stimulatory effects on the system are reduction of spermatozoa insemination concentration, or drastic reduction of sperm-egg coincubation time.[13, 14] Recently Pomeroy et al.[15] reported the enhancement of mouse ova in vitro fertilization by treatment of spermatozoa with caffeine. This effect was only demonstrable when the sperm-egg coincubation time was reduced to 15 minutes.[15] In this study we note a twofold increase in the in vitro fertilization and cleavage rate after a very short exposure of oocytes to spermatozoa in the presence of platelet activating factor and a decreased fertilization rate in the presence of platelet activating factor inhibitor CV3988. Harper et al.[16] reported a statistically significant reduction in the number of rabbit oocytes fertilized in vivo when does were artificially inseminated with 10^{-5} and 10^{-4} mol/L CV3988-treated ejaculated semen. In their study they observed moderate spermatozoa agglutination at 10^{-5} mol/L CV3988 with reduction in motility. After treatment with 10^{-4} mol/L CV3988 they noted severe sperm agglutination and a large decrease in motility but still observed a 77% fertilization rate.[16] In our study with mice we did not observe sperm agglutination at 10^{-5} mol/L CV3988 or a decrease in motility. After 10^{-4} mol/L CV3988 treatment we noted 10% to 15% spermatozoa agglutination, with a mild decrease in motility. The reduction in mouse oocyte fertilization in the presence of 10^{-4} mol/L CV3988 is unlikely to be a result of the mild sperm agglutination or reduced motility. These discrepancies with regard to agglutination and motility reduction may be species specific or a result of the presence of seminal fluid in the rabbit study. In a recent report Kuzan et al.[17] also noted a twofold to threefold increase in the fertilization rate of mouse oocytes in the presence of 1×10^{-8} mol/L platelet activating factor using low sperm concentration (1×10^3/ml). A significant reduction in the fertilization rate of oocytes in the presence of Webb 2086, a specific platelet activating factor receptor antagonist, was also observed by them.[17] These data indicate a direct role for platelet activating factor in the fertilization process. Platelet activating factor binding to target cells is followed by an increase in phosphatidylinositol-4,5-bisphosphate breakdown and the subsequent formation of diacylglycerol, an important second messenger.[18] Phosphatidylinositol-4,5-bisphosphate breakdown generates inositol-1,4,5-triphosphate, which is involved in calcium mobilization.[19] It is possible that calcium mobilization may promote the capacitation and acrosome reaction of spermatozoa. From this study we conclude that platelet activating factor, in addition to its involvement in ovulation, implantation, and parturition, may also have an important role in sperm-egg interaction during fertilization. Previous studies from our laboratory with regard to the presence of platelet activating factor in human spermatozoa and its enhancement of human sperm motility, in conjunction with the preliminary data noting increased fertilization rates of mouse and rabbit oocytes in the presence of platelet activating factor, are highly suggestive for the potential therapeutic value of platelet activating factor in the treatment of various forms of human infertility.[3, 4, 19] Further research will be directed to elucidate the involvement of platelet activating factor in gamete interaction from a mechanistic point of view.

REFERENCES

1. Kumar R, Hanahan DJ. Diversity of the biochemical and biological behavior of platelet activating factor. In: Snyder F, ed. Platelet activating factor. New York: Plenum Press, 1987:239.
2. Abisogun AO, Braquet T, Tsafriri A. The involvement of

platelet activating factor in ovulation. Science 1989; 243:381.
3. Minhas BS, Kumar R, Dodson MG, Palmer TV, Harrill JL, Robertson JL. The presence of platelet activating factor (PAF)–like activity in human spermatozoa and its implications concerning male infertility. In: Proceedings of the forty-fourth annual meeting of the American Fertility Society, October, 1988. Atlanta: American Fertility Society, 1988:S22.
4. Ricker DD, Minhas BS, Kumar R, Robertson JL, Dodson MG. The effects of platelet activating factor on the motility of human spermatozoa. Fertil Steril 1989 [In press].
5. Collier M, O'Neill C, Ammit AJ, Saunders DM. Biochemical and pharmacological characterization of human embryo–derived platelet activating factor. Hum Reprod 1988;3:993.
6. O'Neill C. Partial characterization of the embryo-derived platelet activating factor in mice. J Reprod Fertil 1985; 75:375.
7. O'Neill C. Examination of the causes of early pregnancy-associated thrombocytopenia in mice. J Reprod Fertil 1985;73:567.
8. O'Neill C, Gidley-Baird AA, Pike IL, Porter RN, Sinosich MJ, Saunders EM. Maternal blood platelet physiology and luteal phase endocrinology as a means of monitoring pre- and post-implantation embryo viability following in vitro fertilization. J In Vitro Fert Embryo Transf 1985;2:87.
9. O'Neill C, Gidley-Baird AA, Pike IL, Saunders DM. Use of a bioassay for embryo-derived platelet activating factor as a means of assessing quality and pregnancy potential of human embryos. Fertil Steril 1987;47:969.
10. Spinks NR, O'Neill C. Antagonists of embryo-derived platelet activating factor prevent implantation of mouse embryos. J Reprod Fertil 1988;84:89.
11. Acker G, Hecquet F, Etienne A, Braquet P, Mencia-Huerta JM. Role of platelet activating factor (PAF) in the ova implantation in the rat: effect of the specific PAF-acether antagonist, BN52021. Prostaglandins 1988;35: 233.
12. Angle MJ, Jones MA, McManus LM, Pinckard RN, Harper MJK. Platelet activating factor in the rabbit uterus during early pregnancy. J Reprod Fertil 1988;83:711.
13. Wolf DP, Inoue M. Sperm concentration dependency in the fertilization and zona sperm binding properties of mouse eggs inseminated in vitro. J Exp Zool 1976;196:27.
14. Fraser LR. Accelerated mouse sperm penetration in vitro in the presence of caffeine. J Reprod Fertil 1979;57:377.
15. Pomeroy KD, Dodds JF, Seidel GE Jr. Caffeine promotes in vitro fertilization of mouse ova within 15 minutes. J Exp Zool 1988;248:207.
16. Harper MJK, Woodard DS, Norris CJ. Spermicidal effect of antagonists of platelet activating factor. Fertil Steril 1989;51:890.
17. Kuzan FB, Geissler FT, Henderson WR Jr. Role of platelet activating factor (PAF) in mouse in vitro fertilization. Theriogenology 1989;31:214.
18. Braquet P, Touqui L, Shen TY, Vargaftig BB. Perspectives in platelet activating factor research. Pharmacol Rev 1987;39:97.
19. Roudebush WE, Minhas BS, Ricker DD, Palmer TV, Dodson MG. Platelet activating factor treatment of rabbit spermatozoa induces hyperactivated motility and fertilizability in vitro. Biol Reprod 1989;40(suppl 1):343.

Release of tumor necrosis factor-α by human peritoneal macrophages in vivo and in vitro

Jouko Halme, MD, PhD

Los Angeles, California, and Chapel Hill, North Carolina

Tumor necrosis factor is a product of activated monocytes, macrophages, and lymphocytes that exerts a variety of effects in the host. Its cytotoxicity toward gametes has been suggested as a mechanism of how activated macrophages may cause subfertility, inasmuch as detectable levels of tumor necrosis factor have been reported in the peritoneal fluid of infertile patients. To further examine this issue we measured tumor necrosis factor activity in peritoneal fluid and its release in vitro from monocytes or peritoneal macrophages of patients undergoing laparoscopy because of either tubal ligation or infertility. Cytolytic activity was determined with a bioassay with sensitized mouse fibrosarcoma cells as target. Significant tumor necrosis factor activity (>5 U/ml) was detected in 38% of the peritoneal fluid samples. A total of 43% of the peritoneal macrophage samples released significant tumor necrosis factor activity in vitro and all samples tested including peripheral monocytes released high levels of activity in the presence of bacterial endotoxin. The activity was released in vitro in a time-dependent manner. Thermal stability characteristics and neutralization with a specific antibody indicated that most of the activity in peritoneal fluid or released in vitro was that of tumor necrosis factor-α. Statistical analysis of tumor necrosis factor activity in peritoneal fluid in various diagnostic categories revealed a significantly elevated level in patients with endometriosis, as compared with fertile women. Endometriosis and pelvic adhesions were also significantly more likely to be associated with measurable levels of tumor necrosis factor activity released by peritoneal macrophages in vitro. Overall, because tumor necrosis factor-α levels were frequently detectable, it suggests that activation of peritoneal macrophages either by bacterial products or inflammatory conditions can occur in vivo. Moreover, these cells remain competent to release tumor necrosis factor in vitro. (Am J Obstet Gynecol 1989;161:1718-25.)

Key words: Tumor necrosis factor, monocytes, macrophages, endometriosis

Several constituents of the peritoneal fluid environment have been suggested to possess the potential to adversely (or positively) influence fertility.[1] Increased numbers of peritoneal macrophages and lymphocytes in addition to soluble enzymes and inflammation mediators have been documented in infertile patients with and without endometriosis.[2-5] Second, direct experiments have documented adverse effects on the viability of gametes or preimplantation embryos by peritoneal fluid and media from incubated (peritoneal) inflammatory cells.[6-9] Third, several purified products of activated macrophages or constituents of peritoneal fluid have been shown to exert adverse influence on gametes, fertilization, or embryonic development when tested in vivo.[10-12] Such products include interleukin-1, interleukin-2, interferon-gamma, and tumor necrosis factor (TNF-α).

TNF-α (cachectin) is a cytotoxic protein released by stimulated monocytes and macrophages that is an important component of the host inflammatory response.[13] This compound activates neutrophils,[14] alters the properties of vascular endothelial cells,[15] and regulates the metabolic activity of several tissues.[16] Because TNF-α is cytotoxic against some tumor cells[17] and has been shown to significantly reduce the motility of human sperm in vitro, it has been postulated to be a local mediator of subfertility.[11, 18] Indeed, TNF-α has been detected in peritoneal fluid in approximately one third of asymptomatic infertility patients undergoing laparoscopy, and significantly elevated levels were shown to be associated with advanced endometriosis.[19]

This article reports our studies on the presence of TNF-α in peritoneal fluid in vivo and production of this compound in vitro by peritoneal macrophages from healthy patients. We also examined factors that control secretion of TNF-α in vitro such as macrophage activation and adherence.

From the Divisions of Reproductive Endocrinology, Departments of Obstetrics and Gynecology, University of California, Los Angeles, and University of North Carolina at Chapel Hill.

Supported by National Institute of Child Health and Development Grant No. R01-HD21546.

Presented in part at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.

Reprint requests: Jouko Halme, MD, PhD, Division of Reproductive Endocrinology, Department of Obstetrics and Gynecology, University of North Carolina, Campus Box 7570, MacNider Building, Chapel Hill, NC 52699.

6/6/15666

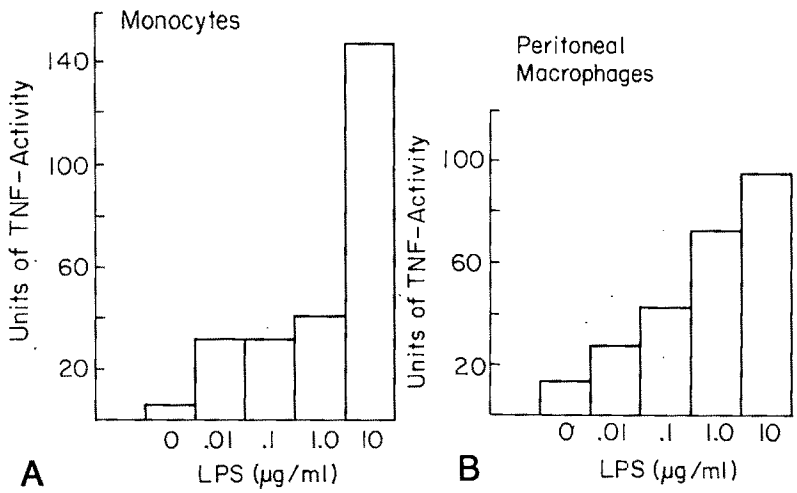


Fig. 1. Release of TNF by monocytes and peritoneal macrophages in response to bacterial endotoxin (lipopolysaccharide) in vitro. *Bars* represent means of triplicate measurements.

Material and methods

Patient population. Peritoneal fluid was obtained with prior consent from a total of 78 patients undergoing either diagnostic laparoscopy or elective laparoscopic tubal sterilization. The age range was from 23 to 42 years and the laparoscopic procedure was performed under general anesthesia between days 2 to 29 of the menstrual cycle. Of the 29 patients who had normal pelvic findings, 9 were undergoing tubal sterilization and the remainder has unexplained infertility. A total of 15 women had tubal occlusion or pelvic adhesions. There were 34 women who were found to have endometriosis, which according to the American Fertility Society classification was stages I to II in 23 women, stage III in 7 women, and stage IV in 4 women. All fertile women had regular menstrual cycles and were neither taking oral contraceptives nor using an intrauterine contraceptive device. The infertility patients had ≥1 year of primary or secondary infertility and had undergone a basic infertility evaluation before laparoscopy.

Collection and isolation of peritoneal cells and peripheral blood monocytes. Peritoneal fluid was collected at laparoscopy with a laparoscopic cannula from the cul-de-sac before any manipulative procedures or tubal insufflation. Peritoneal fluid samples were centrifuged to separate cells from the supernatant, which was stored in aliquots at −70° C until assayed for cytotoxic activity.[20] The macrophages were isolated by discontinuous Ficoll-Paque centrifugation gradient, as previously described.[20] Peripheral blood mononuclear cells were subjected to further purification by a stepwise Percoll gradient to obtain approximately a 90% pure monocyte population.

Monocyte-macrophage cultures. Peritoneal macrophages or monocytes (usually 500,000 cells) were plated in 1.5 cm diameter tissue culture wells (Costar, Cambridge, Mass.) in 1.0 ml of serum-free Dulbecco's modified Eagle's medium-high glucose (DMEM-H) (Irvine Scientific, Irvine, Calif.) for 1 hour. The media was then replaced with 1.5 ml of fresh DMEM-H and the macrophages were incubated as an adherent monolayer for 24 hours in 5% carbon dioxide at 37° C in a humidified incubator. The cells were incubated either in the absence or presence of 0.01 to 10 μg/ml of lipopolysaccharide from *Escherichia coli* 026:B6 (Sigma Chemical Company, St. Louis, Mo.). Cell viability was assessed at the end of incubation by microscopic visualization of cell morphology and adherence. After incubation the monocyte or macrophage culture medium was rendered cell free by centrifugation at 1500 rpm for 10 minutes to remove any cellular debris and then stored in aliquots at −70° C until assayed for cytotoxic activity.

To demonstrate the time dependency of TNF-α release by peritoneal macrophages, either 500,000 or 1 million cells per well were plated as described above and incubated in DMEM-H at 37° C. The medium was harvested and replaced with fresh medium after various time intervals of incubation. Other wells were incubated for the same intervals without replacement of the medium to ascertain that inactivation of cytolytic activity did not occur during prolonged incubation in assay medium.

In experiments to test the role of cell adherence on the release of cytotoxic activity, 500,000 isolated peritoneal macrophages were incubated in triplicate with DMEM-H in polypropylene tubes with continuous agitation to prevent adherence. After incubation for various intervals, the cells were centrifuged at 1500 rpm for 10 minutes and supernatants were assayed for cytotoxic activity.

Assay for TNF activity. A neutral red stain cytotox-

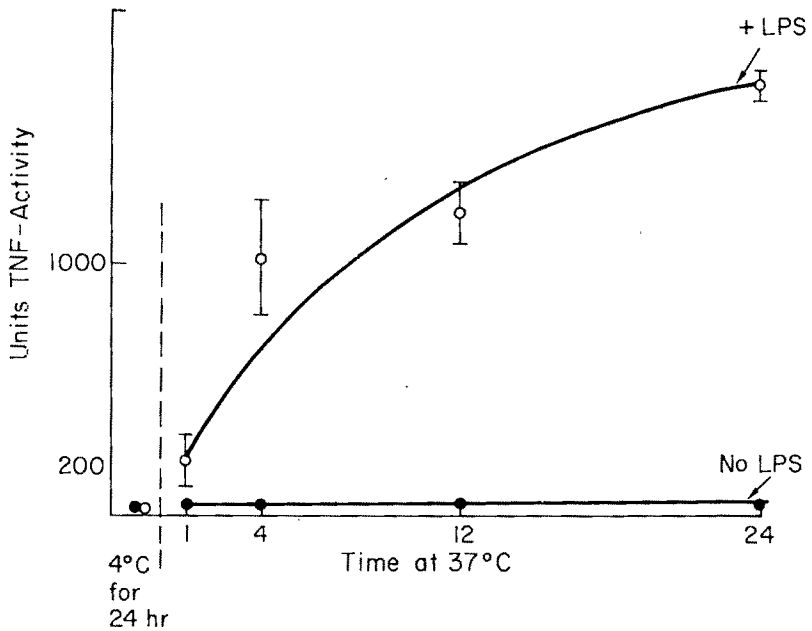


Fig. 2. Time-dependent release of TNF by peritoneal macrophages in vitro. In the example, lipopolysaccharide induces a significant release of activity. *Open* or *closed circles* denote means of triplicate wells. *Vertical lines* represent ±SE.

icity assay was used to measure the percent of surviving target cells.[21] In brief, target cells (WEHI-164 mouse fibrosarcoma cells were seeded in 96 well microwell plates at a concentration of 20,000 cells per well in 100 μl of growth medium (Roswell Park Memorial Institute with 10% calf serum) for 18 hours and incubated overnight at 37° C. To sensitize the cells, the medium was replaced with new medium that contained actinomycin D at a concentration of 1 μg/ml and incubation was continued for 2 hours. Medium was again replaced with fresh medium that contained different dilutions of macrophage culture medium or standard dilutions of recombinant TNF-α, which ranged from 0.1 to 3000 U/ml. The detection limit of the assay was 1 U/ml. The recombinant TNF used as a standard has a specific activity 2.39×10^7 U/mg. After an 18-hour incubation at 37° C, the medium with nonattached target cells was aspirated, and 100 μl of 0.006% neutral red stain in ethanol was added to each well. The plates were agitated and then read on a semiautomatic microplate reader with a test wavelength of 550 nm. Samples were considered to have significant activity if >5 U/ml.

Immunoinhibition. Samples of macrophage-conditioned media or peritoneal fluid were incubated at 22° C for 24 hours with neutralizing rabbit antihuman TNF-α (Olympus Corporation, Lake Success, N.Y.). The antibody contained a gamma globulin concentration of 1 μg/ml and neutralized recombinant TNF at a dilution of 1:100 to 1:100,000. As a control, nonimmune serum was preincubated with macrophage culture medium. Aliquots of macrophage culture medium were then assayed for cytotoxicity on WEHI-164 cells as described above.

Statistical analysis. Statistical evaluation of the data was performed with the unpaired Student *t* test or χ^2 analysis. Data are expressed as means ± SE.

Results

To determine whether TNF activity is produced in vitro, both peripheral monocytes and several samples of peritoneal macrophages were incubated in the presence and absence of lipopolysaccharide and the conditioned medium was assayed for TNF activity. Nonstimulated monocytes produced a negligible amount of TNF activity during a 24-hour incubation. The effect of lipopolysaccharide on production of tumor necrosis factor was dose-dependent with the maximal increase in the presence of 10 μg/ml (Fig. 1, *A*). Incubation of peritoneal macrophages resulted in release of TNF activity in some samples, but in others this was seen only after lipopolysaccharide stimulation. Incubation with increasing concentrations of lipopolysaccharide greatly augmented the production of TNF-α activity by most peritoneal macrophages tested (Fig. 1, *B*).

To establish whether cytolytic activity is produced in vitro in a time-dependent manner, representative samples of peritoneal macrophages were incubated for various intervals at 37° C in the presence or absence of

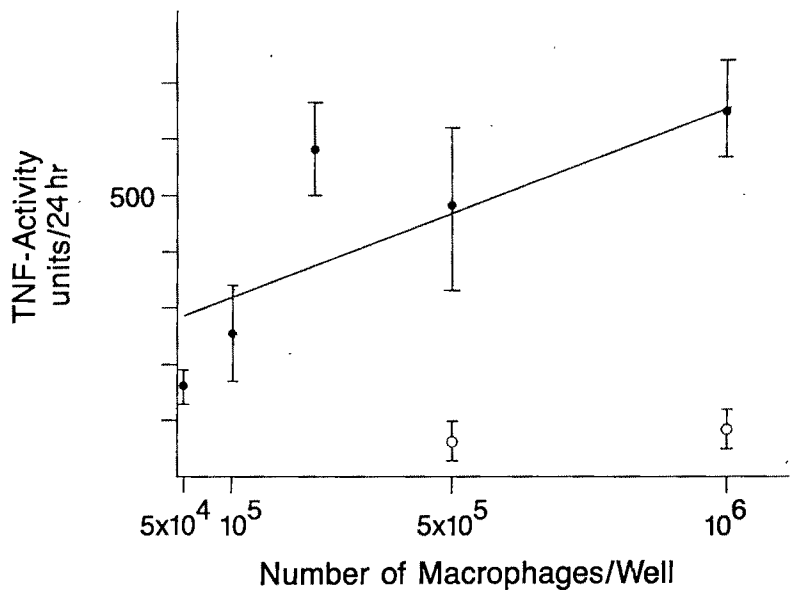


Fig. 3. Release of TNF by varying concentrations of peritoneal macrophages in vitro. *Open circles* denote means of triplicate wells not including lipopolysaccharide. *Closed circles* with 10 μg/ml of lipopolysaccharide. *Vertical lines* represent ±SE. *Regression line* is drawn to indicate relationship between number of incubated macrophages and stimulated release of TNF activity ($r = 0.817$).

lipopolysaccharide (Fig. 2). TNF-α activity was released during the 24-hour period in the absence of lipopolysaccharide. There was no detectable activity without lipopolysaccharide or if the incubation with lipopolysaccharide was carried out at 4° C.

In a similar fashion, increasing numbers of peritoneal macrophages were incubated for 24 hours at 37° C to determine the influence of cell concentration on the release of cytolytic activity (Fig. 3). There was a significant linear correlation between these parameters in the range of 5×10^4 to 1×10^6 cells per well when incubations were carried out in the presence of lipopolysaccharide.

To assess correlation between spontaneous (basal) and lipopolysaccharide–induced TNF-α release in vitro, samples of peritoneal macrophages from a variety of patients were incubated with and without lipopolysaccharide (Fig. 4). Great variability was seen in the level of basal activity and all samples released high levels of activity in response to lipopolysaccharide. However, there was no significant correlation between spontaneous release and induced release (correlation coefficient, $r = 0.2$).

Peritoneal macrophages are obtained and isolated as nonadherent cells. After incubation they become adherent to plastic. Because the adherence to plastic may induce an artificial stimulus, TNF-α production by adherent peritoneal cells was compared with cells that were incubated in identical conditions in a nonadherent state. There was no statistically significant difference in baseline TNF activity between adherent and nonadherent conditions. In some samples lipopolysaccharide–induced activity was significantly higher in adherent conditions, whereas in others this was not the case. However, there appeared to be no difference in heat resistance characteristics of TNF activity produced in either condition. The activity was resistant to heat at 56° C but was generally destroyed at temperatures of 80° C (data not shown). These heat stability characteristics observed in our macrophage culture medium supernatants are consistent with physical properties of human TNF previously published.[22]

To identify the cytolytic activity released by peritoneal macrophages in vitro as TNF activity, neutralization with a specific antibody to human TNF-α was performed. In these experiments, 96% of the cytolytic activity of recombinant human TNF-α was abolished by preincubation with antihuman TNF (data not shown). Furthermore, 82% of the activity in macrophage-conditioned medium and 76% of that in peritoneal fluid was inhibited by the same antibody. These results suggest that the cytolytic activity produced by peritoneal macrophages in vivo or in vitro is, for the most part, identical to human TNF-α.

The cytotoxic activity present in peritoneal fluid in patients in different diagnostic categories was compared, and is depicted in Fig. 5. Thirty of 78 peritoneal fluid samples had detectable activity, with the maximum cytotoxicity found in an infertile patient with pelvic adhesions. There was no significant difference between the mean levels of activity in fertile versus infertile patients ($p < 0.1$) or in the presence versus absence of

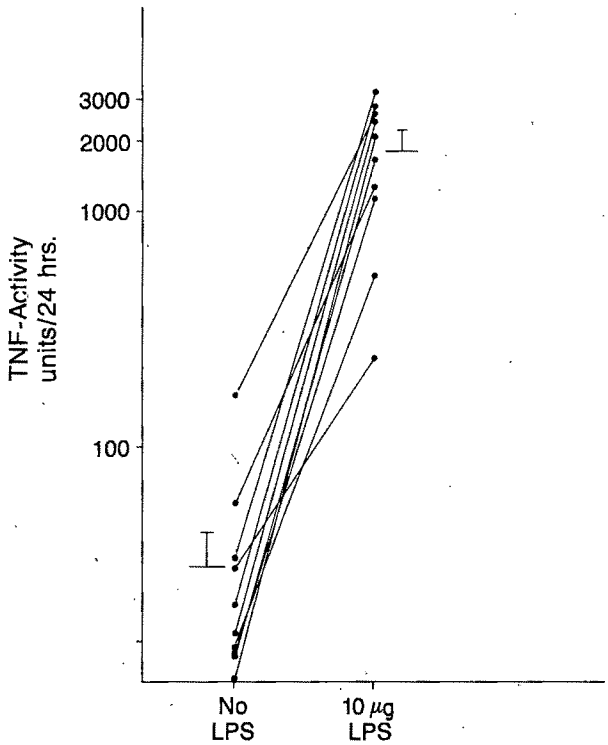


Fig. 4. Spontaneous and lipopolysaccharide–induced release of TNF in vitro by peritoneal macrophages from 10 patients. *Circles* represent means of duplicate wells. *Horizontal lines* are means + SE of group.

endometriosis ($p < 0.2$). However, the mean level of cytotoxic activity was higher in patients with endometriosis than that in fertile women ($p < 0.05$).

Significant TNF activity was also released by peritoneal macrophages in vitro (Fig. 6). Of 71 samples tested, 28 samples had measurable activity. Macrophages from 10 of 38 patients without endometriosis released TNF-α in vitro, as compared with 18 out of 33 patients with endometriosis. A statistical evaluation revealed significant differences in the frequency of detectable TNF release between fertile patients versus those with adhesions ($p < 0.025$), fertile patients versus those with endometriosis ($p < 0.01$), and between all patients with and those without endometriosis ($p < 0.025$). Thus it appears that the presence of cytolytic activity is quite common in peritoneal fluid and that peritoneal macrophages are often capable of releasing TNF activity even without appreciable in vitro exposure to lipopolysaccharide.

Comment

Tumor necrosis factor is a secretory protein that is increasingly recognized as an important biologic mediator.[13] It induces a variety of inflammatory responses in vivo in experimental animals, and recent reports have implicated TNF in critically ill cancer patients,[23] in endotoxin shock,[24] and in several inflammatory states.[25-27] Monocytes and macrophages, which are known to be also present in the peritoneal cavity have been shown to release TNF-α and may respond to a variety of inflammatory stimuli in vivo. The peritoneal cavity represents an environment in which several potentially inflammatory stimuli are often present. Retrograde menstruation, ovulation-associated rupture of follicular contents, and antigenic components of sperm, are all such stimuli. In addition, pelvic infections (both clinical and subclinical) may promote TNF-α production after macrophage activation by a variety of microorganisms.[28]

In this article, we have shown that human peritoneal macrophages from healthy women with or without evidence of some pelvic abnormality often are capable of producing a factor(s) cytotoxic for WEHI-164 cells, a TNF-sensitive cell line. This factor is stable when heated at 56° C for 30 minutes, but it is inactivated by heating at 86° C for 30 minutes. Furthermore, more than 80% of this activity is neutralized by monoclonal antihuman TNF-α antibody. These data suggest that this activity is identical to TNF-α.

Under normal circumstances little biologically active tumor necrosis factor is present within circulating peripheral monocytes, because the gene or genes that control its expression are repressed.[29] This was shown in this study by the nondetectable release of TNF in vitro by nonstimulated monocytes, whereas a dose-dependent TNF release was seen after exposure to lipopolysaccharide in vitro. In contrast, a large proportion of human peritoneal macrophages released TNF in vitro during the 24-hour incubation period without any additional stimulus such as lipopolysaccharide. This suggests that peritoneal macrophages have had exposure in vivo (in the peritoneal cavity) to priming effects of substances such as interleukin-1 or granulocyte-macrophage–colony stimulating factor and possibly bacterial endotoxin. The variable increase of TNF release by the peritoneal macrophages in vitro may reflect the amount and type of the previous in vivo stimulus.

The results of this study suggest that cell adherence is a factor that determines macrophage TNF release and sensitivity to lipopolysaccharide, although some peritoneal macrophages were capable of releasing TNF without a stimulus in nonadherent conditions. However, the control of TNF release by the peritoneal cells appears less dependent on adherence than that of production of macrophage-derived growth activity, which is entirely dependent on adherence.[30]

This study also demonstrates that cytolytic activity is detectable in a significant number of peritoneal fluid samples from patients who have little evidence of gross pelvic pathology. Most of this activity (at least in rep-

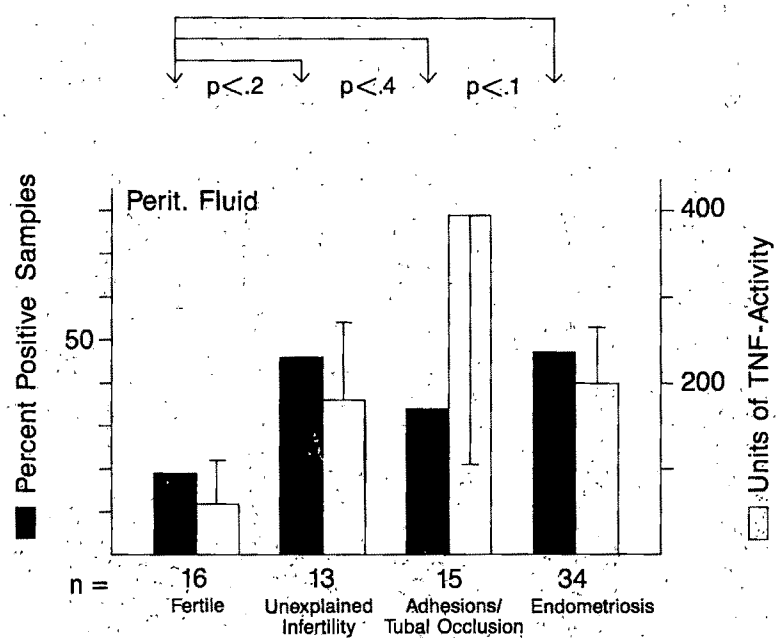


Fig. 5. Comparison of TNF activity in peritoneal fluid of 78 patients. *Open bars* denote means + SE. Statistical evaluation revealed significant difference only between fertile and endometriosis samples (log transformed values). *Closed bars* indicate frequency of positive samples. χ^2 analysis did not reveal significant differences between groups as depicted.

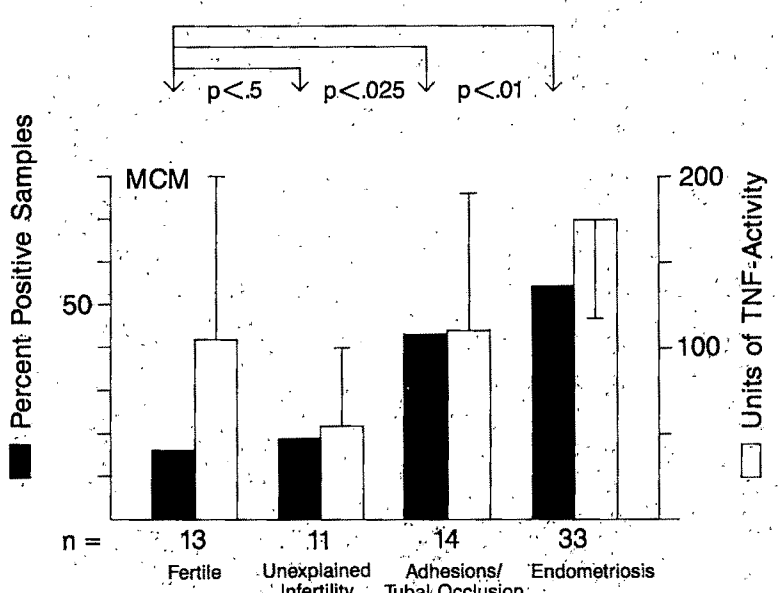


Fig. 6. Comparison of TNF activity released in vitro by peritoneal macrophages of 71 patients. *Open bars* denote means + SE or means − SE. Statistical analysis revealed significant differences only between unexplained infertility and endometriosis samples (log transformed values). *Closed bars* indicate frequency of positive samples. χ^2 analysis revealed significant differences between groups as depicted.

resentative samples) also is neutralized by monoclonal antibody against human TNF-α. The remaining cytolytic activity may represent TNF-β, a cytolytic factor with identical bioactivity but only 28% homology to TNF-α. This factor is secreted by T-lymphocytes in response to antigen challenge to viruses or lymphocyte mitogens.[31] Alternatively, the nonneutralizable cytolytic activity in macrophage media and peritoneal fluid may be some undetermined type of cytolytic factor that antigenically and structurally is unrelated to TNF. Several

studies have identified "cytotoxic activity" in peritoneal fluid with other types of assays for cytotoxicity such as embryo culture,[9, 12] sperm motility,[11] and antifertilization effects,[6] and it remains to be seen whether these activities are identical to the activity detected in this study. Although the WEHI cell line has been widely used in the detection of TNF-α activity, it is not specific for TNF-α.[21]

What is the biologic implication of the presence of significant cytolytic activity in the peritoneal environment? It is obvious that such activity could have potential adverse effects on reproductive processes such as fertilization and early development of the conceptus, but are there any advantages or benefits from such substances? Release of cytolytic activity by monocytes is considered to be part of their role in immunosurveillance and host response to bacteria and viruses. The peritoneal environment is often exposed to such microorganisms in view of the frequent presence of sperm in the peritoneal fluid. Sperm can serve as vectors for bacteria and viruses (this has been recently demonstrated even for human immunodeficiency virus).[32] This stimulus for peritoneal macrophages to release TNF-α is perhaps a vital component of the local host response while being detrimental to fertility.

Although there is great variability in the level of cytolytic activity in the peritoneal fluid and that released by peritoneal macrophages in vitro, these results are in agreement with a previous report that showed detectable levels of TNF in peritoneal fluid of 35% of 74 patients.[19] As a diagnostic group, only patients with moderate to severe endometriosis had significantly elevated levels in that study. Our results indicate a statistically significant increase in TNF levels released in vitro and in peritoneal fluid in patients with endometriosis. The small number of patients with moderate to severe endometriosis in this study does not allow adequate separate statistical evaluation for that group. Indeed, TNF activity measured either in vitro or in vivo does not appear specific for any particular diagnostic category although macrophages from patients with endometriosis are significantly more likely to release TNF in vitro. Furthermore, it is not clear whether the activity is dependent on levels of circulating or local hormonal factors. Another cytokine, interleukin-1, has been shown to have such a hormonal dependency.[33] Certainly further studies are warranted to determine which factors control the production of these cytokines and to elucidate their role and impact in the female pelvis and reproductive physiology.

The recombinant human TNF-α was a gift from Genentech, Co., San Francisco, Calif., and the WEHI-164 mouse fibrosarcoma cells were a gift from Susanne Becker, PhD. The excellent technical assistance of Eufemia Tsui is acknowledged.

REFERENCES

1. Syrop CH, Halme J. Peritoneal fluid environment and infertility. Fertil Steril 1987;48:1-9.
2. Haney AF, Muscato JJ, Weinberg JB. Peritoneal fluid cell populations in infertility patients. Fertil Steril 1981; 35:696-8.
3. Halme J, Becker S, Hammond MG, Raj S. Pelvic macrophages in normal and infertile women: the role of patent tubes. Am J Obstet Gynecol 1982;142:890-5.
4. Olive DL, Weinberg JB, Haney AF. Peritoneal macrophages and infertility. Fertil Steril 1985;44:772-7.
5. Hill JA, Faris HMP, Schiff J, Anderson DJ. Characterization of leukocyte subpopulations in the peritoneal fluid of women with endometriosis. Fertil Steril 1988;50:216-22.
6. Chacho KJ, Stronkowski-Chacho M, Andresen PJ, Scommegna A. Peritoneal fluid in patients with and without endometriosis: prostanoids and macrophages and their effect on the spermatozoa penetration assay. Am J Obstet Gynecol 1986;154:1290-9.
7. Oak MK, Chantler EN, Vaughan-Williams CA, Elstein M. Sperm survival studies in peritoneal fluid from infertile women with endometriosis and unexplained infertility. Clin Reprod 1985;3:297-303.
8. Hahn DW, Carraher RP, Foldesy RG, McGuire JL. Experimental evidence for failure to implant as a mechanism of infertility associated with endometriosis. Am J Obstet Gynecol 1986;155:1109-13.
9. Morcos RN, Gibbons WE, Findley WE. Effect of peritoneal fluid on in vitro cleavage of 2-cell mouse embryos: possible role in infertility associated with endometriosis. Fertil Steril 1985;44:678-83.
10. Fakih H, Baggett B, Holtz G, Tsang KY, Lee JC, Williamson HO. Interleukin 1: a possible role in the infertility associated with endometriosis. Fertil Steril 1987;47: 213-7.
11. Hill JA, Haimovici F, Politch JA, Anderson DJ. Effects of soluble products of activated lymphocytes and macrophages (lymphokines and monokines) on human sperm motion parameters. Fertil Steril 1987;47:460-5.
12. Hill JA, Haimovici F, Anderson D. Products of activated lymphocytes and macrophages inhibit mouse embryo development in vitro. J Immunol 1987;139:2250-4.
13. Beutler B, Cerami A. Cachectin: more than a tumor necrosis factor. N Engl J Med 1987;316:379-85.
14. Shalaby MR, Aggarwal BB, Rinderknecht E, Svedersky LP, Finkle BS, Palladino MA. Activation of human polymorphonuclear neutrophil functions by interferon-alpha and tumor necrosis factors. J Immunol 1985;135:2069-74.
15. Nawroth PP, Bank I, Handley D, Cassimeris J, Chess L, Stern D. Tumor necrosis factor/cachectin interacts with endothelial cell receptors to induce release of interleukin 1. J Exp Med 1986;163:1363-75.
16. Warren RS, Starnes HF Jr. Gabrilove JL, Oettgen HF, Brennan MF. The acute metabolic effects of tumor necrosis factor administration in humans. Arch Surg 1987; 122:1396-400.
17. Ziegler-Heitbrock HWL, Moller A, Linke RP, Haas JG, Rieber EP, Riethmuller G. Tumor necrosis factor as effector molecule in monocyte mediated cytotoxicity. Cancer Res 1986;46:5947-52.
18. Eisermann J, Register K, Strickler RC, Collins JL. Tumor necrosis factor mediates spermicidal activity of peritoneal fluid from infertile women with endometriosis. In: Proceedings of the 44th Annual Meeting of the American Fertility Society. Birmingham, Alabama: The American Fertility Society. 1988:32.
19. Eisermann J, Gast MJ, Pineda J, Odem RR, Collins JL. Tumor necrosis factor in peritoneal fluid of women undergoing laparoscopic surgery. Fertil Steril 1988;50: 573-9.
20. Halme J, Becker S, Hammond MG, Raj MHG, Raj S.

Increased activation of pelvic macrophages in infertile women with mild endometriosis. AM J OBSTET GYNECOL 1983;145:333-7.
21. Bersani L, Colotta F, Mantovani A. Involvement of tumour necrosis factor in monocyte-mediated rapid killing of actinomycin D-pretreated WEHI 164 sarcoma cells. Immunology 1986;59:323-6.
22. Ruff MR, Gifford GE. Tumor necrosis factor, lymphokines. New York: Academic Press, 1981:235-73. (vol 2.)
23. Balkwill F, Burke F, Talbot D, et al. Evidence for tumour necrosis factor/cachectin production in cancer. Lancet 1987;1:1229-32.
24. Beutler B, Milsark IW, Cerami AC. Passive immunization against cachectin/tumor necrosis factor protects mice from lethal effect of endotoxin. Science 1985; 229:869-71.
25. Bachmich PR, Lynch JP, Larrick J, Spengler M, Kunkel SL. Tumor necrosis factor production by human sarcoid alveolar macrophages. Am J Pathol 1986;125:421-5.
26. Dayer J-M, Beutler B, Cerami A. Cachectin/tumor necrosis factor (TNF) stimulates collagenase and PGE_2 production by human synovial cells and dermal fibroblasts. J Exp Med 1985;162:2163-8.
27. Aderka D, Holtmann H, Toker L, Hahn T, Wallach D. Tumor necrosis factor induction by Sendai virus. J Immunol 1986;136:2938-42.
28. Girardin E, Grau GE, Dayer J-M, Roux-Lombard P, Lambert P-H. Tumor necrosis factor and interleukin-1 in the serum of children with severe infectious purpura. N Engl J Med 1988;319:397-400.
29. Nedwin GE, Svedersky LP, Bringman TS, Palladino MS Jr, Goeddel DV. Effects of interleukin 2, interferon gamma, and mitogens on the production of tumor necrosis factors alpha and beta. J Immunol 1985;135:2492-7.
30. Halme J, White C, Kauma S, Estes J, Haskill S. Peritoneal macrophages from patients with endometriosis release growth factor activity in vitro. J Clin Endocrinol Metab 1988;66:1044-9.
31. Nedwin GE, Naylor SL, Sakaquchi AY, et al. Human lymphotoxin and tumor necrosis factor genes: structure, homology and chromosomal localization. Nucleic Acids Res 1985;13:6361-73.
32. Bagasra O, Freund M, Weidman J, Harley G. Interaction of human immunodeficiency virus with human sperm in vitro. J Acquir Immune Defic Syn 1988;1:431-435.
33. Polan ML, Daniele A, Kuo A. Gonadal steroids modulate human monocyte interleukin-1 (IL-1) activity. Fertil Steril 1988;46:964-8.

Isolation and sequencing of a complementary deoxyribonucleic acid clone encoding human placental 17β-estradiol dehydrogenase: Identification of the putative cofactor binding site

Michael J. Gast, MD, PhD,[a] Harold F. Sims, BS,[c] Gary L. Murdock, PhD,[a] Peter M. Gast,[a] and Arnold W. Strauss, MD[b, c]

St. Louis, Missouri

17β-Estradiol dehydrogenase (EC 1.1.1.62) catalyzes the interconversion of estradiol and estrone in human term placenta. We have raised a specific polyclonal antibody to this abundant placental enzyme to study its role in late pregnancy events and its molecular biologic characteristics. In this work the 17β-estradiol dehydrogenase antibody was used to isolate and sequence a complementary deoxyribonucleic acid clone encoding about 98% of the amino acid sequence of the 17β-estradiol dehydrogenase molecule. This sequence verifies previous sequence data on the molecule's steroid binding site and also localizes a putative nicotinamide adenine dinucleotide binding region similar to that of many other pyridine nucleotide–dependent dehydrogenases. Isolation of the complementary deoxyribonucleic acid for 17β-estradiol dehydrogenase expands our knowledge of the structure-function relationships of the enzyme and is a major step in our understanding of its biologic function in pregnancy. (AM J OBSTET GYNECOL 1989;161:1726-31.)

Key words: Estradiol dehydrogenase, nicotinamide adenine dinucleotide binding, placenta

Abnormalities of labor, especially when associated with the problems of prematurity and postmaturity, represent the single most common cause of cerebral palsy and mental retardation in the U.S. today. Much of the recent work in this area centers on the critical role of estrogen in the uterine cellular mechanisms responsible for the initiation of labor.[1] The steroid converting enzyme 17β-estradiol dehydrogenase occupies a central position in that enzymatic cascade responsible for the generation of sex steroids in the woman. The enzyme catalyzes the terminal step of estrogen biosynthesis and is required for the interconversion of estradiol, the major female sex hormone, with its weaker, related steroid estrone. 17β-Estradiol dehydrogenase is found in uncommon abundance in the human placenta where its probable role is in the maintenance and regulation of appropriate estradiol levels in the mother and fetus as delivery approaches. Gaining an understanding of the enzyme's structure and mechanisms of action will be necessary to define and correct any steroid-related abnormalities in the process of labor initiation that lead either to preterm labor or to the failure to properly initiate the labor process (postmaturity).

Although it may be isolated in milligram quantities from the placenta, little is known about the physiologic role of 17β-estradiol dehydrogenase in the pregnancy process. Even less is known about the molecular biologic characteristics of the enzyme. We have long been interested in the study of this pyridine nucleotide–dependent enzyme, both because of its potential regulatory role in the dynamics of placental steroid metabolism and because of our interest in the structural and functional concomitants of the nicotinamide adenine dinucleotide– and nicotinamide adenine dinucleotide phosphate–dependent dehydrogenases. Steroid-converting enzymes in different tissues may share some degree of estradiol dehydrogenase–like activity,[2] leading to confusion about specificity and functional relationships within this class of enzymes. For that reason, reliance on activity measurement and gel chromatography have proved inadequate to probe intracellular sites of action and enzyme regulation. Thus recent advances in cloning and nucleic acid sequencing provide a realistic, currently available tool to evaluate periodic changes in the enzyme's levels and to produce the large quantitites of pure enzyme necessary for crystallographic and stereochemical study.

From the Departments of Obstetrics and Gynecology,[a] Pediatrics,[b] and Biochemistry and Molecular Biophysics,[c] Washington University School of Medicine.

Presented in part at the Thirty-sixth Annual Meeting of the Society For Gynecologic Investigation, San Diego, California, March 15-18, 1989.

Reprint requests: Michael J. Gast MD, PhD, Department of Obstetrics and Gynecology, Washington University School of Medicine, 4911 Barnes Hospital Plaza, St. Louis, MO 63110.

6/6/15965

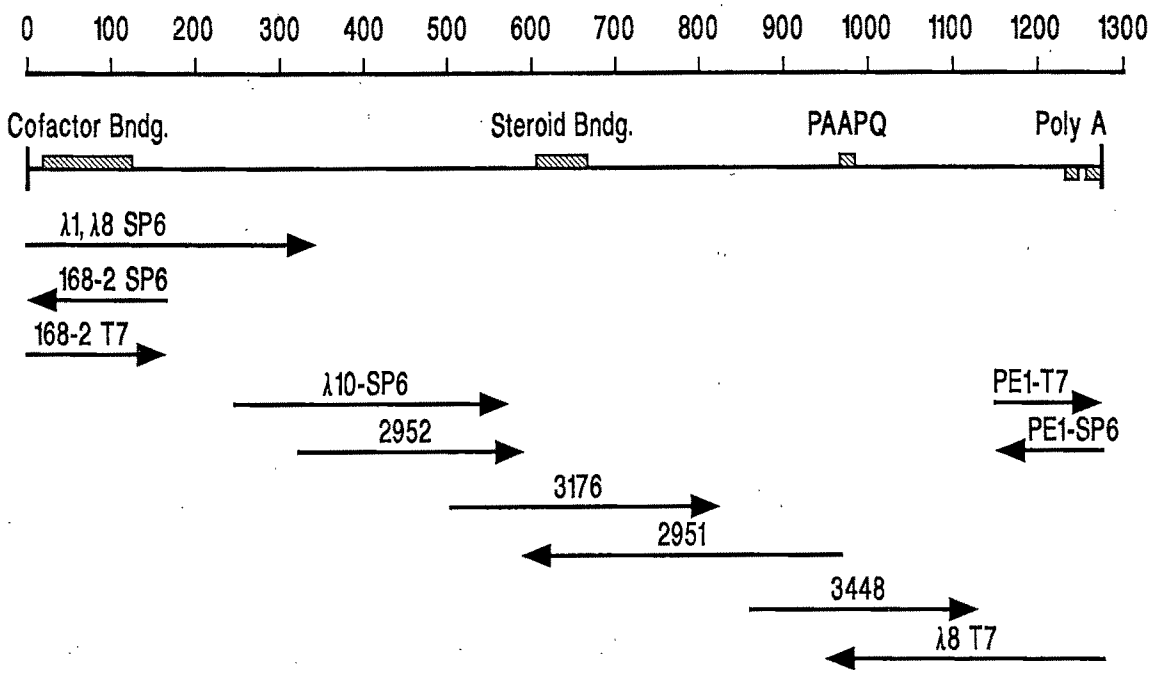


Fig. 1. Sequencing strategy for the 17β-estradiol dehydrogenase cDNA clone. Sequencing was carried out by a modified dideoxy-nucleotide technique[12] that used α[^{35}S]5′-deoxyadenosine triphosphate. Universal primers (SP6 and T7) and specific synthetic oligonucleotides were used as primers for the sequencing reactions. Multiple sequence overlaps were performed for all regions of the clone. The locations of the steroid and cofactor binding sites, as well as the polyadenylation site, may be seen. PAAPQ denotes the carboxyl terminal of the 17β-estradiol dehydrogenase coding sequence.

In the last several years we have extensively studied active site structure and function in the 17β-estradiol dehydrogenase molecule. We have characterized the biochemical and biophysical properties of the purified enzyme, established the kinetics of substrate binding and product inhibition, and probed the active site topography with affinity alkylating steroids and cofactors.[3-5] Collaboration between our department and Dr. Ralph Bradshaw (University of California, Irvine) has produced a putative amino acid sequence for the catalytic site and amino terminus of the 17β-estradiol dehydrogenase molecule.[6] To determine the complete primary structure of the human placental enzyme and to expand our understanding of the polypeptide's substrate and cofactor binding domains, we undertook the isolation and nucleic acid sequencing of a complementary deoxyribonucleic acid (cDNA) clone encoding 17β-estradiol dehydrogenase. Using this clone we hoped to establish the entire primary structure of estradiol dehydrogenase, to verify previously established amino acid sequences for the enzyme's substrate binding domain and its amino terminus, and to identify a putative cofactor binding site. Finally, we wished to compare the nicotinamide adenine dinucleotide binding site's primary and secondary structures with those of other pyridine-dependent dehydrogenases.

Material and methods

Radiolabeled protein A (50 μCi/ml) and high-specific-activity α[^{35}S]5′-deoxyadenosine triphosphate (>1000 Ci/mmol) were obtained from Amersham (Amersham, United Kingdom). pGEM4Z plasmid-DNA was obtained from Promega, Inc. (Madison, Wis.). All reagent-grade chemicals were obtained from Sigma Chemical Co. (St. Louis). 20α-Hydroxysteroid dehydrogenase was generously provided by Dr. Jorge Pineda (Washington University, St. Louis).

To screen a human placental cDNA library for estradiol dehydrogenase clones, it was first necessary to produce a highly specific polyclonal antibody to the enzyme. Highly purified enzyme was isolated by the affinity purification technique of Chin and Warren[7] and evaluated for activity by the method of Langer and Engel.[8] The protein was then used to raise antiserum in male New Zealand White rabbits by a technique described previously.[9] Whole serum was evaluated for sensitivity and specificity of antibody response during the development of a sensitive indirect enzyme immunosorbent assay (data not shown). This enzyme-linked immunosorbent assay proved capable of accurately measuring known quantities of 17β-estradiol dehydrogenase as small as 10 ng. When other proteins, such as bovine serum albumin, creatine phosphokinase, and

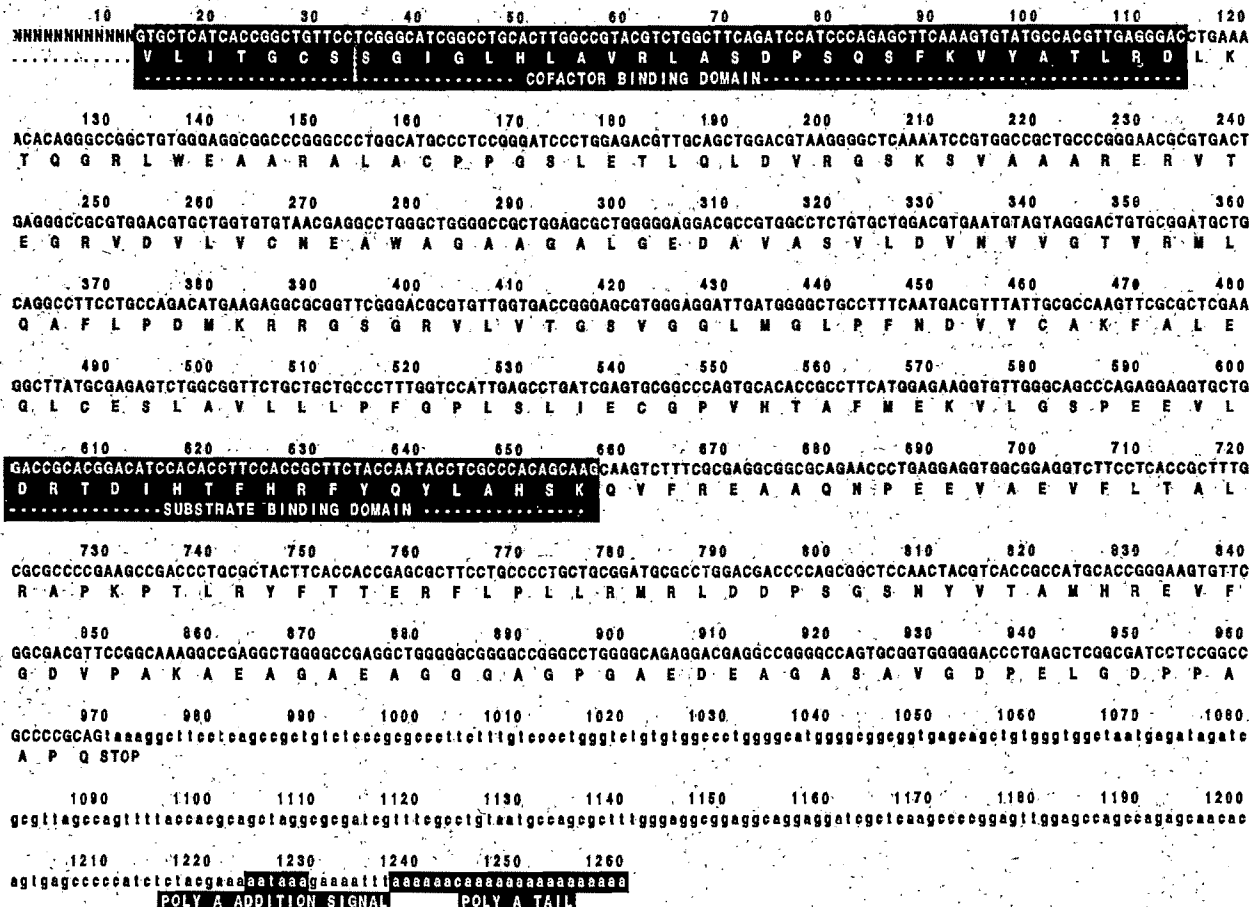


Fig. 2. Translation of 17β-estradiol dehydrogenase into protein. 17β-Estradiol dehydrogenase is a protein of approximately 323 amino acids. This figure delineates the primary structure of the molecule for all but its first four amino acids. The area marked *cofactor binding domain* is extrapolated from the structural motifs derived by Birktoft and Banaszak.[13] The substrate binding domain is the 20-amino acid peptide fragment affinity labeled by Murdock et al.[14] in 1986. The consensus polyadenylation addition signal AATAAA may be seen near nucleic acid 1230.

20α-hydroxysteroid dehydrogenase from bull testes, were used as the preincubation immunosorbent in the enzyme-linked immunosorbent assay, no specific estradiol dehydrogenase—like protein was measured. Having satisfied ourselves that the antibody we had generated was indeed 17β-estradiol dehydrogenase–specific, we decided to identify and purify a series of clones encoding the enzyme from a human placental cDNA library prepared by Dr. Evan Sadler.[10] This library was size-selected for cDNA inserts of >1000 bases. The inserts were ligated into the *Eco*RI site of the bacteriophage expression vector λgt11. The library was transfected into the nonrestrictive ampicillin-resistant host, *Escherichia coli* Y1090, and plated out at an initial concentration of 5×10^4 plaque-forming units of recombinant phage. Multiple plates were screened with the specific 17β-estradiol dehydrogenase antiserum according to the technique of Huynh et al.[11] This method uses the ability of λgt11 to synthesize immunologically competent β-galactosidase–estradiol dehydrogenase fusion proteins from in-frame/coding region cDNAs inserted adjacent to the phage's lacZ promoter. Plaques containing the fusion protein were identified by sequential exposure to a 1:200 dilution of the estradiol dehydrogenase–specific antiserum and iodine 125–labeled *Staphylococcus* protein A (approximately 1 μCi per filter). The labeled plaques were lifted and further purified by rescreening at progressively lower plaque densities until several homogeneous 17β-estradiol dehydrogenase–positive phage populations were identified. These clones were then amplified and their phage DNA was subjected to *Eco*RI digestion. The resulting inserts were subcloned into the double-stranded plasmid pGEM4Z (Promega). DNA sequencing was carried out according to a modification of the dideoxy-chain termination method of Sanger et al.[12]

The cDNA inserts used for futher study were found to hybridize both to a 1400 base pair placental ribonucleic acid and to human genomic DNA by Northern and Southern blot analysis, respectively (data not shown).

Results

Eighteen 17β-estradiol dehydrogenase–positive clones were identified by antibody screening and later by oligonucleotide screening. All or parts of five of these clones were used in the DNA sequencing process. Fig. 1 represents the sequencing strategy used to establish the nucleic acid sequence of the longest of the clones. Universal primers and a variety of specific oligonucleotides were used to provide a series of overlapping sequence data. These established a cDNA of 1270 bases, some 970 of which make up the 17β-estradiol dehydrogenase coding sequence. Also shown in this figure are the relative locations of the substrate and cofactor binding regions within the cDNA sequence. Although a poly(A)tail was identified at the end of the noncoding 3′region of the clone, no ATG start codon was identified in our cDNA.

Fig. 2 details the translation of our nucleic acid sequence into protein. The polyadenylation site and its consensus start sequence AATAAA (located 8 bases upstream) may be seen at the 3′-end of the sequence. The carboxy terminal of the enzyme (proline-alanine-alanine-proline-glutamine) is located at approximately nucleic acid 970.

In 1984, Warren et al.[6] presented a putative *N*-terminal amino acid sequence for estradiol dehydrogenase derived by standard protein chemistry techniques. That sequence began with the amino acids alanine-arginine-threonine-valine. The next series of amino acids from their sequence corresponds to the first 19 amino acids deduced from our cDNA clone and may be seen at its 5′-end. Since no ATG codon was identified by our group, we conclude that this clone ends at least five amino acids shy of the amino terminus of the enzyme.

Two other areas of the estradiol dehydrogenase amino acid sequence also have been published. In 1973, Nicolas and Harris[13] detailed a 17-amino acid fragment that they affinity labeled with a halogenated nicotinamide adenine dinucleotide phosphate derivative. The sequence of this fragment was confirmed by translation of bases 150 to 200 in our nucleic acid sequence. In 1986, Murdock et al.[14] published a 20-amino acid partial sequence from the putative steroid-binding site of 17β-estradiol dehydrogenase after purification with a series of radiolabeled derivatized enzyme substrates. This region contains three histidine residues thought to be critical to the enzyme's activity. DNA sequencing of the region, also present in our cDNA, confirms the accuracy of their amino acid sequence. It corresponds roughly to amino acids 195 to 214 in the estradiol dehydrogenase molecule.

Birktoft and Banaszak[15] have analyzed and compared the nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate binding domains of more than a dozen of the >250 pyridine-dependent dehydrogenases and their isoforms that have been identified to date. They suggested that the dinucleotide-binding domains of these enzymes contain a common structural motif. This motif consists of several, nearly parallel β-sheets with a single α-helix forming the connection between the first two β-strands. Several invariate glycine residues permit close proximity of the two ribose rings of cofactor to the β-sheet and the pyrophosphate to the α-helix. An acidic group, usually aspartic acid, also interacts with the adenine ribose 2-hydroxyl group of the nicotinamide adenine dinucleotide. Fig. 3 offers a comparison of the amino terminal portion of estradiol dehydrogenase with the nicotinamide adenine dinucleotide–binding regions of several of these other dehydrogenases. When compared with the nicotinamide adenine dinucleotide–binding sites of lactic dehydrogenase, malate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, and others, the characteristic β-, α-, β-pattern, multiple proximate glycine residues, and typical acidic amino acid for ribose binding all may be seen within the 34-amino terminal residues of our cDNA-derived protein sequence. The amino terminal location of this portion of the estradiol dehydrogenase molecule is a signature location for the dinucleotide binding regions of other dehydrogenases.

Comment

We have isolated and sequenced a cDNA clone encoding nearly 98% of the protein sequence of 17β-estradiol dehydrogenase. On the basis of this work, estradiol dehydrogenase is a polypeptide made up of approximately 323 amino acids. This corresponds closely to previous molecular weight estimates that were based on gel chromatography[16] and denaturing gel electrophoresis.[16, 17] The relative frequency of individual amino acids in our putative sequence also correlates with earlier estimates that were based on compositional analysis.[16, 17] Finally, the cDNA-derived sequence is nearly identical to a 17β-estradiol dehydrogenase sequence derived in the laboratory of Dr. Ralph Bradshaw using standard protein chemistry techniques (personal communication). This sequence is also in almost complete agreement with the DNA sequence of a human placental 17β-estradiol dehydrogenase cDNA published earlier this year by Peltoketo et al.[18] The six

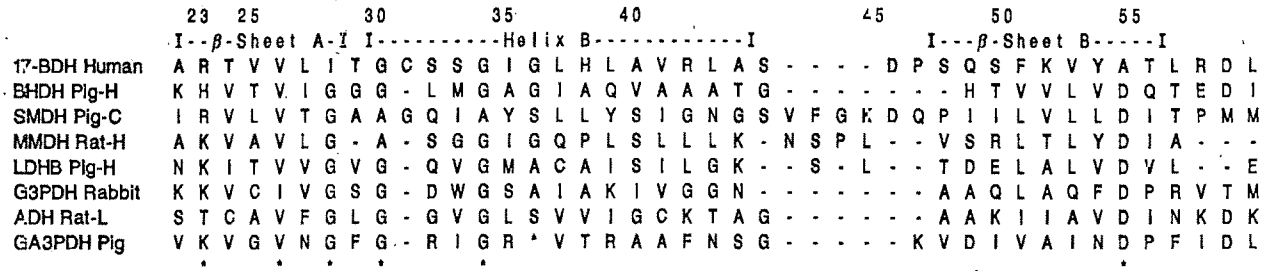


Fig. 3. Comparison of the nicotinamide adenine dinucleotide–binding sites of selected pyridine nucleotide–dependent enzymes with that of 17β-estradiol dehydrogenase. Amino acid sequences of the core dinucleotide-binding domains of various nicotinamide adenine dinucleotide–binding enzymes are presented. The secondary structure assignments of the proteins are indicated at the top of the figure. The exact starting and ending points for the β-strands and the α-helix vary among proteins. The *stars* at the bottom of the figure indicate the locations of critical or highly conserved residues. Abbreviations for enzyme names are as follows: *17β-BDH,* 17β-Estradiol dehydrogenase; *BHDG,* β-hydroxyacyl-coenzyme A dehydrogenase; *SMDH,* cytosolic malate dehydrogenase; *MMDH,* mitochondrial malate dehydrogenase; *LDHB,* lactic dehydrogenase B; *G3PDH,* glycerol-3-phosphate dehydrogenase; *ADH,* alcohol dehydrogenase; *GA3PDH,* glyceraldehyde-3-phosphate dehydrogenase. The source of each enzyme is included after its abbreviation.

differences in the amino acid composition of our sequence and that of Peltoketo et al. can, in general, be explained by differences in a single nucleic acid within a given area of the DNA sequence. This most likely occurs because the two groups have sequenced slightly different allelic forms of the cDNA.

Since the mid-1970s, identification of the putative nicotinamide adenine dinucleotide binding site of 17β-estradiol dehydrogenase has remained elusive. Sequence analysis, as inferred from our DNA sequencing technique, identifies a putative cofactor binding site of approximately 40 amino acids located in the molecule's amino terminus that is similar in primary and secondary structure to other pyridine-dependent dehydrogenases. This sequence does not contain the 17-amino acid fragment labeled by Nicolas and Harris[13] with derivatized cofactor analog. We believe that this cofactor analog in fact binds at the site that we have identified but that the derivatized ring selectively labels an adjacent area of protein sequence. As may be seen from the sequence of our cloned cDNA, the Nicolas and Harris peptide, which contains none of the structural motifs identified by Birktoft and Banaszak,[15] also lies in the amino terminal half of the molecule and thus is most likely in close proximity to the authentic cofactor binding site. Definitive elucidation of the mechanics of cofactor binding and amino acid residues that are critical for hydrogen transfer awaits crystallographic analysis and study of enzymatic activity after site-directed mutagenesis.

Identification and comparison of steroid-binding domains for several steroid-converting enzymes with the use of affinity labeling with substrate derivatives have suggested similarities in the presumed active sites of these enzymes.[14, 19] However, specific characteristics of these active sites and the importance of individual amino acids within the sites (e.g., histidine or cysteine residues) have yet to be elucidated. The technique of gene modification combined with existing affinity labeling technology now offers an opportunity to study the comparative biologic features of normal and modified active sites and thus the function of specific amino acids and steric influences in the mechanism of steroid conversion.

This work confirms the sequence of the substrate binding domain elucidated by Warren et al.[6] and places its location in the first half of the molecule's carboxy terminal portion. This location is analogous to the locations of the substrate binding regions of other nicotinamide adenine dinucleotide–dependent dehydrogenases. We presume this region includes those amino acid residues that are responsible for catalytic hydrogen ion transfer at the D ring of the steroid substrate. Mutagenesis in this region, accompanied by studies with derivatized substrate, offers the opportunity to determine which residues are required for the catalytic event and the molecular mechanisms involved in their participation.

Although the techniques of cDNA cloning and sequencing and gene modification have been popular tools for the evaluation of proteins and hormones for a decade, their use as investigative tools for the class of pyridine nucleotide–dependent dehydrogenases has been limited. The steroid dehydrogenases offer a unique opportunity to study structure-function relationships because of our ability to combine probes of active site topography (using the tool of alkylation with derivatized substrate) with genetically modified point changes in amino acids located at the enzyme's active site. In the future we hope to isolate a full-

length cDNA, which will allow us to sequence the remaining coding region of the molecule and its untranslated 5′-region. We also hope to use the clone to identify the biologic concomitants of 17β-estradiol dehydrogenase expression and to transfect 17β-estradiol dehydrogenase–bearing plasmid into prokaryotic and eukaryotic expression vectors to explore structure-function relationships of the molecule through the techniques of site-directed mutagenesis and affinity alkylation with derivatized substrates. Kinetic and crystallographic study of the high-quantity, homogeneous material produced by transformed cell lines is a first step in the understanding and possible modification of the role of estrogen in clinical conditions characterized by abnormal labor.

REFERENCES

1. Walsh SW, McCarthy MS. Selective placental secretion of estrogens into fetal and maternal circulations. Endocrinology 1981;109:2152-9.
2. Marovitz W, Loucopoulos A, Satyaswaroop PG, Gurpide E, Todd R, Naftolin F. Apparent immunologic nonidentity of human placental and endometrial 17β-estradiol dehydrogenase. Am J Obstet Gynecol 1980; 138:643-7.
3. Chin CC, Pineda J, Warren JC. Spatial relationships of steroid and cofactor at the active site of human placental 17β-estradiol dehydrogenase. J Biol Chem 1982;257: 2225-9.
4. Murdock GL, Chin CC, Offord RE, Bradshaw RA, Warren JC. Human placental 17β-estradiol dehydrogenase. Identification of a single histidine residue affinity labelled by both 3-bromoacetoxyestrone and 12β-bromoacetoxy-4-estrene-3,17-dione. J Biol Chem 1983;258:11460-4.
5. Murdock GL, Warren JC, Sweet F. Human placental estradiol 17β dehydrogenase: evidence for inverted substrate orientation (wrong-way binding) at the active site. Biochemistry 1988;27:4452-8.
6. Warren JC, Murdock GL, Fang J, Bradshaw RA. In: Proceedings of the thirty-first annual meeting of the Society for Gynecologic Investigation [Abstract 319]. San Francisco: Society for Gynecologic Investigation, 1984.
7. Chin CC, Warren JC. Purification of estradiol-17β dehydrogenase from human placenta by affinity chromatography. Steroids 1973;22:373-8.
8. Langer LJ, Engel LL. Human placental estradiol 17β dehydrogenase. I. Concentration, characterization, and assay. J Biol Chem 1958;233:583-8.
9. Gast MJ. Characterization of preprorelaxin by tryptic digestion and inhibition of its conversion to prorelaxin by amino acid analogs. J Biol Chem 1983;258:9001-4.
10. Ye RD, Wun T-C, Sadler JE. cDNA cloning and expression in *Escherichia coli* of a plasminogen activator inhibitor from human placenta. J Biol Chem 1987;262:3718-25.
11. Huynh TV, Young RA, Davis RW. Constructing and screening cDNA libraries in λgt10 and λgt11. In: Glover DM, ed. DNA cloning: a practical approach. Oxford: IRL Press, 1985:49-78.
12. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci 1977;74: 5463-7.
13. Nicolas JC, Harris JI. Human placental 17β-oestradiol dehydrogenase. Sequence of a tryptic peptide containing an essential cysteine. FEBS Lett 1973;29:173-6.
14. Murdock GL, Chin CC, Warren JC. Human placental 17β-estradiol dehydrogenase: sequence of a histidine-bearing peptide in the catalytic region. Biochemistry 1986;25: 641-6.
15. Birktoft JJ, Banaszak LJ. Structure-function relationships among nicotinamide-adenine dinucleotide dependent oxidoreductases. In: Hearn MTW, ed. Peptide and protein reviews. New York: Marcel Dekker, 1984 vol 4:1-46.
16. Engel LL, Groman EV. Human placental 17β estradiol dehydrogenase: characterization and structural studies. Recent Prog Horm Res 1974;30;139-69.
17. Burns DJW, Engel LL, Bethune JL. Amino acid composition and subunit structure. Human placental 17β estradiol dehydrogenase. Biochemistry 1972;11:2699-703.
18. Peltoketo H, Isomaa V, Mäentausta O, Vihko R. Complete amino acid sequence of human placental 17β-hydroxysteroid dehydrogenase deduced from cDNA. FEBS Lett 1988;239:73-7.
19. Rossman MG, Garavito RM, Eventoff W. Conformational adaptations among dehydrogenases. In: Sund H, ed. Pyridine nucleotide dependent dehydrogenases. New York: Walter deGruyter, 1977:3-30.
20. Birktoft JJ, Fernley RT, Bradshaw RA, Banaszak LJ. Amino acid sequence homology among 2-hydroxy acid dehydrogenases: mitochondrial and cytoplasmic malate dehydrogenases form a homologous system with lactic dehyrogenase. Proc Natl Acad Sci USA 1982;79:6166-70.

Molecular scanning of Yq11 (interval 6) in men with Sertoli-cell-only syndrome

Mark D. Johnson, MD,[a] Sandra P. T. Tho, MD,[a] Ali Behzadian, PhD,[a, b] and Paul G. McDonough, MD[a]

Augusta, Georgia

Data suggesting that probes pDP105/B and 50f2/C,E may identify sequences on distal Yq11 (interval 6) that are critical for spermatogenesis stimulated a study of this region by means of these two probes in azoospermic 46,XY men with biopsy-proved Sertoli-cell-only syndrome. Deoxyribonucleic acid samples from controls and study subjects were digested with the restriction enzymes *Taq*I, *Eco*RI, and *Bam*HI. These samples were blotted and hybridized with pDP105/B, 50f2/C,E, and two more proximal Yq11 probes 4B-2 and pAS1. The sequence hydridizing to 50f2/C was absent in one study subject. No deletions were detected with pDP105/B and the two more proximal probes. (AM J OBSTET GYNECOL 1989;161:1732-7.)

Key words: Spermatogenesis, Y chromosome, Sertoli-cell-only syndrome, DNA

Spermatogenesis is one of the most complicated and poorly understood differentiation processes in higher eukaryotes.[1] Genes on the Y chromosome that regulate spermatogenesis are highly conserved in evolution from *Drosophila* through mouse to man. Chromosomal deletions and rearrangements in the Yq11 region of azoospermic men with germ cell aplasia (Sertoli-cell-only syndrome) and maturation arrest suggest that sequences critical for spermatogenesis are present within the Yq11 region.[2-4]

An ordinal mapping of the human Y chromosome by Vergnaud et al.[5] has divided it into seven deletion intervals with each interval being defined by hybridization of contained dexoyribonucleic (DNA) restriction fragments with specific probes. On the basis of Yq deletions, intervals 5, 6, and 7 are assigned to Yq with deletion interval 6 defining a region of distal Yq11.[5] A molecular deletion of distal Yq11 (interval 6) was reported in a 45,X,14p+[45,X,t(Y;14)] azoospermic man with biopsy-proved germ cell aplasia (Sertoli-cell-only syndrome)[6] and an azoospermic 45,X,15p+[45,X,t(Y;15)] man without histologic diagnosis[7] by means of interval 6–specific probes pDP105/B and 50f2/C,E. These two probes distinguished fertile men from some infertile azoospermic men. On the basis of these data, Andersson et al.[6] hypothesized that DNA sequences critical for spermatogenesis are located on distal Yq11 within interval 6.

From the Departments of Obstetrics and Gynecology[a] and Anatomy,[b] Medical College of Georgia.

Presented in part at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.

Reprint requests: Paul G. McDonough, MD, Department of Obstetrics and Gynecology, Medical College of Georgia, Augusta, GA 30912-3360.

6/6/15960

The present study screened for molecular deletions in distal Yq11 (interval 6) in cytogenetically intact 46,XY men with germ cell aplasia (Sertoli-cell-only syndrome) using probes pDP105/B[6] and 50f2/C,E.[8] Two other probes, 4B-2[9] and pAS1,[10] screened for the integrity of Yq11 sequences proximal to interval 6.

Material and methods

The methods used in this study had prior approval of the Human Assurance Committee of the Medical College of Georgia.

Study subjects. Six azoospermic men with normal G-banded 46,XY karyotypes and biopsy-proved germ cell aplasia (Sertoli-cell-only syndrome) were enrolled in this study. The study subjects were from 24 to 44 years old and had no history of significant chronic medical illnesses, previous genital injury, infection, or orchitis, toxic environmental exposures, or urologic surgical procedures before biopsy and vasography. Physical examination revealed a normal male phenotype without evidence of anomalous genitalia or gynecomastia. Testes were in the normal range for volume and consistency in four patients; two individuals had smaller than normal testes. Serum testosterone levels were within the normal range except for one individual with a mildly decreased level (testosterone = 316 ng/dl). Luteinizing hormone levels ranged from 3.3 to 21.5 mIU/ml. Follicle-stimulating hormone levels ranged from 14.8 to 40.6 mIU/ml, the last value being elevated in only one individual. Semen analyses were all azoospermic at the time of biopsy with normal seminal volumes. Vasography revealed intact ductal systems in all individuals. Bilateral testicular biopsies were done in all individuals except for one.

DNA probes. Probe pDP105(DYZ4) is a 5.5 kb *Hin*dIII genomic fragment inserted into the *Hin*dIII

site of pUC13. It detects multiple Y-specific sequences in deletion interval 3 on Yp (pDP105/A) and in interval 6 on distal Yq11 (pDP105/B) (Page DC, personal communication). A 5.2 kb *Taq*I fragment (pDP105/B) is a marker for deletion interval 6.[7]

Probe 50f2[8] is a 1.7 kb *Eco*RI fragment from a Y-chromosome library inserted into the *Eco*RI site of plasmid pBR322. It defines five Y-specific *Eco*RI loci and one autosomal locus. It detects *Eco*RI fragments of 10 kb (50f2/A) and 7.5 kb (50f2/B) in deletion interval 3 on Yp. Probes 50f2/C and 50f2/E hybridize with *Eco*RI fragments of 6 and 1.7 kb, respectively, in deletion interval 6 of distal Yq11. The 50f2/D probe is a marker for an *Eco*RI 4.5 kb centromeric fragment. The autosomal locus is defined by an *Eco*RI 2.4 kb fragment.[5]

Probe 4B-2 (DYZ-15) is a recombinant phage construct developed from a Y-DNA library inserted into plasmid pBR322 at the *Eco*RI site. It contains a 3.3 kb *Eco*RI fragment that maps to the subcentromeric portion of Yq11 distal to the pAS1 sequence.[9]

Probe pAS1 represents an arginosuccinate synthetase pseudogene and is a 1.55 kb sequence constructed from a complementary DNA library inserted into the plasmid pBR322 at the *Pst* I site. It detects a 4.3 kb *Eco*RI fragment and a 2.45 kb *Bam*HI sequence, both of which map to Ycen-Yq11.

These recombinant plasmids were amplified and isolated by conventional methods.[11] The corresponding inserts were released from their vectors by restriction digests with the *Hin*dIII, *Eco*RI, and *Pst*I enzymes, respectively. They were purified by electrophoresis, phenol extraction, and ethanol precipitation. Before hybridization the DNA probes were labeled with phosphorus 32 by oligonucleotide random priming (Amersham International pic, Amersham, United Kingdom) to a high specific activity of 10^8 cpm/μg DNA.[12]

DNA analysis. Genomic DNA from the six study subjects, two normal fertile control men, and two normal control women, was digested with the restriction enzymes *Taq*I, *Eco*RI, and *Bam*HI. Samples of 10 μg DNA per well were electrophoresed in 0.8% high-melting-point agarose gels. DNA was transferred to Gene Screen Plus (New England Nuclear Research Products, Boston) by methods described by Southern[13] and baked in a vacuum for 2 hours at 80° C.

Transferred *Taq*I digests were hybridized with ^{32}P-labeled pDP105. *Eco*RI blots were separately hybridized with ^{32}P-labeled 50f2 and 4B-2 probes. The ^{32}P-labeled pAS1 probe was hybridized with separate *Eco*RI and *Bam*HI blots. All hybridizations were performed at 65° C for 2 days' duration.

The pDP105, 4B-2, and pAS1 hybridized blots were washed at high-stringency conditions at 65° C with decreasing concentrations of sodium chloride–sodium citrate (2 × SSC to 0.1 × SSC) and 1% sodium dodecyl sulfate. The 50f2 hybridized blots were washed at moderate-stringency conditions at 65° C with 2 × SSC and 1% sodium dodecyl sulfate. Autoradiographs were exposed at −80° C with intensifying screens from 1 to 4 days on Kodak XAR-5 film (Eastman Kodak Company, Rochester, N.Y.).

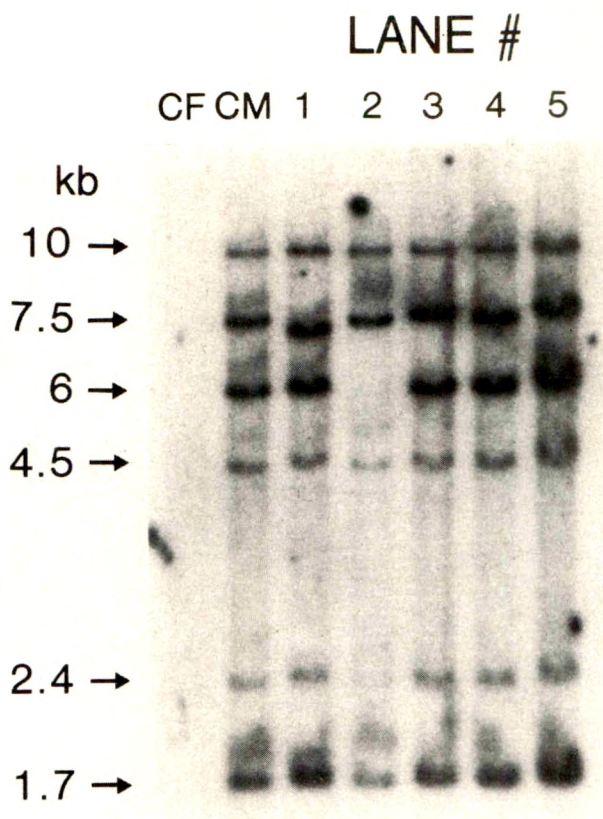


Fig. 1. Probe 50f2 hybridization with a Yq11-specific 6 kb *Eco*RI sequence (50f2/C) and a Yq11-specific 1.7 kb *Eco*RI sequence (50f2/E). Lane *CF* is DNA from a control woman. Lane *CM* is DNA from a fertile control man. Lanes *1* to *5* are DNA from men with Sertoli-cell-only syndrome.

Results

DNA from the azoospermic study subjects with germ cell aplasia (Sertoli-cell-only syndrome) was contrasted with DNA from normal fertile control men and normal control women. Analyses with 50f2/C,E were performed on only five of the six subjects since one azoospermic individual withdrew from the study. Probing distal Yq11 (interval 6) with 50f2/C,E in five azoospermic study subjects revealed absence of the *Eco*RI 6 kb 50f2/C band in only one individual (Fig. 1, lane 2). All other Y-specific bands and one autosomal band were present in the remaining azoospermic individuals and control men. The *Eco*RI 2.4 kb autosomal band was present at very low intensity in the control woman.

Six azoospermic men with Sertoli-cell-only syndrome and the normal fertile control men were studied with pDP105/B and exhibited the *Taq*I 5.2 kb band specific for distal Yq11 (interval 6) when hybridized with probe

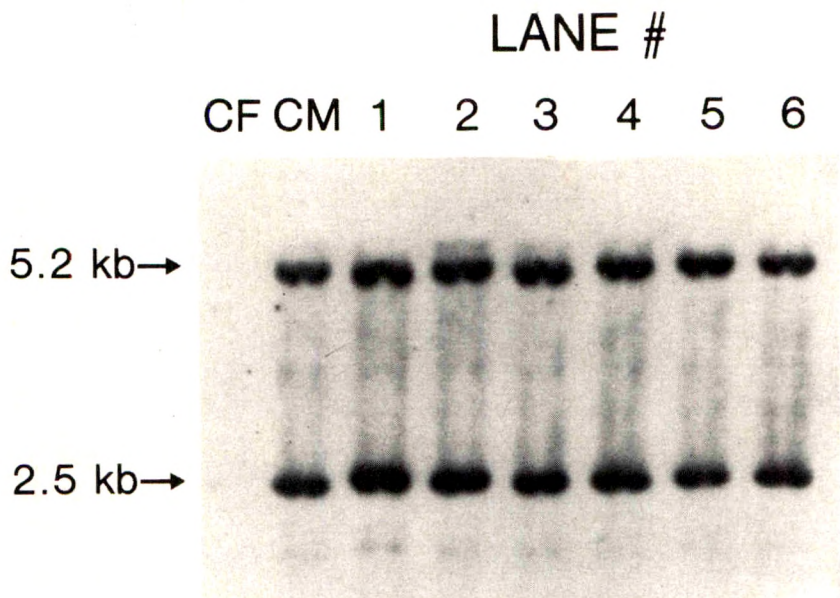


Fig. 2. Probe pDP105 hybridization with a Yq11-specific 5.2 kb *Taq*I sequence (pDP105/B) and a Yp-specific 2.5 kb *Taq*I sequence (pDP105/A). Lane *CF* is DNA from a control woman. Lane *CM* is DNA from a fertile control man. Lanes *1* to *6* are DNA from men with Sertoli-cell-only syndrome.

pDP105/B (Fig. 2). The same six study subjects and normal control men showed the presence of *Eco*RI 3.3 kb (Fig. 3), *Eco*RI 4.3 kb (Fig. 4), and *Bam*HI 2.45 kb (Fig. 5) Yq11-specific bands when hybridized with their respective probes 4B-2 and pAS1. These Yq11-specific hybridization bands were not seen in the DNA from the normal control woman.

Comment

This preliminary molecular scan of cytogenetically intact 46,XY men with biopsy-proved germ cell aplasia (Sertoli-cell-only syndrome) identified one individual (Fig. 1, lane 2) with absence of the *Eco*RI 6 kb 50f2/C band specific for distal Yq11 (interval 6). The significance of the absent 50f2/C band in this azoospermic individual awaits analysis of family members to confirm either a polymorphism or a de novo deletion. The remaining azoospermic subjects and fertile control men had the 50f2/C,E sequences present. Further scanning of Yq11 in these azoospermic study subjects and fertile control men with interval 6–specific pDP105/B and other probes (4B-2 and pAS1) that hybridize to more proximal regions of Yq11 revealed conservation of all intact sequences without evidence of deletion. As noted in Figs. 2 to 5, the probes pDP105/B, 4B-2, and pAS1 hybridized with Yq11-specific 5.2kb *Taq*I, 3.3 kb *Eco*RI, 4.3 kb *Eco*RI, and 2.45 kb *Bam*HI sequences, respectively.

Cytogenetic and molecular evidence in the study of mutations in *Drosophila*, mouse, and man supports the hypothesis that the deletion of certain Y-DNA sequences results in aberrant spermatogenesis.

Genetic loci on the Y chromosome of *Drosophila* responsible for spermatogenesis have been recognized for many years. Brosseau[14] studied X-irradiated male sterile *D. melanogaster* mutants. By using a marked Y chromosome for complementation tests, he defined seven fertility genes. His genetic map of the Y chromosome described two genes on the Y short arm and five genes on the Y long arm.[14] Hardy et al.[15] produced deletions of specific male fertility loci in *D. melanogaster* and correlated specific anatomic lesions in spermatogenic cell development with segmental Y-chromosome deficiencies.[15]

In mice, as in humans, the presence of spermatogenesis genes on the Y chromosome has been based on the study of sterile mutants. Intriguing evidence has accumulated from the study of sex-reversed XO males and associated variant strains.[16-18] In classic Sxr mutants a small segment of the Y-chromosome-containing testis determinants and H-Y antigen expression determinants is meiotically transferred to the X chromosome, thereby creating sex-reversed mice.[16, 19] Adult XO,Sxr male mice have normal-sized testes with all stages of spermatogenesis but are sterile because of defective meiotic divisions postulated to result from the absence of the Y chromosome and abnormal pairing in meiosis.[16]

Sxr′ is another Sxr mutant, which carries Y-chromosome testicular determinants but does not carry H-Y antigen expression determinants.[17] Adult, XO,Sxr′ mice are H-Y antigen negative by T-cell-restricted assay and have severely decreased spermatogonia with severe impairment of developmental stages before meiosis.[18]

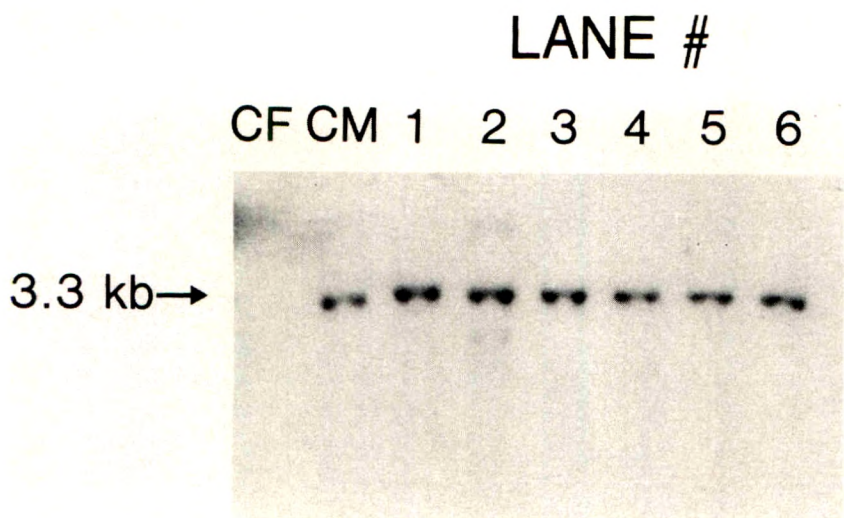


Fig. 3. Probe 4B-2 hybridization with a Yq11-specific 3.3 kb *Eco*RI sequence. Lane *CF* is DNA from a control woman. Lane *CM* is DNA from a fertile control man. Lanes *1* to *6* are DNA from men with Sertoli-cell-only syndrome.

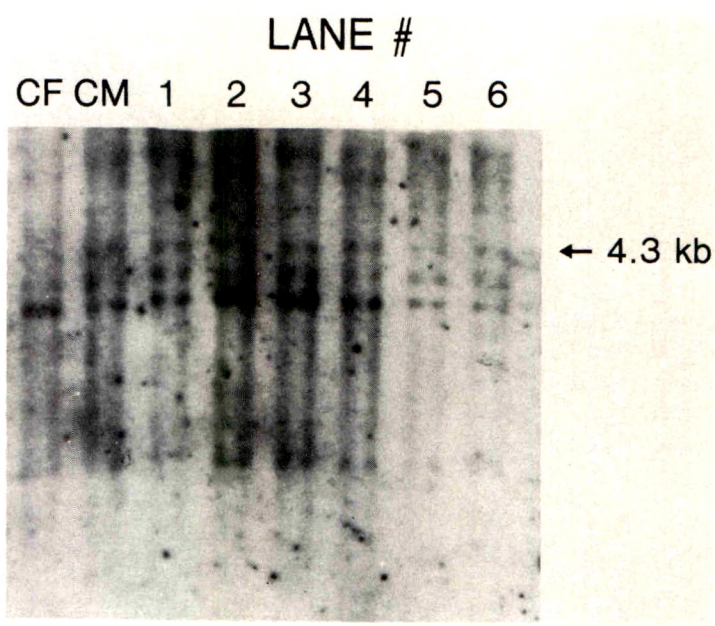


Fig. 4. Probe pAS1 hybridization with a Yq11-specific 4.3 kb *Eco*RI sequence. Lane *CF* is DNA from a control woman. Lane *CM* is DNA from a fertile control man. Lanes *1* to *6* are DNA from men with Sertoli-cell-only syndrome.

The premeiotic defect in spermatogenesis seen in these XO,Sxr′ mice contrasts with the abnormal meiotic divisions in spermatogenesis seen in adult XO,Sxr males with H-Y antigen expression. Burgoyne et al.[18] hypothesized that H-Y antigen may be a product of a "spermatogenesis" gene; however, it is possible that there are separate genes for spermatogenesis and H-Y antigen expression that are closely linked. This work by Burgoyne et al. with the Sxr′ variant clearly separates the genetic loci for Tdy and H-Y antigen and suggests that the protein encoded by H-Y antigen may be involved in the regulation of spermatogenesis.[17, 18]

Some humans with azoospermia have associated abnormalities of the Y chromosome. In 1976, Tiepolo and Zuffardi[2] reported on six azoospermic men with a distal deletion of Yq11. On the basis of these six azoospermic men, as well as two cases of ring Y chromosome with loss of Yq12 and part of Yq11, Tiepolo and Zuffardi[2]

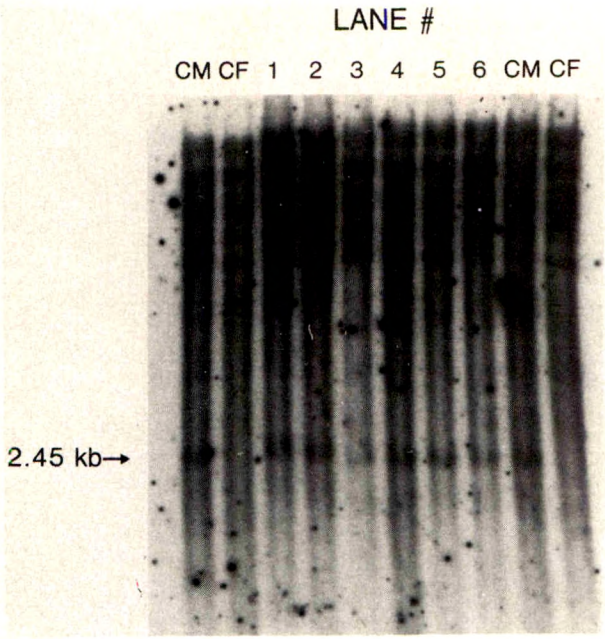


Fig. 5. Probe pAS1 hybridization with a Yq11-specific 2.45 kb *Bam*HI sequence. Lanes *CM* are DNA from fertile control men. Lanes *CF* are DNA from control women. Lanes *1* to *6* are DNA from men with Sertoli-cell-only syndrome.

proposed the presence of spermatogenesis-controlling factors at the distal portion of Yq11. Numerous reports of men with similar deletions of distal Yq11 and azoospermia are in the literature.[2-4, 20-25] Several azoospermic men with ring Y chromosomes [46,X,r(Y) and 45,X/46,X,r(Y)] also have been reported.[4]

Germ cell aplasia (Sertoli-cell-only syndrome) is a frequent histopathologic diagnosis in azoospermic men with absence of the distal Yq11 region. Six of nine azoospermic men with cytogenetic deletions of the distal Yq11-Yq12 region and three of five azoospermic men with r(Y) had histopathologic diagnoses of germ cell aplasia; the remainder had presumptive evidence of this diagnosis.[2, 3, 20-23, 25]

Recent molecular studies support the concept that sequences critical for spermatogenesis are present on distal Yq11 and that deletion of some or all of the sequences can result in extreme conditions such as germ cell aplasia (Sertoli-cell-only syndrome). As described earlier, Andersson et al.[6] and Gal et al.[7] reported on two separate azoospermic individuals with molecular deletions of distal Yq11 (interval 6). The azoospermic individual described in the former study had germ cell aplasia (Sertoli-cell-only syndrome) whereas the azoospermic individual in the latter study lacked a histologic diagnosis. Chandley et al.[25] described an azoospermic man with Yq deletion involving loss of interval 6 that was confirmed by absence of hybridization with pDP105/B. The individual has advanced tubular hyalinization in both testes with minimal germ cell elements.

Probing distal Yq11 (interval 6) with 50f2/C,E and pDP105/B and scanning more proximal Yq11 with 4B-2 and pAS1 screened only finite sequences of several-kilobase lengths within Yq11. It is apparent from these data that further investigation of the potential role of Yq11 deletions in spermatogenesis requires scanning with more Yq11 DNA probes and probing much larger DNA segments of Yq11 fractionated by pulsed-field gel electrophoresis. These techniques will enable us to identify starting points for restriction site mapping of this macroregion of DNA and subsequently assist in characterizing DNA sequences critical in spermatogenesis.

We acknowledge Dr. David Page for providing probe pDP105, Dr. Jean Weissenbach for providing probe 50f2, Dr. Kirby Smith for providing probe 4B-2, and Dr. Arthur Beaudet for providing probe pAS1. We thank Dr. J Rogers Byrd for his support in cytogenetics.

REFERENCES

1. Hennig W, ed. Results and problems in cell differentiation. Spermatogenesis: genetic aspects. Berlin: Springer-Verlag, 1987 vol 15:V.
2. Tiepolo L, Zuffardi O. Localization of factors controlling spermatogenesis in the nonfluorescent portion of the human Y chromosome long arm. Hum Genet 1976;34:119-24.
3. Fitch N, Richer C, Pinsky L, Kahn A. Deletion of the long arm of the Y chromosome and review of Y chromosome abnormalities. Am J Med Genet 1985;20:31-42.
4. Buhler EM. A synopsis of the human Y chromosome. Hum Genet 1980;55:145-75.
5. Vergnaud G, Page DC, Simmler M-C, et al. A deletion map of the human Y chromosome based on DNA hybridization. Am J Hum Genet 1986;38;109-24.
6. Andersson M, Page DC, Pettay D, et al. Y; autosome translocations and mosaicism in the aetiology of 45,X maleness: assignment of fertility factor to distal Yq11. Hum Genet 1988;79:2-7.
7. Gal A, Weber B, Neri G, et al. A 45,X male with Y-specific DNA translocated onto chromosome 15. Am J Hum Genet 1987;40;477-88.
8. Guellaen G, Casanova M, Bishop C, et al. Human XX males with Y single-copy DNA fragments. Nature 1984; 307:172-3.
9. Burk Rd, Ma P, Smith KD. Characterization and evolution of a single copy sequence from the human Y chromosome. Mol Cell Biol 1985;5:576-81.
10. Daiger SP, Wildin RS, Su TS. Sequences on the human Y chromosome homologous to the autosomal gene for argininosuccinate synthetase. Nature 1982;298:682-4.
11. Maniatis T, Fritsch ER, Sambrook J, ed. In: Molecular cloning: a laboratory manual. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory, 1982:383.
12. Feinberg AP, Vogelstein B. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 1983;132:6-13.
13. Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 1975;98:503-17.
14. Brosseau GE Jr. Genetic analysis of the male fertility factors on the Y chromosome of *Drosophila melanogaster*. Genetics 1960;45:257.

15. Hardy RW, Tohuyasu KT, Lindsley DL. Analysis of spermatogenesis in *Drosophila melanogaster* bearing deletions for Y chromosome fertility genes. Chromosoma (Berlin) 1981;83:593.
16. Cattanach BM, Pollard CE, Hawkes SG. Sex-reversed mice: XX and XO males. Cytogenetics 1971;10:318-37.
17. McLaren A, Simpson E, Tomonari K, Chandler P, Hogg H. Male sexual differentiation in mice lacking H-Y antigen. Nature 1984;312:552-5.
18. Burgoyne PS, Levy ER, McLaren A. Spermatogenic failure in male mice lacking H-Y antigen. Nature 1986; 320:170-2.
19. Bennett D, Mathieson BJ, Scheid M, et al. Serological evidence for H-Y antigen in *Sxr*, XX sex reversed phenotypic males. Nature 1977;265:255.
20. Interlandi JW, Russell MH, Kirchner F, Rabin D. Genetic and endocrine findings in a 31-year-old 45,X/46,X del(Y) (q12) male. J Clin Endocrinol Metab 1981;53:1047-55.
21. Kosztolanyi G, Trixler M. Yq deletion with short stature, abnormal male development, and schizoid character disorder. J Med Genet 1983;20:393.
22. Martin MJ, Rodriguez MT, Allen V, Abrisqueta JA, Rojo JM. Yq deletion (q 11.21) in a H-Y+ azoospermic male. Clin Genet 1985;28:80.
23. Rivas F, Garcia-Esquivel L, Diaz M, Rivera H, Cantu JM. Cytogenetic evaluation of 163 azoospermics. J Genet Hum 1987;35:291.
24. Montali E, Guazzelli R, Piazzini M, et al. Anomalies du chromosome Y et azoospermie. J Genet Hum 1987; 35:195.
25. Chandley AC, Gosden JR, Hargreave TB, Spowart G, Speed RM, McBeath S. Deleted Yq in the sterile son of a man with a satellited Y chromosome (Yqs). J Med Genet 1989;26:145-53.

Steroid biosynthesis in the Sertoli-Leydig cell tumor: Effects of insulin and luteinizing hormone

Manubai Nagamani, MD, Charles A. Stuart, MD, and Tung Van Dinh, MD
Galveston, Texas

In vitro steroid production by a virilizing Sertoli-Leydig cell tumor of the ovary was studied. For comparison, stromal tissue from the opposite normal ovary was also incubated under similar conditions. The tumor fragments secreted significantly more testosterone (527 ± 168 versus 48 ± 29 pg/mg tissue, $p < 0.001$), androstenedione (1188 ± 400 versus 40 ± 10 pg/mg tissue, $p < 0.001$), and dehydroepiandrosterone (419 ± 132 versus 73 ± 25 pg/mg tissue, $p < 0.004$) than that of normal ovarian stroma. Measurement of steroids in the ovarian venous serum draining the tumor indicated a peripheral ovarian gradient for both Δ^4 and Δ^5 steroids. Incubation of tumor fragments with luteinizing hormone alone resulted in a significant increase in the secretion of androstenedione and dehydroepiandrosterone ($p < 0.05$). Addition of insulin to luteinizing hormone resulted in significantly greater release of androstenedione than that of treatment with luteinizing hormone alone ($p < 0.04$). Addition of insulin had no effect on the release of dehydroepiandrosterone. Luteinizing hormone and insulin, either alone or in combination, failed to produce any change in the secretion of testosterone. We conclude that (1) increased testosterone secretion by Sertoli-Leydig cell tumor resulted from increased availability of precursors from both Δ^4 and Δ^5 pathways; (2) the tumor was responsive to luteinizing hormone with an increase in the secretion of androstenedione and dehydroepiandrosterone; (3) insulin acts synergistically with luteinizing hormone to increase secretion of androstenedione; (4) the tumor has specific binding sites for insulin; and (5) the increased levels of insulin and luteinizing hormone in polycystic ovarian disease may play a role in the pathogenesis of Sertoli-Leydig cell tumor. (Am J Obstet Gynecol 1989;161:1738-43.)

Key words: Arrhenoblastoma, insulin receptors, steroid biosynthesis

Sertoli-Leydig cell tumors, or arrhenoblastomas, are sex-cord stromal tumors of the ovary with testicular-directed differentiation.[1] Testosterone is the principal androgen secreted by these tumors, which are usually discovered during the investigation of androgen excess as manifested by masculinization, hirsutism, and amenorrhea. Opportunities to study the steroid biosynthesis in these tumors have been limited because of their rarity. Study of their pattern of steroidogenesis will help to identify their origin. Predominance of a Δ^4 pathway of steroidogenesis indicates stromal cell origin of the tumor, whereas predominance of the Δ^5 pathway indicates Leydig cell origin. Ullery[2] has speculated that the arrhenoblastoma may be derived from male cells that persist in the medullary portion of the ovary. The histogenesis of these tumors is not known. Sertoli-Leydig cell tumors have been reported in women with polycystic ovarian disease.[3-6] It is possible that the levels of constantly elevated luteinizing hormone (LH) or hyperinsulinism that are present in polycystic ovarian disease play a role in the pathogenesis of Sertoli-Leydig cell tumors of the ovary.

We undertook this study to investigate (1) the pathway of steroid biosynthesis in the Sertoli-Leydig cell tumors by in vitro and in vivo studies; (2) the effect of LH and insulin on in vitro steroidogenesis by the tumor tissue, and (3) whether the tumor has specific binding sites for insulin.

Material and methods

Patient. The patient was a 44-year-old nulliparous woman with a 10-year history of amenorrhea and progressive hirsutism because of which she had to shave daily. Her cycles had been irregular from menarche at age 12. She also had undergone deepening of her voice, temporal balding, and decrease in breast size.

Physical examination revealed a severely hirsute obese woman with male body habitus. She weighed 220 pounds and was 5 feet 1 inch tall. There was coarse dark hair on her face, chest, and abdomen and marked temporal balding. The clitoris was enlarged to 1 cm in

From the Departments of Obstetrics and Gynecology and Medicine, University of Texas Medical Branch.
Supported by Grant No. DHHS-1-R01-CA45181-01A1 from the National Institutes of Health and Clinical Research Center Grant No. RR-73.
Presented at the Thirty-sixth Annual Meeting of the Society for Gynecologic Investigation, San Diego, California, March 15-18, 1989.
Reprint requests: Manubai Nagamani, MD, Department of Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, TX 77550.
6/6/15968

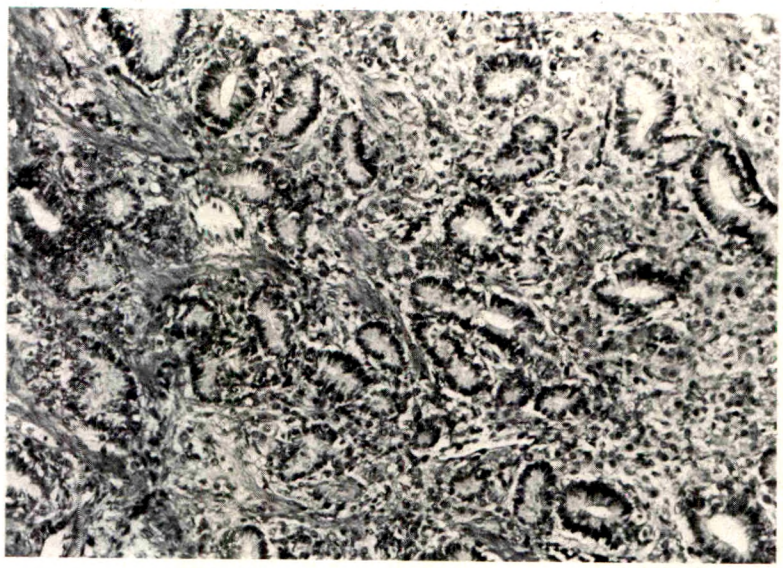


Fig. 1. Microscopic sections of tumor show tubules and prominent interstitial cells.

diameter. Pelvic and ultrasonographic examinations revealed an enlarged right ovary (6.5 × 4 cm); the left ovary was within normal limits.

The patient's serum testosterone level was 420 ng/dl (normal, 30 to 100 ng/dl), dehydroepiandrosterone (DHEA) sulfate was 350 ng/ml (normal, 500 to 3000 ng/ml), LH was 19 mIU/ml, and follicle-stimulating hormone was 10 mIU/ml. An oral glucose tolerance test (GTT) with insulin levels showed a fasting insulin level of 33 μU/ml (normal, 5 to 22 μU/ml). Insulin response to the oral GTT was increased, but glucose levels were normal. Insulin level 1 hour after glucose administration was 310 μU/ml (normal, 20 to 95 μU/ml); at 2 hours, 215 μU/ml (normal, 10 to 50 μU/ml); and, at 3 hours, 146 μU/ml (normal, 3 to 22 μU/ml).

At laparotomy, the right ovary was 7 × 4 × 2 cm. A total abdominal hysterectomy with bilateral salpingo-oophorectomy was performed. Simultaneous blood samples were obtained from the ovarian and peripheral veins at the time of surgery. Histologic examination revealed a well-differentiated Sertoli-Leydig cell tumor of the right ovary. The tumor comprised two major components: small tubules that were lined by a single layer of cells with acidophilic cytoplasm and a proliferation of interstitial Leydig cells (Fig. 1). There was evidence of polycystic ovarian disease in the left ovary and there were nests of luteinized thecal cells in the stroma. The uterus was normal in size; the endometrium was weakly proliferative and showed mild adenomatous hyperplasia and focal dysplastic changes. Six weeks after surgery, the patient's testosterone level was 35 ng/dl.

Tissue culture. Immediately after oophorectomy, a portion of the tissue removed from the center of the tumor was placed in the incubation medium. Under sterile techniques the tissue was sectioned into pieces <2 mm in diameter. All dissections were performed under a laminar flow hood. A portion of the tumor tissue (about 100 mg) was frozen to assess basal steroid content. About 100 mg of tissue was placed with 2 ml of medium in six-well tissue culture plates (Falcon, Becton-Dickinson, Rutherford, N. J.). The culture plates were incubated at 37° C in 5% carbon dioxide in air. For comparison, stromal tissue obtained from the left ovary immediately after its removal was incubated under similar conditions. Dissections of this ovary were carefully performed to obtain only the stromal tissue and to exclude thecal tissue and follicles.

All sections from the tumor were incubated either in medium alone or in medium to which was added LH at 50 ng/ml, insulin at 500 ng/ml, or a combination of the two. These amounts of LH and insulin were selected on the basis of previous dose-response studies reported in experiments with the ovarian stroma.[7, 8] All incubations were done in quadruplicate. Because of a lack of tissue, the stroma from the left ovary was incubated with only incubation medium and no experiments were performed with LH or insulin. After a 4-hour incubation the medium was removed from each well and stored at −72° C until analysis. The tissue sections from each well were weighed at this time, fixed in 10% formalin, and examined histologically with hematoxylin and eosin stain. Tissue fragments were found to be viable with intact cell membranes and the nuclei at the end of incubation.

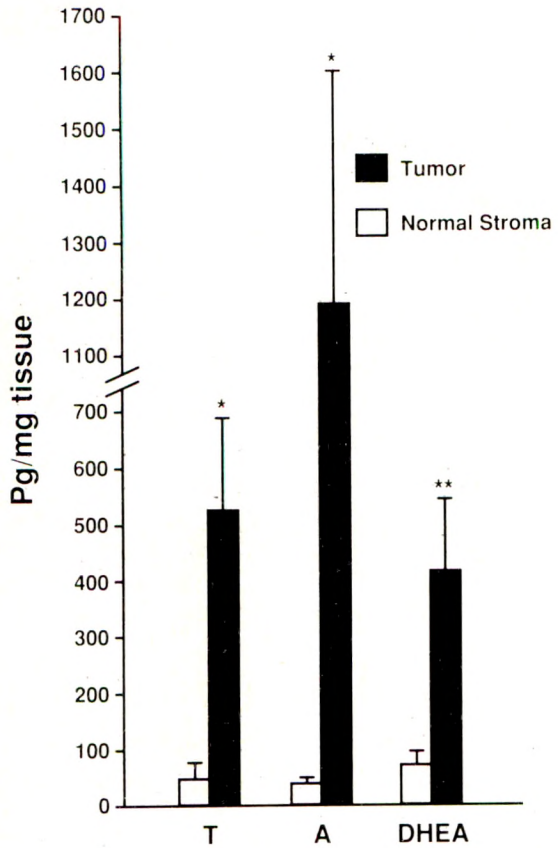


Fig. 2. Testosterone *(T)* androstenedione *(A)*, and dehydroepiandrosterone *(DHEA)* production in vitro by ovarian stromal tissue from left ovary and by Sertoli-Leydig cell tumor (mean ± SD; $n = 4$). *Asterisk*, $p < 0.001$; *double asterisks*, $p < 0.004$.

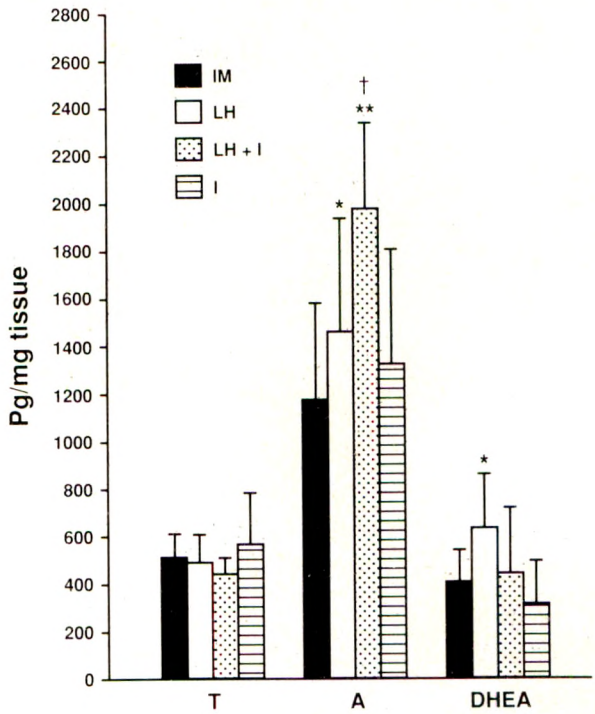


Fig. 3. Effects of LH and insulin on mean (±SD; $n = 4$) testosterone, androstenedione, and DHEA production by Sertoli-Leydig cell tumor tissue in vitro. *IM*, Incubation medium (control); *LH*, LH 50 ng/ml; *I*, insulin 500 ng/ml; *LH + I*, LH 50 ng/ml and insulin 500 ng/ml. *Asterisk*, $p < 0.05$; *double asterisks*, $p < 0.002$ (compared with control); *dagger*, $p < 0.04$ (compared with LH alone). For testosterone, the F ratio was $1.29_{3,12}$ (not significant).

Incubation medium. McCoy's 5a medium with 5% newborn calf serum, penicillin (100 U/ml), streptomycin (100 μg/ml) and L-glutamine (0.29 mg/ml), was used as the medium for all incubations. All media were bubbled in 95% oxygen and sterilized through 0.2 and 0.45 μm filters before use.

Human LH was obtained from the National Hormone and Pituitary Program and human insulin was obtained from Eli Lilly & Co. (Indianapolis, Ind.). McCoy's 5a medium and calf serum were supplied by Gibco (Grand Island, N. Y.). The newborn calf serum did not have detectable levels of LH or insulin.

Studies of insulin binding in the tumor tissue and in the ovarian stroma from 11 normal premenopausal women were performed in accordance with procedures described previously.[9, 10] Serum insulin levels were measured in duplicate by double antibody radioimmunoassay (RIA) as previously described.[10, 11] All steroids were measured by RIA after fractionation by Celite column chromatography as described elsewhere.[12, 13] Serum or incubation medium was extracted three times with three volumes of ether after tritiated tracers were added for recovery calculations. Extracts were dried under nitrogen and subjected to chromatography on micro-Celite columns. The various fractions were collected, dried under nitrogen, and reconstituted with assay buffer. Different volumes were used in the assay and 0.1 ml was used for recovery calculations. All the steroids were measured by specific RIAs as previously described.[12, 13] Recoveries ranged from 70% to 90%. DHEA sulfate was measured by direct RIA.

Steroid secretion by the control ovary and the tumor tissue was compared by the Student t test after log transformation of the data to stabilize heterogenous variances. Steroid production by the tumor tissue under control and different experimental conditions were compared by one-way analysis of variance.

Results

The basal steroid content of the tumor tissue was <5 pg/mg of tissue for all steroids, and the stroma from the left ovary contained <1 pg of steroids per milligram tissue. When incubated for 4 hours, the tumor tissue secreted significantly more testosterone [527 ± 168

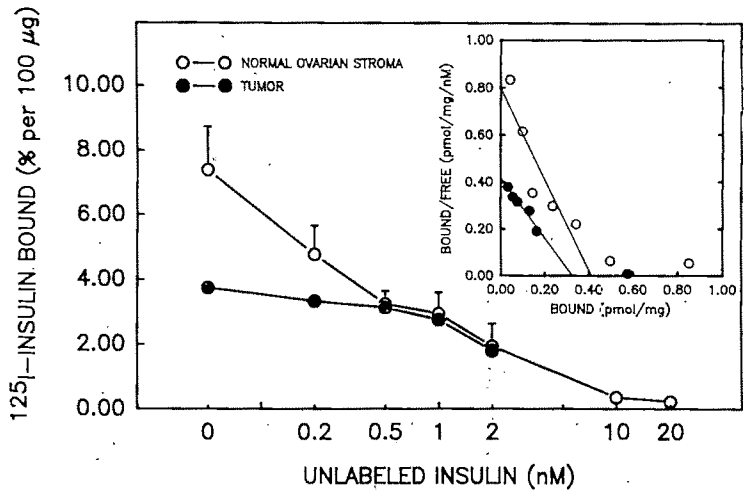


Fig. 4. Insulin binding to membranes from Sertoli-Leydig cell tumor and normal premenopausal ovarian stroma ($n = 11$). Shown are mean (±SE) for studies in normal ovarian stroma. Tumor data points represent means of triplicate determinations. *Inset* shows Scatchard analysis of same data shown in competition curves.

Table I. Peripheral and ovarian vein concentrations of steroids (pg/ml)

Steroid	*Peripheral vein (patient)*	*Right ovarian vein (tumor)*	*Left ovarian vein*	*Normal women (peripheral vein)*
Progesterone	7,407	24,246	5,915	100-350
17OHP	955	80,000	16,941	100-300
17OHΔ⁵P	13,000	110,000	—	1,500-10,000
DHEA	10,105	69,444	3,550	1,600-7,000
Androstenedione	3,325	66,000	7,188	340-1,650
Testosterone	4,337	43,956	6,190	500-900
Dihydrotestosterone	92	290	62	30-220
DHEA sulfate (ng/ml)	350	380	300	500-3,000
17β-Estradiol	26	1,113	51	50-200

(±SD) versus 48 ± 29 pg/mg tissue, $p < 0.001$], androstenedione (1188 ± 400 versus 40 ± 10 pg/mg tissue, $p < 0.001$), and DHEA (419 ± 132 versus 73 ± 25 pg/mg tissue, $p < 0.004$) than that of the ovarian stroma from the opposite ovary (Fig. 2).

Incubation of tumor fragments with LH alone resulted in a significant increase in the secretion of androstenedione (1188 ± 400 versus 1482 ± 483 pg/mg tissue, $p < 0.05$). The addition of insulin alone resulted in a small statistically insignificant increase in the secretion of androstenedione (1349 ± 493 pg/mg tissue). The addition of LH and insulin resulted in a significantly greater release of androstenedione (1956 ± 373 pg/mg tissue) compared with the increase after treatment with LH alone ($p < 0.04$). Incubation of the tumor tissue with LH resulted in a significant increase in DHEA secretion, as compared with the increase after incubation with medium alone (419 ± 132 versus 626 ± 219 pg/mg tissue, $p < 0.05$). The addition of insulin, whether alone or with LH, had no effect on DHEA release (Fig. 3). LH and insulin, either alone or in combination, failed to produce any change in testosterone secretion.

Peripheral and ovarian venous steroid levels are shown in Table I. Peripheral levels of progesterone, 17α-hydroxyprogesterone (17OHP), 17α-hydroxypregnenolone (17OHΔ⁵P), DHEA, androstenedione, and testosterone were increased. Levels of all these steroids were several times higher in the right ovarian venous serum draining the tumor than levels in the left ovarian venous serum. Thus there was a peripheral ovarian gradient for both Δ^4 and Δ^5 steroids. Concentrations of 17OHΔ⁵P and DHEA in the ovarian vein serum were relatively higher than those of progesterone and 17OHP. Although peripheral levels were in the normal range, 17β-estradiol and dihydrotestosterone concentrations in the ovarian vein serum draining the tumor were increased, which indicates the presence of minimal aromatase and 5α-reductase activity in the tumor.

Binding studies with insulin labeled with iodine 125 showed the presence of specific insulin binding sites in

the tumor tissue. The percentage of specific total binding of ^{125}I insulin in tumor tissue was 3.7%/100 μg protein, which is below the normal range seen in premenopausal ovarian stroma (4.3% to 13.0%/100 μg protein). Fig. 4 shows the competition curves for binding of ^{125}I insulin to ovarian microsomal membranes prepared from ovarian stroma from 11 premenopausal women and the membranes from tumor tissue, in the absence and presence of unlabeled insulin at the concentrations shown on the X axis. Shown are the mean and SE for studies in ovarian membranes from 11 premenopausal women. The tumor data points represent the means of triplicate determinations. The Scatchard plots of the high-affinity segments of the data (first five points) were parallel (inset of Fig. 4), which indicates that the difference in insulin binding was a result of a difference in the concentrations of receptors rather than differences in affinity. The affinity constants for the specific high-affinity insulin receptors from these preparations were 1.28×10^9L/mol for the tumor and 2.7 ± 0.5 gm $\times 10^9$L/mol for the normal ovarian stroma.

Comment

There is considerable controversy about the origin of Sertoli and Leydig cells in the arrhenoblastoma of the ovary. Inasmuch as the hilum of the normal ovary contains cells that resemble testicular Leydig cells, it has been speculated that arrhenoblastomas and hilus cell tumors of the ovary arise from these cells. Kurman and colleagues[14] studied the site production of testosterone and 17β-estradiol in Sertoli-Leydig cell tumor tissue with the use of immunohistochemical techniques. They observed that primitive spindle cells, which resemble cells found in the embryonic gonad, are present in Sertoli-Leydig cell tumor and suggested that these cells could be precursors of both Sertoli and Leydig cells. Ultrastructural studies by Genton[15] suggest that the arrhenoblastoma arises from ovarian stroma. The pathogenesis of Sertoli-Leydig cell tumors from these cells is also not well understood. An arrhenoblastoma is often seen in association with polycystic ovarian disease as it was in our patient. Most patients with an arrhenoblastoma have, as did our patient, a long history of obesity and irregular menstrual cycles that preceeded the development of signs of virilization.[16] Our patient had hyperinsulinemia with normal glucose levels, which indicated insulin resistance. Moreover, the tumor removed from our patient had specific binding sites for insulin and responded to insulin and LH with an increase in the release of androstenedione. Sternberg and colleagues[17] observed that the Leydig-like hilus cells of the ovary became more prominent and showed evidence of mitosis when treated with gonadotropins. It is possible that the elevated LH levels and hyperinsulinemia of polycystic ovarian disease may play a role in the histogenesis and development of these tumors.

Both the in vivo and in vitro studies of the tumor indicate that the increase in testosterone secretion resulted from increased availability of precursors from both the Δ^4 and the Δ^5 pathways. The relatively higher levels of 17OHΔ^5P and DHEA in the ovarian venous serum, as compared with levels of progesterone and 17OHP, indicate the predominance of the Δ^5 pathway. These findings are in agreement with the in vitro studies of Maschler and colleagues[18] who observed a decreased conversion of Δ^5P to progesterone and 17OHP in an arrhenoblastoma. With the use of 7^3H-pregnenolone as a substrate, Kase and Conrad[19] observed greater incorporation of radioactivity in DHEA than in progesterone, and concluded that the Δ^5-3βOH pathway was the predominant pathway of steroidogenesis in this tumor. With the use of ^{3}H-Δ^5P and ^{14}C-progesterone as substrates, YoungLai and colleagues[20] observed that arrhenoblastoma tissue used ^{3}H-Δ^5P preferentially via the Δ^5 pathway in the synthesis of androstenedione, which is the direct precursor of testosterone. Although predominance of the Δ^5 pathway indicates a certain similarity between arrhenoblastoma and testicular Leydig cells, the associated increase in Δ^4 precursors that we found supports the contention of Genton and colleagues[15] that an arrhenoblastoma arises from ovarian stromal cells. We believe arrhenoblastomas have a dual origin and probably derive both from ovarian hilus cells and from steroidogenically active stromal cells.

We found no report of previous studies in vitro of the effects of insulin on steroidogenesis in Sertoli-Leydig cell tumors. Because insulin did not affect DHEA secretion, it probably stimulates only the Δ^4 pathway of steroidogenesis. The decreased concentration of insulin receptors in the tumor, as compared with concentrations in normal ovarian stroma, could be a result of down regulation in response to the elevated insulin levels we found in our patient. Because only a small percentage of insulin receptors are occupied at insulin concentrations that have maximal biologic effects, a decrease in receptor binding would not be expected to change the maximal effect of insulin.[21]

Among previous studies of Sertoli-Leydig cell tumor, reports on effects of LH on testosterone secretions in vitro are conflicting. Specific LH binding sites have been demonstrated in Leydig cell tumors of the ovary.[18] Brunsted et al.[22] observed that in doses ≤100 mIU/ml, human chorionic gonadotropin failed to stimulate testosterone secretion by a Sertoli-Leydig cell tumor in vitro. However, Lamberts et al.[23] observed a doubling of testosterone secretion by dispersed Sertoli-Leydig cell tumor cells when 2 μg/ml of LH was added to the medium. It is of interest that, in our patient, there was no increase in testosterone secretion with LH treatment

but there was an increase in androstenedione secretion. This finding could indicate that the final step of the synthesis of testosterone, namely the conversion of androstenedione to testosterone by 17β-hydroxysteroid dehydrogenase, is probably not LH-dependent. Kondo and colleagues[24] did not observe stimulation of 17β-hydroxysteroid dehydrogenase activity in the hypophysectomized rat ovary when treated with LH. In another study,[25] in vitro treatment of *Xenopus* follicles with human chorionic gonadotropin induced a 40% decrease of 17β-hydroxysteroid dehydrogenase activity. The lack of increase in testosterone secretion could also be a result of the limitations of the in vitro system. With in vitro studies, conditions and availability of cofactors may not be the same as in physiologic conditions in vivo.

Our results indicate the following conclusions:

1. Increased testosterone secretion by the Sertoli-Leydig cell tumor results from increased availability of precursors from both Δ^4 and Δ^5 pathways.

2. The tumor responded to LH with an increase in release of androstenedione and DHEA.

3. Insulin had no effect on DHEA release—a result that may indicate that insulin stimulates steroidogenesis in the tumor only via the Δ^4 pathway.

4. The tumor had specific binding sites for insulin.

5. The increased insulin and LH levels that are present in polycystic ovarian disease may play a role in the pathogenesis of Sertoli-Leydig cell tumors.

REFERENCES

1. Young RH, Scully RE. Ovarian Sertoli-Leydig cell tumors. Am J Surg Pathol 1985;9:543-69.
2. Ullery JG, Boutselis JG. The gross pathology, diagnosis and clinical management of ovarian enlargement. Obstet Gynecol Surv 1959;14:635.
3. Betson RJ, Marshall RA, Chiffele TL. Scleropolycystic (Stein-Leventhal) ovary with an arrhenoblastoma of the opposite gonad. AM J OBSTET GYNECOL 1962;83:93-100.
4. Case records of the Massachusetts General Hospital. N Engl J Med 1965;272:365-71.
5. Zourlas PA, Jones HW. Stein-Leventhal syndrome with masculinizing ovarian tumors. Obstet Gynecol 1969;34: 861-6.
6. Cruikshank PD, Chapler FK. Arrhenoblastomas and associated ovarian pathology. Obstet Gynecol 1973;43:539-43.
7. McNatty KP, Anastasia M, Osathanondh R. Effects of luteinizing hormone on stercidogenesis by thecal tissue from human ovarian follicles in vitro. Steroids 1980; 36;53.
8. Barbieri RL, Makris A, Randall WR, Daniels G, Kistner RW, Ryan KJ. Insulin stimulates androgen accumulation in incubations of ovarian stroma from women with hyperandrogenism. J Clin Endocrinol Metab 1986;62:904.
9. Stuart CA. Phylogenetic distance from man correlates with immunologic cross-reactivity among liver insulin receptors. Comp Biochem Physiol 1986;84B:167.
10. Nagamani M, Hannigan EV, Dinh TV, Stuart CA. Hyperinsulinemia and stromal luteinization of the ovaries in postmenopausal women with endometrial cancer. J Clin Endocrinol Metab 1988;67:144.
11. Soeldner JS, Slone D. Critical variables in the radioimmunoassay of serum insulin using the double antibody technique. Diabetes 1965;14:771.
12. Parker CR, Ellegood JO, Mahesh VB. Methods for multiple steroid radioimmunoassay. J Steroid Biochem 1975; 6:1.
13. Nagamani M, McDonough PG, Ellegood JO, Mahesh VB. Maternal and amniotic fluid steroids throughout human pregnancy. AM J OBSTET GYNECOL 1979;134:674.
14. Kurman RJ, Andrade D, Goebelsmann U, Taylor CR. An immunohistological study of steroid localization in Sertoli-Leydig tumors of the ovary and testis. Cancer 1978;42: 1772-83.
15. Genton CY. Ovarian Sertoli-Leydig cell tumors. Arch Gynaec 1980;230:49-75.
16. Pedowitz P, O'Brien FB. Arrhenoblastoma of the ovary. Obstet Gynecol 1960;16:62-77.
17. Sternberg WH, Segaloff A, Cornelia J Gaskill. Influence of chorionic gonadotropin on human ovarian hilus cells (Leydig like cells). J Clin Endocrinol Metab 1952;13:139-53.
18. Maschler I, Weidenfeld I, Fleischner B, et al. Seroidogenesis in virilizing ovarian tumor. Clin Endocrinol (Oxf) 1975;4:427.
19. Kase N, Conrad SH. Steroid synthesis in abnormal ovaries. AM J OBSTET GYNECOL 1964;90:1251-61.
20. YoungLai EV, Richmond H, Atyeo R, Johnson FL. Arrhenoblastoma: in vivo and in vitro studies. AM J OBSTET GYNECOL 1973;116:401-7.
21. Konot, Barham FW. The relationship between the insulin binding capacity of fat cells and the cellular response to insulin studies with intact and trypsin treated fat cells. J Biol Chem 1971;426:6210.
22. Brumstead JR, Chapitis J, Riddick D, Gibson M. Norethindrone inhibition of testosterone secretion by an ovarian Sertoli-Leydig cell tumor. J Clin Endocrinol Metab 1987; 65:194-7.
23. Lamberts SWJ, Timmers JM, Oorterom R, Verlium T, Rommerts FG, DeJong FH. Testosterone secretion by cultured arrhenoblastoma cells: suppression by a luteinizing hormone-release hormone agonist. J Clin Endocrinol Metab 1982;54;450.
24. Kondo K, Aono T, Furubayashi Y, Terada N, Matsumoto K. Hormonal regulation of activities of 4-ene-5 alpha-reductase and 17 beta-ol-dehydrogenase in adult androgen-sterilized rat ovaries. J Steroid Biochem 1980; 13:703-5.
25. Thibier C, Özon R. Regulation of 17 beta-hydroxysteroid oxydoreductase of the *Xenopus leavis* follicle by gonadotropin and prolactin. Gen Comp Endocrinol 1983;49: 352-7.

CORRESPONDENCE

Fetomaternal hemorrhage—Possible cause of fetal distress and death

To the Editors:

We read with interest the article by Stafford et al. (Stafford PA, Biddinger PW, Zumwalt RE. Lethal intrauterine fetal trauma. AM J OBSTET GYNECOL 1988;159:485-9). In their study they concluded on the basis of eight cases that lethal placental or direct fetal injury may occur with even minor maternal injury. Indeed, abruptio placentae of a severe degree may cause fetal death acutely and direct fetal trauma (i.e., skull fracture may also result in intrauterine fetal death); however, the authors neglect to mention fetomaternal hemorrhage as a possible cause of fetal distress and fetal death.

Rose et al.[1] found significant fetomaternal hemorrhage 28% of the time in gravid women who had undergone recent trauma. Fetal death, anemia, and paroxysmal atrial tachycardia occurred in the group of patients who had fetomaternal hemorrhage. Significant fetomaternal hemorrhage may also be associated with abruptio placentae.[2] The degree of the fetomaternal hemorrhage may be quantitated with Mollison's formula.[3]

We concur with Rose et al. that fetomaternal hemorrhage should be assumed in all cases of maternal trauma. Kleihauer-Betke stains are useful in the detection of fetomaternal hemorrhage and may aid in the management of such cases. Lastly, let us beware that insignificant maternal trauma may lead to significant medicolegal damage as a result of unsuspected fetomaternal hemorrhage.

Michael S. Cardwell, MD
Scott W. Snyder, MD
Department of Obstetrics and Gynecology
University of Missouri-Columbia
1 Hospital Drive
Columbia, MO 65212

REFERENCES

1. Rose PG, Strohm PL, Zuspan FP. Fetomaternal hemorrhage following trauma. AM J OBSTET GYNECOL 1985; 153:844.
2. Cardwell MS. Ultrasound diagnosis of abruptio placentae with fetomaternal hemorrhage. AM J OBSTET GYNECOL 1987;157:358.
3. Mollison PL. Quantitation of transplacental hemorrhage. Br Med J 1972;3:31.

Reply

To the Editors:

In our article we focused on the extent of maternal, fetal, and placental injuries associated with motor vehicle accidents. In every case, some type of placental abnormality was detected, usually abruption or infarction. We regard fetomaternal hemorrhage as a potential complication of placental injury and a mechanism for fetal distress and fetal death. Although we did not specifically discuss this mechanism, we readily acknowledge its possibility and agree that the Kleihauer-Betke test is useful in the detection of fetomaternal hemorrhage. Nevertheless, we are pleased that Drs. Cardwell and Snyder seem to agree with our major conclusion that lethal intrauterine fetal trauma can occur even though maternal injuries are minor or insignificant.

Paul W. Biddinger, MD
Patricia A. Stafford, MD
Ross E. Zumwalt, MD
Department of Pathology and Laboratory Medicine
College of Medicine
University of Cincinnati Medical Center
Mail Location 529
231 Bethesda Ave.
Cincinnati, OH 45267

Dosage and duration differences lead to diverse conclusions

To the Editors:

The very interesting article (Smarr SC, Wing R, Hammond MG. Effect of therapy on infertile couples with antisperm antibodies. AM J OBSTET GYNECOL 1988;158:969-73) raises some important questions. Their well-documented results indicate that the use of prednisone medication in patients who have an infertility problem and who also have shown the presence of sperm antibody activity, was not effective in the improvement of pregnancy rates. Whereas this conclusion is quite valid for the management that they used, the conclusion may be misleading, especially because they seem to be contrasting their results with the highly successful results that we observed with the use of corticosteroid medication in such cases. Nonetheless, their conclusion is quite reasonable, as was ours; this difference in efficacy is a consequence of the tremendous difference in dosage of the steroid and also in the duration.

In our first report of the use of steroid for suppression of the antibody problem,[1] we suggested the use of 96 mg per day of methylprednisolone (Medrol) for a period of 7 days. This was subsequently modified to be a repeated regimen, with the same 7-day course given at monthly intervals. Durations up to 6 months might be used. A large group was studied over several years, and a success rate of 44% in terms of pregnancies achieved was reported.[2] Others also used this intermittent, *high-dose method,* as it became termed, with very satisfying results, as has been reviewed.[3] However, Smarr et al. used a *low-dose method* and gave only 15 mg per day of the steroid prednisone for a duration ≤3 months. This procedure has some similarity to the ear-

lier work of Hendry and his colleagues,[4] although they often continued their regimen (15 mg per day of prednisone) for as long as 12 months. They found that this method could be successful, although not as often as the high-dose method. It may be important to note that Hendry et al. were treating men, and women were treated in the report by Smarr et al. Nonetheless, there may be some importance in a treatment duration longer than 3 months.

We also must consider the methods for the detection of sperm antibody. Smarr et al. have used a particular method of passive hemagglutination[5] and a method of cytotoxicity,[6] both developed by Mathur and colleagues. Whereas these methods may be reasonable and useful, most of the earlier work has been done with the standard methods of sperm agglutination. There has not been any extensive comparison between these two groups of methods. All these methods have been extensively reviewed and described.[7,8] I urge that future studies in the detection of this antibody activity be done with the well-established agglutination techniques to allow comparison with the former findings.

I should also comment on the value of the agglutination techniques. If these are properly done with regard to serum dilution levels, control sera, length of incubation, nature of the reading for minimal reactivity, and repetitions of each test, they have proved to be as reliable and reproducible as any other method.[9] Smarr et al. quote Mettler et al.,[10] "Some fertile women have been shown to have agglutination antibodies; however, immobilizing and cytotoxic antibodies are found almost exclusively in infertile couples." However, in our work published in this JOURNAL many years ago,[11] we showed that very few women from fertile couples had agglutinating antibody and these were at low titers, whereas a much higher number of infertile women showed this activity and at higher titers.

Smarr et al. also quote Hekman and Rümke,[12] "Agglutination assays are less specific in that many factors other than antibodies . . . are known to cause sperm agglutination." This philosophic concept should also be laid to rest as being rather unlikely in the vast majority of serum studies because there are, in fact, low incidences of positive results when sera from fertile individuals are tested; it would be difficult to see why the perturbing and nonspecific factors should only occur in sera from people afflicted with infertility.

Sidney Shulman, PhD
Sperm Antibody Laboratory
Fertility Antibody Diagnostics, Inc.
945 West End Ave.
New York, NY 10025

REFERENCES

1. Shulman S. Treatment of immune male infertility with methylprednisolone. Lancet 1976;2:1243.
2. Shulman JF, Shulman S. Methylprednisolone treatment of immunologic infertility in the male. Fertil Steril 1982; 38:591-9.
3. Shulman S. Therapy of immunological infertility. EOS 1986;2:95-7.
4. Hendry WF, Stedronska J, Hughes L, Cameron KM, Pugh RCB. Steroid treatment of male subfertility caused by antisperm antibodies. Lancet 1979;2:498-500.
5. Mathur S, Williamson HO, Landgrebe SC, Smith CL, Fudenberg HH. Application of passive hemagglutination for the evaluation of antisperm antibodies and a modified Coombs' test for detecting male autoimmunity to sperm antigens. J Immunol Methods 1979;30:381-93.
6. Mathur S, Williamson HO, Derrick FC, et al. A new microassay for spermocytotoxic antibody: comparison with passive hemagglutination assay of antisperm antibodies in couples with unexplained infertility. J Immunol 1981; 126:905-9.
7. Shulman S. Human sperm antibodies and their detection. In: Rose NR, Friedman H, eds. Manual of clinical laboratory immunology. 3rd ed. Washington: American Society for Microbiology. 1985:771.
8. Shulman S. Autoimmune aspects of human infertility. in vivo 1988;2:57-60.
9. Shulman S. Sperm antibodies detected by classical methods. In: Perspectives in immunoreproduction. Mathur S, Fredericks CM, eds. New York: Hemisphere Publishing Corporation, 1988:43-60.
10. Mettler L, Gradl T, Scheidel P. Humoral and cellular response to sperm components. In: Hafez ESE, ed. The human semen and fertility regulation in the male. St. Louis: CV Mosby, 1978:268-75.
11. Shulman S, Jackson H, Stone ML. Antibodies to spermatozoa. VI. Comparative studies on sperm agglutination activity in groups of infertile and fertile women. AM J OBSTET GYNECOL 1975;123:139-44.
12. Hekman A, Rümke P. Seminal antigens and autoimmunity. In: Hafez ESE, ed. The human semen and fertility regulation in the male. St. Louis: CV Mosby, 1976:245-57.

Reply

To the Editors:

I would like to respond to Dr. Shulman's insightful comments with regard to our article on treatment of antisperm antibodies. Dr. Shulman has raised several important questions with regard to the diagnosis and treatment of sperm antibody related infertility.

The first question has to do with the appropriate assessment of the results of treatment. In our article we report pregnancy rates that varied between 32% and 48% for patients who received prednisone or other forms of treatment, excluding in vitro fertilization. This "pregnancy rate" is a raw pregnancy rate that determines the number of patients who conceived but does not introduce a factor of time. Dr. Shulman[1] reports a similar pregnancy rate in his treatment groups, again, uncorrected for time. Both studies would benefit from life table analysis, which could report a per cycle pregnancy rate and would eliminate the variable of varying length of treatment and length of follow-up. It is of interest that Dr. Shulman's pregnancy rate is similar to ours but is also similar to our control group of untreated patients. It is important in all studies of infertility treatment to identify and follow up a control group of patients who do not receive treatment to demonstrate a benefit of treatment. There is as yet no controlled study that shows benefit for methylprednisone therapy.

The second area of concern has to do with the di-

agnosis of antisperm antibodies and indeed this is an area of active controversy. Each center has antibody testing procedures that they prefer. A number of methods are used and new methods for the detection of antibodies appear on a regular basis. It is difficult to make a case that one method is better than another. It is most important that the method selected be adequately standardized with local normal populations and performed with care. One of the difficulties in the evaluation of the literature is the fact that no center performs all tests; thus comparison of titers and test results is not possible.

Apparently it was not clear from our article that we treated both male and female partners in our study, treating whichever partner was antibody positive. However, the data were not divided along these lines.

To clarify our statement that agglutinating assays are less specific because of other factors that cause positive sperm agglutination, we refer only to seminal plasma. Males with infections will frequently have positive antibody titers in the semen.

I hope that our article and this discussion will stimulate others to design appropriate evaluation of antisperm antibody treatments that involve the use of a control group, either a well-standardized and widely used assay or multiple antisperm antibody assays, and life table analysis of the results.

Mary G. Hammond, MD
Department of Obstetrics and Gynecology
Old Clinic Building 226-H
The University of North Carolina at Chapel Hill
Chapel Hill, NC 27514

REFERENCE

1. Shulman JF, Shulman S. Methylprednisolone treatment of immunologic infertility in the male. Fertil Steril 1982; 38:591.

Number clarification requested

To the Editors:

I read with interest the article by Wilkins et al. (Wilkins IA, Lynch L, Mehalek K, Berkowitz GS, Berkowitz RL. Efficacy and side effects of magnesium sulfate and ritodrine as tocolytic agents. Am J Obstet Gynecol 1988;159:685-9). The authors attempt to assess the relative efficacy and side effects of these two agents (given alone or in combination). I have a specific question with regard to the number of patients presented in Tables III and IV. In Table IV, the "Total no. of patients receiving drugs" in the magnesium sulfate category is listed as 70. However, if the number of patients listed in Table II (n = 66) and the number of patients who received magnesium sulfate as a second-line drug in Table III (n = 6) are combined, the total number should be 72. Similarly, the total number of patients who received both drugs intravenously in Table IV is listed as 13. However, from Table III, the total number of patients who received both drugs together intravenously should be 11. It would be very helpful if the authors would clarify these discrepancies and, if necessary, re-evaluate the statistical analysis of their data, should the numbers reported be in error.

Although we agree that systemic tocolysis is associated with potentially serious side effects, our data concerning combination therapy[1,2] do not suggest that the incidence of side effects from combination therapy is markedly different from that seen with ritodrine alone. Continuing experience with this type of treatment with the involvement of greater numbers of patients might answer the questions raised.

Christos G. Hatjis, MD
Riverside Hospital
3535 Olentangy River Road
Columbus, OH 43214

REFERENCES

1. Hatjis CG, Nelson LH, Meis PJ, Swain M. Addition of magnesium sulfate improves effectiveness of ritodrine in preventing premature delivery. Am J Obstet Gynecol 1984; 150:142-50.
2. Hatjis CG, Swain M, Nelson LH, Meis PJ, Ernest JM. Efficacy of combined administration of magnesium sulfate and ritodrine in the treatment of premature labor. Obstet Gynecol 1987;69:317-22.

Reply

To the Editors:

We are greatly flattered by the close attention that Dr. Hatjis paid to our article. He is entirely correct that there is an error in Table III. Although 10 patients who received ritodrine therapy required second-line therapy, 4 of those received magnesium sulfate and 6 received both drugs together. The next column regarding delivery should also be reversed. All of the patients in both groups achieved a 48-hour delay. The remainder of the table is correct, as is Table IV. Because of the small numbers in Table III, we did not perform any statistical analysis; therefore the remainder of our results and the Comment section are unchanged.

Of course, we are aware of Dr. Hatjis' work with regard to combination tocolytic therapy and agree wholeheartedly that more work needs to be done to clarify this issue.

Isabelle Wilkins, MD
Lauren Lynch, MD
Department of Obstetrics and Gynecology and Reproductive Science
Mount Sinai Medical Center
Mount Sinai School of Medicine
One Gustave L. Levy Place
New York, NY 10029

Pulmonary artery agenesis

To the Editors:

The title of the article recently published by Stiller et al. (Stiller RJ, Soberman S, Turetsky A, Lockwood C, Haddad R. Agenesis of the pulmonary artery: An unusual cause of dyspnea in pregnancy. Am J Obstet

GYNECOL 1988;158:172-3) attracted my attention because I was unfamiliar with the entity of pulmonary artery agenesis. However, in describing their patient's lesion, the authors stated that the computerized tomographic scan revealed the main right pulmonary artery and proximal descending portions to be "hypoplastic" compared with the left side. The authors reviewed findings in other reports of patients with pulmonary artery *agenesis* and referred to their own patient's lesion as such.

In 1978, a working group on nomenclature published proposed definitions to describe abnormal biologic developmental processes.[1] Agenesis is defined as "absence of a part of the body due to an absent anlage (primordium)"; hypoplasia is "underdevelopment of normally structured and formed organism, organ or tissue." The definition of agenesis is essentially the same in *Dorland's Medical Dictionary*, 25th edition, 1974, and *Webster's Seventh New Collegiate Dictionary*, 1972. In addition, the references cited by Stiller et al. clearly describe "absence" of a pulmonary artery rather than hypoplasia.[2, 3]

Whereas other dictionaries may define agenesis somewhat differently, I believe that the medical community uses the terms as defined above and that it is useful to maintain the distinction between agenesis and hypoplasia.

Marion S. Verp, MD
Department of Obstetrics and Gynecology
The University of Chicago
5841 S. Maryland Ave.
Chicago, IL 60637

REFERENCES

1. Benirschke K, Lowry RB, Opitz JM, Schwarzacher HG, Spranger JW. Developmental terms—some proposals: first report of an international working group. Am J Med Genet 1979;3:297.
2. Gluck MC, Moser KM. Pulmonary artery agenesis. Diagnosis with ventilation and perfusion scintiphotography. Circulation 1970;41:859.
3. Buhler RC, Carson P, Traks E, Levene A, Gillespie D. Absent right pulmonary artery. Problems in diagnosis and management. Am J Med 1969;46:64.

Other routes charted after description of adventurous journey of CA 125

To the Editors:

With great interest I read the article by Quirk et al. (Quirk JG, Brunson GL, Long CA, et al. CA 125 in tissues and amniotic fluid during pregnancy. AM J OBSTET GYNECOL 1988;159:644-9). I agree with the authors that the decidua is one of the sites of CA 125 synthesis. However, to "explain the distribution of CA 125 in maternal blood, decidua, and amniotic fluid during gestation" a hypothesis describing a rather adventurous journey of CA 125 through the fallopian tubes to the peritoneal cavity is presented. This mechanism of tubal reflux was published earlier by others[1] and served as an explanation for elevated serum levels during menstruation. With the aforementioned hypothesis, raised maternal CA 125 concentrations that occur after the first trimester of gestation[2] can hardly be explained.

A wide distribution of CA 125 in benign and malignant tissues and body fluids has been reported.[3-6] Furthermore, except in ovarian carcinoma, elevations in the serum of women with different benign[1, 2, 5] and malignant[5, 6] disorders were reported. In agreement with Fleuren et al.[7] it is concluded that in malignant tumors CA 125 gains access to the circulation by a leakage of the antigen. In addition, a diffusion of antigen from body fluids (ascites, cervical mucus, amniotic fluid) into veins and lymph vessels during infiltrative growth, inflammation, and gestation is conceivable.

Thus elevations of CA 125 in patients with advanced cervical and endometrial carcinoma,[6] carcinoma and cirrhosis of the liver,[5] and pelvic inflammation[2] can be explained. Consistent with this assumption would be an antigen transfer into the maternal circulation from the amniotic fluid and the decidua.

L. C. Fuith, MD
Department of Obstetrics and Gynecology
University of Innsbruck
Anichstrasse 35
A-6020 Innsbruck, Austria

REFERENCES

1. Mastropaolo W, Fernandez Z, Miller EL. Pronounced increases in the concentration of an ovarian tumor marker, CA-125, in serum of a healthy subject during menstruation. Clin Chem 1986;32:2110-1.
2. Halila H, Stenman UH, Seppälä M. Ovarian cancer antigen CA 125 levels in pelvic inflammatory disease and pregnancy. Cancer 1986;57:1327-9.
3. Kabawat SE, Bast RC Jr, Bhan AK, Welch WR, Knapp RC, Colvin RB. Tissue distribution of a coelomic-epithelium-related antigen recognized by the monoclonal antibody OC 125. Int J Gynecol Pathol 1983;2:275-85.
4. De Bruijn HW, van Beeck Calkoen-Carpay T, Jager S, Duk JM, Aalders JG, Fleuren GJ. The tumor marker CA 125 is a common constituent of normal cervical mucus. AM J OBSTET GYNECOL 1986;154:1088-91.
5. Bergmann JF, Bidart JM, George M, Beaugrand M, Levy VG, Bohuon C. Elevation of CA 125 in patients with benign and malignant ascites. Cancer 1987;59:213-7.
6. Fuith LC, Daxenbichler G, Dapunt O. CA 125 in the serum and tissue of patients with gynecological disease. Arch Gynecol Obstet 1987;241:157-64.
7. Fleuren GJ, Nap M, Aalders J, Trimbos JB, de Bruijn HWA. Explanation of the limited correlation between tumor CA 125 content and serum CA 125 antigen levels in patients with ovarian tumors. Cancer 1987;60:2437-42.

Reply

To the Editors:

We appreciate Dr. Fuith's thoughtful reading of our article and agree that during infiltrative growth and inflammation, CA 125 likely gains access to the circulation directly from its source. Indeed, the elevated levels of CA 125 found in maternal serum in the first trimester may simply reflect infiltrative growth of the

immature placenta within the decidua. The "adventurous journey" that we hypothesized for CA 125 in pregnancy needs to be tested, but we were pleased to learn that Bast thinks "tubal reflux" is a possible mechanism for the rise in serum CA 125 observed during menses.[1]

With respect to understanding the biologic function of CA 125, its method of entry into the circulation is probably of little significance. However, we do not think nor do the available data support the notion that CA 125 simply diffuses into the maternal serum from the amniotic fluid and the decidua. Rather, it seems that for most of pregnancy, CA 125 of putative decidual origin gains access to the amniotic fluid compartment and is excluded from the maternal circulation.[2-4] Ultimately, it will be important to discover why this large-molecular-weight glycoprotein of decidual origin is found in such high concentrations in amniotic fluid throughout pregnancy.

J. Gerald Quirk, Jr., MD, PhD
Timothy J. O'Brien, PhD
Department of Obstetrics and Gynecology
University of Arkansas for Medical Sciences
4301 W. Markham
Mail Slot 518
Little Rock, AK 72205-7199

REFERENCES

1. Mastropaolo W, Fernandez Z, Miller EL. Pronounced increases in the concentration of an ovarian tumor marker, CA-125, in serum of a healthy subject during menstruation. Clin Chem 1986;32:2110-1.
2. Niloff JM, Knapp RC, Schaetzl E, Reynolds C, Bast RC. CA 125 antigen levels in obstetric and gynecologic patients. Obstet Gynecol 1984;64:703-7.
3. Takahashi K, Yamane Y, Yoshino K, Shibukawa T, Matsunaga I, Kitao M. Studies in serum CA 125 levels in pregnant women. Acta Obstet Gynaecol Jpn 1985;37:1931-4.
4. Seki K, Kikuchi Uesato T, Kato K. Increased serum CA 125 levels during the first trimester of pregnancy. Obstet Gynecol Scand 1986;65:583-5.

Perinatal death as a result of social factors

To the Editors:

The perinatal death rate of normally formed infants at or after term pregnancy as a result of social conditions is nearly 40% in the article by Delke et al. (Delke I, Hyatt R, Feinkind L, Minkoff H. Avoidable causes of perinatal death at or after term pregnancy in an inner-city hospital: medical versus social. AM J OBSTET GYNECOL 1988;159:562-6). The authors conclude, "It would seem reasonable to assume that efforts to improve perinatal mortality rates will need to be played out in a broader arena than the hospital. Indeed, these efforts may be dependent on society's willingness to address the problem of endemic poverty."

The social problems that need to be addressed go beyond education, which the authors also advocate, and elimination of poverty. Substance abuse, pregnancy in teenagers, multiparity much greater than three or four (which should satisfy the fictional parity of 2.2 required for population maintenance and account for those who remain childless either by choice or infertility), pregnancy in the single unemployed mother, pregnancy in the woman with chronic life-shortening disease, and pregnancies in others unable to provide adequate nourishment, clothing, shelter, education, and love until the child reaches maturity all need to be addressed. An ounce of contraceptive prevention may well be worth far more than several pounds of a bouncing newborn who may survive the perinatal period only to suffer when growing up and be destined to repeat the cycle.

Is it reasonable to conclude that if society has an interest in addressing the problem of perinatal death as a result of social factors that it may as well have an interest, both logically and legitimately, in addressing the question of who gets pregnant, how often, and when?

This is a personal communication of the author's and is not to be construed as representing either Kaiser Permanente or the Southern California Permanente Medical Group.

Sylvain Fribourg, MD
Obstetrics and Gynecology Department
Kaiser Permanente
Southern California Permanente Medical Group
13652 Cantara St.
Panorama City, CA 91402

Reply

To the Editors:

We must confess to being somewhat taken aback by what we perceive to be the subtly laid out agenda of Dr. Fribourg's letter. Hidden beneath laudable sentiments about the "clothing, shelter, education, and love" necessary to rear children are comments that come dangerously close to having undertones of racism and social engineering. Society does *not* have a moral "interest" in addressing the question of who gets pregnant beyond empowering women to choose their family size. Once we develop criteria to link family size to employment status and marital status, we approach the edge of a slippery slope that augers poorly for reproductive rights.

Isaac Delke, MD
Howard L. Minkoff, MD
Department of Obstetrics and Gynecology
State University of New York Health Science Center at Brooklyn
450 Clarkson Ave.
Box 24
Brooklyn, NY 11203-2098

Techniques differ in use of Doppler ultrasonography

To the Editors:

We are pleased that Lee et al. confirmed our findings that Doppler technique is an accurate means to noninvasively measure cardiac output in pregnancy (Lee

W, Rokey R, Cotton DB. Noninvasive maternal stroke volume and cardiac output determinations by pulsed Doppler echocardiography. AM J OBSTET GYNECOL 1988;158:505-10). With growing acceptance of the method, a great potential exists to broaden our understanding of hemodynamics in pregnancy. However, we must take exception to their criticisms of our technique.

Dr. Lee suggests on theoretic grounds that his method is superior to ours. We measure the aortic diameter with A-mode ultrasonography just distal to the excursion of the valve leaflets. The velocity profile is measured with continuous Doppler ultrasonography from a suprasternal window.[1] Dr. Lee measures the aortic diameter at the valve annulus with a two-dimensional imaging system. He measures the velocity profile with pulsed Doppler ultrasonography from an apical five-chamber window.

Notwithstanding theoretic concerns, the accuracy of a method must be determined on the basis of data. Although Dr. Lee's study produced results very similar to ours, our regression line more closely approximates the ideal line, $y = x$. The following examples generated from each regression line demonstrate the differences:

	Cardiac output (L/min)	
Thermodilution	5.0	11.0
Easterling et al.	4.6	11.2
Lee et al.	4.3	12.4

We believe that Doppler technique is the best available noninvasive method to measure cardiac output. The concordance of Dr. Lee's work with ours with the use of slightly different techniques should enhance the growing acceptance of the method.

Thomas R. Easterling, MD
Thomas J. Benedetti, MD
Department of Obstetrics and Gynecology
University of Washington
Seattle, WA 98195

REFERENCE

1. Easterling TR, Watts DH, Schmucker BC, Benedetti TJ. Measurement of cardiac output in pregnancy: validation of Doppler technique and clinical observation in preeclampsia. Obstet Gynecol 1987;69:845-50.

Reply

To the Editors:

In the process of effectively evaluating new methods it is often important to compare techniques with other reported work. However, it should not be too surprising that both continuous-wave Doppler and pulsed wave Doppler determinations of maternal stroke volume correlate well with thermodilution studies since this application has been extensively evaluated in nonpregnant subjects.[1] In this regard, it is extremely difficult to imply that any particular method is clearly superior over other techniques because of the limited number of pregnant patients who have been simultaneously studied by pulmonary artery catheterization. We do agree that Doppler ultrasonography is probably the best noninvasive method for the estimation of maternal stroke volume and cardiac output. However, if optimal aortic waveform data cannot be obtained because of technical reasons then one should consider a variety of other echocardiographic windows and Doppler techniques. In our experience, this will depend on body habitus, position, patient cooperation, anatomic variation, and availability of appropriate instrumentation. These factors will often determine whether a particular Doppler technique is more effective for a given patient.

There are certain advantages and limitations to both techniques. For example, pulsed-wave Doppler estimations of maternal stroke volume by the apical five-chamber window is primarily useful in the lateral and supine positions only. In contrast, application of continuous-wave Doppler through the suprasternal notch may be applied to several positions as recently demonstrated by Dr. Easterling.[2] Furthermore, there is less cost for a dedicated nonimaging continuous-wave Doppler system as compared with instrumentation with pulsed-wave Doppler capabilities. Occasionally, there may be difficulty with consistent acquisition of good quality aortic velocity waveforms by continuous-wave Doppler from the suprasternal notch when compared with pulsed-wave Doppler through the apical view. However, these discrepancies may simply be a matter of technique.

With the continuous-wave Doppler system, blood flow velocities are measured at all points along the pathway of the Doppler signal and our greatest concern about this technique is related to the difficulty of matching a corresponding section of aorta with the flow velocity waveform to calculate absolute volumetric flow. At our perinatal facility, we use suprasternal notch continuous-wave Doppler ultrasonography to obtain *relative* changes in stroke volume (for instance, before and during maternal exercise). We prefer to use the pulsed-wave Doppler technique to estimate *absolute* stroke volume because real-time imaging of the heart will allow measurement of the aortic annulus at the specific site of Doppler interrogation and associated angle correction factors can also be minimized. Furthermore, the pulsed-wave Doppler sample volume can be specifically placed at the corresponding aortic annulus, which is the limiting orifice that is associated with peak velocity, laminar blood flow, and a flat spatial velocity profile.[3]

Aside from these theoretic considerations, Dr. Easterling and colleagues have nicely demonstrated the application of a valuable technique to pregnancy. Pulsed- and continuous-wave Doppler ultrasonographic techniques should be jointly regarded as important but complementary adjuncts to other echocardiographic methods (e.g., two-dimensional, M-mode) that can be

used to noninvasively evaluate maternal hemodynamic function.

Wesley Lee, MD

Department of Obstetrics and Gynecology
William Beaumont Hospital
3601 West Thirteen Mile Road
Royal Oak, MI 48072

Roxann Rokey, MD

Department of Medicine
Division of Cardiology
Baylor College of Medicine
One Baylor Plaza
Houston, TX 77030

REFERENCES

1. Zoghbi WA, Quinones MA. Determinations of cardiac output by Doppler echocardiography: a critical appraisal. Herz II 1986;5:258.
2. Easterling TR, Schmucker BC, Benedetti TJ. The hemodynamic effects of orthostatic stress during pregnancy. Obstet Gynecol 1988;72:550.
3. Seed WA, Wood NB. Velocity patterns in the aorta. Cardiovasc Res 1971;5:319.

Response by Drs. Easterling and Benedetti. We agree with Drs. Lee and Rokey that Doppler technique is the best noninvasive method to measure cardiac output in pregnant patients. In our hands, continuous-wave Doppler has been demonstrated to correlate well with thermodilution. In Dr. Lee's hands, pulsed-wave Doppler has been demonstrated to work equally well. Neither Dr. Lee nor we use both methods extensively. Therefore, reasonable comparisons cannot be made. Each method with an experienced operator can be expected to accurately predict absolute cardiac output.

Maternal hematocrit and premature labor

To the Editors:

Contrary to Lieberman et al. (Lieberman E, Ryan KJ, Monson RR, Schoenbaum SC. Association of maternal hematocrit with premature labor. AM J OBSTET GYNECOL 1988;159:107-14), I believe that the association of maternal hematocrit with premature labor is simple. There is a physiologic anemia of pregnancy that is most severe in the second and early third trimesters when, by definition, cases of premature labor occur.[1] This decrease in hematocrit is documented by tables 1.3 and 1.5 in *The Physiology of Human Pregnancy* by Hytten and Leitch.[2] Lieberman et al. attempt to refute this simple explanation by reference to only a minimal change in maternal hematocrit. We need to know the hematocrit values of their patients with normal pregnancies in the second trimester and at term. I believe that it is almost a universal experience that maternal hematocrits in pregnant women rise at least 10% between the second trimester and term when they are given additional iron. What information do we have about the iron intake of their subjects?

While the authors did make reference to their very similar article in *The New England Journal of Medicine,* "Risk factors accounting for ratio differences in the rate of premature labor," is this not an example of "repetitive" publications[3]? Given the criticism the authors received by the multiple letters to the editor, as well as the editorial in *The New England Journal of Medicine,* I am surprised that they persisted in their repeat presentation, especially since Fig. 1 of their reply shows that increased hematocrit can be associated with increased percent of prematurity.[4] Is their argument only over the position of the nadir of the hematocrit curve versus percent prematurity?

Robert C. Goodlin, MD

Department of Obstetrics and Gynecology
Denver General Hospital
777 Bannock St.
Denver, CO 80204-4507

REFERENCES

1. Goodlin RC. Why treat "physiologic" anemia of pregnancy? J Reprod Med 1982;27:165-84.
2. Hytten FE, Leitch I. The physiology of human pregnancy. 2nd ed. Oxford: Blackwell, 1971:26,32.
3. Pitkin RM. Repetitive publication [Editorial]. Obstet Gynecol 1988;72:263-4.
4. Lieberman E, Ryan K, Monson R, Schoenbaum S. Reply [Letter]. N Engl J Med 1988;318:786.

Reply

To the Editors:

We are well aware the there are physiologic changes in hematocrit over the course of pregnancy, including a decrease in hematocrit early in pregnancy. Changes in hematocrit in later pregnancy, however, are less well documented. Although Dr. Goodlin believes that it is "... almost a universal experience that maternal hematocrits in pregnant women rise at least 10% between the second trimester and term ...," he cites no references to support this view. The literature shows that when hematocrits are measured serially in individual women, hematocrit values either stabilize by 24 weeks[3] or increase slightly (two percentage points or less) between 25 and 30 weeks and term.[4,5] We have previously shown that after adjusting for such small changes, the association of hematocrit with premature labor remains strong.[6] Dr. Goodlin further inappropriately draws conclusions that are based on a hypothetical model that appeared in our letter to *The New England Journal of Medicine.*[6] This model corrected for possible changes in hematocrit over the course of pregnancy and was constructed solely to demonstrate that the association of hematocrit and premature birth would remain even after adjusting for hematocrit changes that might occur late in pregnancy. Finally, we agree that serial examination of both second-trimester and term hematocrits in the same patients would be useful and we have been collecting such data.

We were puzzled by Dr. Goodlin's accusation of repetitive publication on our part. The two articles to which he referred (1,2) were both published in the literature in leading peer-reviewed journals and were cross-referenced. While both are related to preterm birth, they focus on completely different issues. The article in the JOURNAL[1] focused on hematocrit as a risk factor for preterm labor. The other article[2] considered

a wide variety of socioeconomic and medical variables (including hematocrit) as part of an examination of the increased risk of preterm labor in black women. Its aim was not to determine if variables themselves were risk factors for premature labor but rather to ascertain whether these specific risks accounted for racial differences in the occurrence of premature labor. The analyses of the two articles were conducted independently and the groups of women in the two studies were not identical. We suggest that Dr. Goodlin read both articles and reconsider his accusation.

Ellice Lieberman, MD, DrPH
Brigham and Women's Hospital
Harvard Medical School
75 Francis St.
Boston, MA 02115

Kenneth J. Ryan, MD
Department of Obstetrics and Gynecology
Brigham and Women's Hospital
Harvard Medical School
75 Francis St.
Boston, MA 02115

Richard R. Monson, MD, ScD
Department of Epidemiology
Harvard School of Public Health
677 Huntington Ave.
Boston, MA 02115

Stephen C. Schoenbaum, MD, MPH
Harvard Community Health Plan
10 Brookline Place West
Brookline, MA 02145

REFERENCES

1. Lieberman E, Ryan KJ, Monson RR, Schoenbaum SC. Association of maternal hematocrit with premature labor. AM J OBSTET GYNECOL 1988;159:107-14.
2. Lieberman E, Ryan KJ, Monson RR, Schoenbaum SC. Risk factors accounting for racial differences in the rate of premature birth. N Engl J Med 1987;317:743-8.
3. Lund CJ, Donavan JC. Blood volume during pregnancy: significance of plasma and red cell volumes. AM J OBSTET GYNECOL 1967;98:393.
4. Hytten F. Blood volume changes during normal pregnancy. Clin Haematol 1985;14:601-12.
5. Nilsen ST, Sagen N, Kim HC, Bergsjo P. Smoking, hemoglobin levels, and birth weights in normal pregnancies. AM J OBSTET GYNECOL 1984;148:752-8.
6. Lieberman E, Ryan KJ, Monson RR, Schoenbaum SC. Maternal hematologic status and outcome of pregnancy in blacks and whites [Letter reply]. N Engl J Med 1988; 318:785-6.

Multiple gestation with intrauterine insemination and menotropin therapy

To the Editors:

Dodson et al. recently reported their experience with combined intrauterine insemination and menotropin therapy (Dodson WC, Hughes CL Jr, Haney AF. Multiple pregnancies conceived with intrauterine insemination during superovulation: an evaluation of clinical characteristics and monitored parameters of conception cycles. AM J OBSTET GYNECOL 1988;159:382-5). They concluded that "25% to 30% of the conceptions" will "unavoidably result in multiple pregnancy." (Actually, their data describe pregnancies past the first trimester rather than "conceptions.") We do not believe that their conclusion is warranted.

We had previously reported a similar experience with menotropin/intrauterine insemination therapy[1] but have now been able to reduce the multiple gestation rate substantially. In 1987, only 3 of 35 viable pregnancies in patients with combined menotropin/intrauterine insemination therapy resulted in multiple pregnancies (all twins), for a multiple gestation rate of $<10\%$.

We see three significant differences between our approach to menotropin/intrauterine insemination therapy and that used by Dodson et al., which may contribute to our lower multiple gestation rate: (1) Patient selection. Virtually all patients in our menotropin/intrauterine insemination program represent failures of clomiphene citrate/menotropin/intrauterine insemination therapy. The prior use of the clomiphene citrate/menotropin/intrauterine insemination protocol[2] may preselect patients with a lower fecundity for the menotropin/intrauterine insemination program resulting in lower pregnancy rate per cycle and a decreased number of multiple gestations. (2) Menotropin management. Dodson et al. stated that human chorionic gonadotropin is administered when "one follicle or more" measure >15 mm. Apparently more than one follicle >15 mm was quite common because they reported the mean number of such follicles to be 3.5 to 3.8. Similarly, the mean estradiol concentration at human chorionic gonadotropin administration was about 1000 pg/ml. By contrast, the corresponding numbers for our pregnant patients are as follows: mean follicle number ≥ 15 mm, 1.7; mean serum estradiol concentration, 419 pg/ml (menotropin/intrauterine insemination program, 1987). (3) Sperm preparation. Dodson et al. prepared sperm by simple centrifugation and inseminated the entire cellular component of the specimen, approximately 100 million motile sperm. On the other hand, we use a swim-up procedure and, since our initial experience,[1] deliberately limit the number of motile sperm in the inseminate to <20 million.

Since our initial experience with menotropin/intrauterine insemination was similar to that discussed by Dodson et al. and since reductions in sperm number per inseminate represent the major modification in our treatment protocol, the 1987 drop in our multiple gestation rate may reflect primarily the impact of limiting numbers of sperm in the inseminate.

Whereas we were able to reduce the multiple gestation rate, our menotropin/intrauterine insemination program also demonstrated a lower cycle fecundity (10%) than reported by Dodson et al. Whether the lower pregnancy rate can be attributed to patient selection, more conservative menotropin management, or sperm numbers cannot be ascertained from our data at this time, but it is likely that all three factors play a role.

It is our contention that the 25% to 30% multiple gestation rate with menotropin/intrauterine insemination can be reduced. It is possible that this increase

in safety is at the expense of a lower probability of pregnancy per treatment cycle.

Gregory H. Corsan, MD
Robert M. Shelden, PhD
Ekkehard Kemmann, MD
University of Medicine and Dentistry of New Jersey
Robert Wood Johnson Medical School
CN 19
New Brunswick, NJ 08903

REFERENCES

1. Shelden R, Kemmann E, Bohrer M, Pasquale S. Multiple gestation is associated with the use of high sperm numbers in the intrauterine insemination specimen in women undergoing gonadotropin stimulation. Fertil Steril 1988;49: 607-10.
2. Kemmann E, Bohrer M, Shelden R, Fiasconaro G, Beardsley L. Active ovulation management increases the monthly probability of pregnancy occurrence in ovulatory women who receive intrauterine insemination. Fertil Steril 1987; 48:916-20.

Reply

To the Editors:

We appreciate the interest in our publication shown by Corsan et al. and are grateful for the opportunity to continue the dialogue.

We agree that factors potentially associated with an increased multiple gestation rate might include patient characteristics (such as age, diagnosis, parity), prior treatment experience, and sperm preparation techniques. However, at present no convincing evidence of such associations exists. After reviewing our patient population, we determined that these factors were not overrepresented in the couples with multiple gestations.

Management of superovulation may be different at Duke than at the University of Medicine and Dentistry of New Jersey, and there is no simple way to compare the two programs because Shelden et al. did not monitor the ovarian response to menotropin with pelvic ultrasonography.

Corsan et al. indicate that limiting the numbers of sperm inseminated may reduce the multiple gestation rate. They noticed a decrease in the multiple gestation rate in 1987, after limiting the sperm number per inseminate. Our experience does not confirm that such a relationship exists. In our retrospective report we found no association of the multiple gestation rate and the number of sperm inseminated. Additionally, in the past year we noted a 40% decrease in the multiple gestation rate—without limiting the number of sperm inseminated.

In summary, we still cannot identify any clinical features, monitored parameters, or sperm characteristics of a pregnancy cycle currently used in our program that would indicate which couples are more likely to conceive multiple pregnancies with intrauterine insemination and menotropin–induced superovulation. Furthermore, the currently available retrospective analyses that identify correlated factors need to be substantiated with prospective randomized trials.

William C. Dodson, MD
Claude L. Hughes, PhD, MD
A. F. Haney, MD
Box 3527
Duke University Medical Center
Durham, NC 27710

Allergic vaginitis

To the Editors:

The report by Witkin et al. (Witkin SS, Jeremiahs J, Ledger WJ. Recurrent vaginitis as a result of sexual transmission of IgE antibodies. Am J Obstet Gynecol 1988;159:32-6) proposes that there are subsets of patients with vaginitis caused by immunoglobulin E–mediated reactions, transmitted both actively, through antigens that are present in seminal fluid and react with sensitized vaginal mucosa, and passively, through specific immunoglobulin in seminal fluid that reacts with antigens in the vaginal mucosa. These workers found that the use of a condom eliminates the reactions. The following may be an example of allergic vaginitis caused by atmospheric allergens and suggests an alternative method of treatment of this problem.

The patient, a 26-year-old, married graphic designer, was referred by her gynecologist because of vaginal symptoms that he susepcted might be allergic in nature. In the winter or early spring of 1983, she began to have vaginal itching and burning pain with coitus. The itching, but not the pain, was helped by vinegar douches. In January 1987, vaginal cultures and smears were negative, and she did not respond to empiric treatment with Flagyl. In mid-July she developed symptoms typical of allergic rhinitis, and in September she had increased coital pain. She consulted an ophthalmologist because of itching eyes; allergic conjunctivitis and dry eyes were confirmed by Schirmer's test, and eye symptoms were relieved by topical steroids and cromolyn sodium. By January the nasal, eye, and vaginal symptoms cleared completely, and she was well until mid-July 1988, when she had a recurrence of nasal, eye, and all vaginal symptoms. Examination revealed moderately congested, red, dry nasal membranes, dry, slightly injected conjunctivae, and a clear chest. Results of vaginal examination were unremarkable.

Intradermal tests to inhalant allergens showed significant positive reactions to weeds, grasses, molds, dust, and household environmental agents. Smears of nasal and vaginal secretions showed large numbers of leukocytes (half eosinophils and the rest neutrophils). Impression was allergic rhinitis, conjunctivitis, and possible vaginitis (dry eye syndrome).

For treatment, she was given Opticrom (cromolyn 4%), one drop in each eye, for the eye symptoms and Nasalcrom nasal solution, one puff in each nostril four times daily, for the nasal symptoms. She was instructed to obtain ampules of cromolyn sodium, containing 20 mg in 2 ml, and to inject it intravaginally with a rubber ear syringe four times daily. She returned for follow-up 2 weeks later on August 30, having gone through a menstrual cycle, and reported marked improvement in all symptoms. Specifically, the vaginal symptoms cleared completely, and she no longer had pain or discomfort on coitus.

A smear of nasal secretions was unchanged from the pre-

vious examination. A smear of vaginal secretions showed a moderate number of leukocytes (three fourths neutrophils and the rest eosinophils).

While one can never be certain in an unusual presentation of this kind, the history and response to medication suggest the possibility that this patient did have allergic vaginitis caused by pollen.

Cromolyn sodium acts by stabilizing mast cells, thereby reducing mediator release. Its site of action may involve effects on calcium channels[1] or regulation of phosphorylation of a protein that is essential for degranulation to occur.[2] It has proved useful in preventing allergic conjunctivitis, allergic rhinitis, and asthma caused by allergy, exercise, exposure to cold, and some irritants. Its efficacy in vaginitis remains to be proved by properly controlled studies, but since it is such an innocuous medication, it is reasonable to consider using it to treat vaginitis where allergic mechanisms are suspected.

Since submission of this letter, the patient has gone through the 1989 spring and fall pollen seasons with recurrence of allergic symptoms, including vaginal itching and burning. The vaginal symptoms were again completely controlled with the use of cromolyn instilled intravaginally.

Murray Dworetzky, MD
Cornell University Medical College
Department of Medicine
115 East 61st St.
New York, NY 10021

REFERENCES

1. Mazurek N, Bashkin P, Pecht I. Isolation of a basophilic membrane protein binding the anti-allergic drug, cromolyn. EMBO J 1982;1:585.
2. Theoharides TC, Sieghart W, Greenard P, Douglas WW. Anti-allergic drug, cromolyn, may inhibit histamine secretion by regulating phosphorylation of a mast cell protein. Science 1980;207:80.

Reply

To the Editors:

Dr. Steven Witkin asked me to comment on the letter from Dr. Dworetzky concerning the use of intravaginal cromolyn sodium for treatment of vaginal allergy.

For the past 18 months I have been prescribing intravaginal cromolyn sodium for patients with allergic vaginitis. Diagnosis of allergic vaginitis was based on the presence of immunoglobulin E by immunofluorescence testing of vaginal secretions. Fifteen patients so diagnosed all were advised to douche vaginally with ampules of cromolyn sodium containing 20 mg in 2 ml twice daily. Fifteen patients have reported results to me. Douching with cromolyn sodium has been beneficial in 35%, with the improvement in vaginal symptoms being modest, rated by the patients at a 30% to 50% improvement in symptoms of itching, burning, and discomfort.

Leo Galland, MD
41 East 60th St.
New York, NY 10022

Results of uterine activity studies in agreement, but methods differ

To the Editors:

I read with interest the article by Dr. Nageotte and his coworkers about the spontaneous contraction activity of the pregnant uterus (Nageotte MP, Dorchester W, Porto M, Keegan KA Jr., Freeman RK. Quantitation of uterine activity preceding preterm, term, and postterm labor. Am J Obstet Gynecol 1988;158:1254-9). We agree with the results of the authors but not with the method. The authors prospectively studied the maximum uterine contraction frequency during any 10-minute period in 2446 patients and found significantly higher activity in the premature group. However, the length of observation (i.e., the average number of measurements) also was the highest in the premature group (4.4 ± 1.8 in the preterm group versus 2.2 ± 1.6 in the postterm group). Obviously, anyone has a greater chance to find a 10-minute period with high-frequency activity if the length of the observation is longer. On the other hand, the increased contraction activity of the uterus may clearly appear in a large number of patients in the pathologic group, even with the authors' method, but not in each individual case because a 10-minute period does not represent the whole day's activity.

In a prospective clinical study performed in the past 2 years with a smaller group of pregnant women, we already have observed >600 hours of spontaneous uterine activity of pregnant women between 25 and 37 weeks' gestation.[1] According to our data the activity sometimes suddenly increases, then decreases without any regularity. The difference between "normal" and "abnormal" in some cases is only the number of high-frequency periods per day. So, high-frequency periods may and usually do appear in normal cases, too (e.g., after sexual intercourse or nipple stimulation, or because of a full bladder). Therefore with a 10-minute or even a 30-minute window the physician can and probably will miss most of the pathognomonic periods that appear in some cases one or more times a day, but in another case can register the only high-frequency period of the day.

In light of the above-mentioned facts, we prefer long-time continuous uterine activity monitoring of high-risk patients with portable uterine contraction monitors.[2-4] The predictive value and efficacy of preterm uterine activity monitoring can reach a significant level only by this method.

Miklós Török, MD
István Gáti, PhD, DSc
Postgraduate Medical School
Department of Obstetrics and Gynecology
1135 Budapest XIII Szabolcs u 35, Hungary

REFERENCES

1. Török M, Turi Zs, Gáti I. A new method, useful for long-time quantitative and qualitative external analysis of uter-

ine contraction and home telemetry. Rome: XI European Congress of Perinatal Medicine, 1988.
2. Bell R. The prediction of preterm labor by recording spontaneous antenatal uterine activity. Br J Obstet Gynaecol 1983;90:884.
3. Katz M, Roger R, Gill PJ. Assessment of uterine activity in ambulatory patients at high-risk of preterm labor and delivery. AM J OBSTET GYNECOL 1986;154:44.
4. Nageotte MP, Freeman RK, Porto M. Technology for preventing premature birth. Contemp Obstet Gynecol Tech 1988:9-16.

Reply.

To the Editors:

I would like to take this opportunity to clarify some misunderstandings. The article on uterine activity preceding preterm, term, and postterm delivery addressed the assessment of maximum uterine activity in any 10-minute period *before* uterine stimulation was begun in patients who underwent contraction stress tests. The length of time that preceded the start of uterine stimulation never exceeded 20 minutes according to protocol. Consequently, the maximum contraction rate during any 10 minutes of this 20-minute "window" is what constitutes the study period. It is not any 10-minute period as apparently was the understanding.

Certainly longer periods of monitoring uterine activity will give more information of contraction frequency for a given patient. We do not propose that simply looking at 10 to 20 minutes of uterine activity in a particular patient has any predictive value to time delivery. However, when evaluating a large number of patients it is interesting to note an apparent population-based difference in contraction frequency at a given gestational age for patients delivered of preterm, term, and postterm infants.

Michael P. Nageotte, MD
Women's Hospital
Memorial Medical Center of Long Beach
2801 Atlantic Ave.
Long Beach, CA 90801

Effect of magnesium and preeclampsia on plasma cholinesterase activity

To the Editors:

In a recent report (Kambam JR, Perry SM, Entman S, Smith BE. Effect of magnesium on plasma cholinesterase activity. AM J OBSTET GYNECOL 1988;159:309-11), an approximately 30% reduction in plasma cholinesterase activity was demonstrated in women with preeclampsia as compared with healthy gravid women. There was no difference in plasma cholinesterase activity before and after the administration of magnesium sulfate. It was concluded that the low level of plasma cholinesterase is probably responsible for the prolonged action of succinylcholine in women with preeclampsia who are receiving magnesium sulfate. There are three reasons why this conclusion is questionable. First, the elimination of magnesium sulfate does not depend on plasma cholinesterase; magnesium is excreted primarily by the kidney. Second, there was no difference in the duration of action of succinylcholine between healthy gravid women undergoing cesarean section and women undergoing laparoscopic tubal ligation despite a 20% lower level of plasma cholinesterase in the pregnant women.[1] In healthy puerperal women 2 days post partum, when the plasma cholinesterase level is at its lowest (21% less than at the time of cesarean section),[1] the action of succinylcholine was only 200 seconds longer than in young gynecologic patients, a clinically insignificant prolongation.[2] Third, magnesium sulfate is a depressant of the neuromuscular junction and, as such, prolongs the action of all muscle relaxants, including the nondepolarizing agents, which are not degradated by plasma cholinesterase.[3, 4] Thus the prolongation of the action of succinylcholine in gravid women with preeclampsia can be amply explained by the characteristics of magnesium sulfate alone.

Gertie F. Marx, MD
Department of Anesthesiology—J 1226
Albert Einstein College of Medicine
1300 Morris Park Ave.
Bronx, NY 10461

REFERENCES

1. Blitt CD, Petty WC, Alberternst EE, Wright BJ. Correlation of plasma cholinesterase activity and duration of action of succinylcholine during pregnancy. Anesth Analg 1977;56: 7-83.
2. Ganga CC, Heyduk JV, Marx GF, Sklar GS. A comparison of the response to suxamethonium in post-partum and gynaecological patients. Anaesthesia 1982;37:903-6.
3. Ghoneim MM, Long JP. The interaction between magnesium and other neuromuscular blocking agents. Anesthesiology 1970;32:23-7.
4. Sinatra RS, Philip BK, Naulty JS, Ostheimer GW. Prolonged neuromuscular blockade with vecuronium in a patient treated with magnesium sulfate. Anesth Analg 1985; 64:1220-2.

Reply

To the Editors:

Our plasma cholinesterase data show that there is a mean decrease of 60% (range 40% to 80%) and 30% (range 25% to 55%) in pregnant patients with preeclampsia when compared with healthy nonpregnant and pregnant patients, respectively. We hope to clarify the questions raised by Marx.

There are at least two good reasons why pregnant patients with preeclampsia are different from healthy pregnant patients in relation to the duration of action of succinylcholine: (1) The blood volume and consequently the distribution volume are increased for succinylcholine in healthy pregnant patients when compared with pregnant patients with preeclampsia in whom there is a decrease in blood volume; (2) the decrease in plasma cholinesterase activity in patients with preeclampsia is far greater than that seen in healthy

pregnant patients. To our knowledge, there are no published data to date on the correlation of plasma cholinesterase activity and duration of succinylcholine in patients with preeclampsia. A correlative study published by Viby-Mogensen[1] did show that when there is a decrease of >60% to 80% in mean plasma cholinesterase activity, the duration of apnea after succinylcholine (1 mg/kg intravenously) administration in nonpregnant patients lasts up to 22 minutes. This duration of apnea is approximately what we see clinically in patients with preeclampsia after a single dose of succinylcholine, 1 mg/kg intravenously. It is also possible that an occasional patient with preeclampsia and very low plasma cholinesterase activity may develop a desensitization block, in which case the action of succinylcholine may be prolonged several times the usual duration. We have recently shown such a desensitization block after a single injection of succinylcholine in a patient with approximately 20% of normal plasma cholinesterase activity.[2]

Regarding the comment on magnesium pharmacokinetics, we did not state in our article that the elimination of magnesium is influenced by plasma cholinesterase activity. Magnesium is known to decrease the release of acetylcholine at the neuromuscular junction. While a decrease in acetylcholine release at the neuromuscular junction explains the mechanism for prolongation of a nondepolarizing or curare type of block, this does not explain the reason for prolongation of a depolarizing or succinylcholine-induced block. Data of James et al.[3] clearly show that magnesium has no effect on the duration of action of succinylcholine when tested in healthy nonpregnant patients with presumably normal plasma cholinesterase activity levels. On the basis of the fact that there is a decrease in distribution volume, as well as a decrease in plasma cholinesterase activity in preeclampsia, and in the presence of evidence that magnesium does not prolong the action of succinylcholine in nonpregnant patients with normal level of plasma cholinesterase, we concluded that the low level of plasma cholinesterase activity is probably responsible for the prolonged action of succinylcholine in women with preeclampsia who receive magnesium sulfate.

Jay Kambam, MD
Stephanie M. Perry, MD
Bradley E. Smith, MD

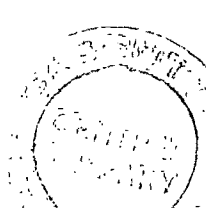
Department of Anesthesiology
Vanderbilt University Medical Center
Nashville, TN 37232

REFERENCES

1. Viby-Mogensen J. Correlation of succinylcholine duration of action with plasma cholinesterase activity in subjects with genotypically normal enzyme. Anesthesiology 1980;53:517-20.
2. Kambam JR, Franks JJ, Naukam RJ, Sastry BVR. The effect of quinidine on plasma cholinesterase activity and succinylcholine neuromuscular blockade. Anesthesiology 1987;67:858-60.
3. James MFM, Cork RC, Dennett JE. Succinylcholine pretreatment with magnesium sulfate. 1986;65:373-6.

Correction

In the article by Burry et al., entitled "Metabolic changes during medical treatment of endometriosis: Nafarelin acetate versus danazol" (Am J Obstet Gynecol 1989;160:1454-61), on page 1461, in the right-hand column, line 7, the sentence beginning "If gonadotropin-releasing hormone analogs, such as danazol . . ." should have been "If gonadotropin-releasing hormone analogs, such as nafarelin . . . ."



ITEMS

Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60.00 U.S. per insertion and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, The C.V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318.

Postgraduate Course in Gynecologic and Obstetric Pathology with Clinical Correlation—1990, April 9-13, 1990, Parker House Hotel, Boston, Massachusetts. Fee for course: $595.00 (residents and fellows, $375.00). Address for further information: Department of Continuing Education, Harvard Medical School, 25 Shattuck St., Boston, MA 02115.

Symposium on Uterine Contractility: Mechanisms of Control, Robert Garfield, PhD, Chairman, March 17-20, 1990, Marriott Pavilion Hotel, St. Louis, Missouri. Sponsored by Serono Symposia, USA. Abstract forms and registration information may be obtained by contacting L. Lisa Kern, PhD, Serono Symposia, USA, 100 Longwater Circle, Norwell, MA 02061. Tel.: (800) 283-8088 or (617) 982-9000.

Thirteenth Annual Mid-Winter Symposium in OB/GYN, February 28, March 1 and 2, 1990, Scottsdale Hilton Hotel, Scottsdale, Arizona. Co-Sponsored by the Phoenix OB/GYN Society and the Department of OB/GYN, Maricopa Medical Center. For information contact: The Secretary, Phoenix OB/GYN Society, 511 W. Montebello, Phoenix, AZ 85013. Tel.: (602) 266-6321.

2nd Annual Review Course in Obstetrics and Gynaecology, May 1-5, 1990, Hilton International Toronto, Toronto, Ontario, Canada. AMA Category 1 credit hours. For further information contact: Continuing Education, Faculty of Medicine, University of Toronto, Medical Sciences Building, Toronto, Ontario, Canada M5S 1A8. Tel.: (416) 978-2718.

Bound volumes available to subscribers

Bound volumes of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY are available to subscribers (only) for the 1989 issues from the Publisher, at a cost of $56.00 ($79.00 international) for Vol. 160 (January-June) and Vol. 161 (July-December). Shipping charges are included. Each bound volume contains a subject and author index and all advertising is removed. Copies are shipped within 60 days after publication of the last issue in the volume. The binding is durable buckram with the JOURNAL name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact The C.V. Mosby Company, Circulation Department, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318, USA; phone (800) 325-4177, ext. 351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular JOURNAL subscription.

American Journal of Obstetrics and Gynecology

Editors

JOHN I. BREWER, *Editor in Chief*
Sandra Fernandez, *Senior Editorial Assistant*

FREDERICK P. ZUSPAN, *Editor*
Donna Peterson, *Editorial Assistant*

E. J. QUILLIGAN, *Editor*
Sherry A. Merryman, *Editorial Assistant*

ALBERT B. GERBIE, *Associate Editor*

Advisory committee on policy

Garland Anderson
William R. Anderson
Mason C. Andrews
E. Forrest Boyd
Robert C. Cefalo
Donald R. Coustan
David C. Figge
A. Jerome Freeland
Edgar O. Horger III
W. Glenn Hurt
James M. Ingram
Walter S. Keifer
Emmet J. Lamb
Roger A. Lobo
Douglas J. Marchant
James H. Maxwell
Arthur M. McCausland
Allan G. McLeod
James A. Merrill
Daniel R. Mishell, Jr.
John C. Morrison
Carl J. Pauerstein
Richard Paul
Karl C. Podratz
Ruth W. Schwartz
James R. Scott
Henry A. Thiede
Carol Trumbold

Board of corresponding editors

Luigi Carenza, *Rome, Italy*
T. K. A. B. Eskes, *Nijmegen, The Netherlands*
Wolfgang Künzel, *Giessen, West Germany*
Matsuto Mochizuki, *Kobe, Japan*
M. Reuben Peyser, *Tel Aviv, Israel*
Nils-Otto Sjöberg, *Malmö, Sweden*
Gordon M. Stirrat, *Bristol, England*
Claude Sureau, *Paris, France*
Carl Wood, *Melbourne, Victoria, Australia*
Olavi Ylikorkala, *Helsinki, Finland*

Publisher
THE C.V. MOSBY COMPANY, 11830 Westline Industrial Drive,
St. Louis, Missouri 63146-3318
Kay G. Goehler, *Senior Manuscript Editor*

Volume 161
July-December, 1989
The C.V. Mosby Company
St. Louis
1989

in addition to those listed on the front cover
the Journal is the official publication
of the following societies

NEW YORK OBSTETRICAL SOCIETY
OBSTETRICAL SOCIETY OF PHILADELPHIA
BROOKLYN GYNECOLOGICAL SOCIETY
ST. LOUIS GYNECOLOGICAL SOCIETY
NEW ORLEANS GYNECOLOGICAL AND OBSTETRICAL SOCIETY
THE OBSTETRICAL AND GYNECOLOGICAL SOCIETY OF MARYLAND
CHICAGO GYNECOLOGICAL SOCIETY
CINCINNATI OBSTETRICAL AND GYNECOLOGICAL SOCIETY
WASHINGTON GYNECOLOGICAL SOCIETY
PITTSBURGH OBSTETRICAL AND GYNECOLOGICAL SOCIETY
BOSTON OBSTETRICAL SOCIETY
LOUISVILLE OBSTETRICAL AND GYNECOLOGICAL SOCIETY
SEATTLE GYNECOLOGICAL SOCIETY
ALABAMA ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS
AKRON OBSTETRICAL AND GYNECOLOGICAL SOCIETY
KANSAS CITY GYNECOLOGICAL SOCIETY
CENTRAL NEW YORK ASSOCIATION OF GYNECOLOGISTS
AND OBSTETRICIANS
NEW JERSEY OBSTETRICAL AND GYNECOLOGICAL SOCIETY
IOWA OBSTETRIC AND GYNECOLOGIC SOCIETY
THE TEXAS ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS
OKLAHOMA CITY OBSTETRICAL AND GYNECOLOGICAL SOCIETY
MEMPHIS OBSTETRICAL AND GYNECOLOGICAL SOCIETY
UTAH OBSTETRICAL AND GYNECOLOGICAL SOCIETY
ROCHESTER OBSTETRICAL AND GYNECOLOGICAL SOCIETY
ARKANSAS OBSTETRICAL AND GYNECOLOGICAL SOCIETY
TENNESSEE STATE OBSTETRICAL AND GYNECOLOGICAL SOCIETY
NEW YORK GYNECOLOGICAL SOCIETY
PACIFIC NORTHWEST OBSTETRICAL AND GYNECOLOGICAL ASSOCIATION
BUFFALO OBSTETRICAL AND GYNECOLOGICAL SOCIETY
SAN FRANCISCO GYNECOLOGICAL SOCIETY
JACKSON GYNECIC SOCIETY
INDIANA OBSTETRICAL AND GYNECOLOGICAL SOCIETY
THE MINNESOTA OBSTETRICAL AND GYNECOLOGICAL SOCIETY
COLUMBUS OBSTETRIC AND GYNECOLOGIC SOCIETY
GREATER HARTFORD OBSTETRICAL AND GYNECOLOGICAL SOCIETY

Volume 161
Copyright © 1989 by The C.V. Mosby Company
(All rights reserved)

Printed in the United States of America

Index to volume 161

Author index*

A

Abe T (*see* Kyodo et al). 1989;161:94-6
Abitbol MM, Monheit AG, Rochelson BL, Stern W, Blyakher L, Saraf V. The use of an indwelling Doppler probe to study acute changes in umbilical artery waveforms in the fetal sheep. 1989;161:1324-31
Abraham A (*see* Kanwar et al). 1989;161:995-6
Abramowicz JS, Jaffe R, Warsof SL. Ultrasonographic measurement of fetal femur length in growth disturbances. 1989;161:1137-40
Abu-Fadil S (*see* Sacks et al). 1989;161:638-41
—— (*see* Sacks et al). 1989;161:642-5
Abulafia O (*see* Feinkind et al). 1989;161:765-70
Adam E (*see* Kaufman et al). 1989;161:1420 (Letter reply)
Adamson SL (*see* Morrow et al). 1989;161:1055-60
Adcock LL (*see* Borazjani et al). 1989;161:1253-7
Adelson M (*see* Baggish et al). 1989;161:60-8
Adler-Storthz K (*see* Kaufman et al). 1989;161:1420 (Letter reply)
Ahn MO (*see* Sarno et al). 1989;161:1508-14
—— (*see* Strong et al). 1989;161:29-32
Ahokas RA, Sibai BM, Anderson GD. Lack of evidence of a vasodepressor role for relaxin in spontaneously hypertensive and normotensive pregnant rats. 1989;161:618-22
Akino T (*see* Hallman et al). 1989;161:965-9
Albazzaz SJ (*see* Hadi and Albazzaz). 1989;161:318-21
Alberda AT (*see* Weyerman et al). 1989;161:1145-6
Alden ER (*see* Cohen et al). 1989;161:1013-4
Ali MM (*see* Hurt et al). 1989;161:530-1
Allen RD (*see* Spinnato et al). 1989;161:742-7
Alonso JG (*see* Valenzuela et al). 1989;161:1566-70
Alvarez RD, Grizzle WE, Smith LJ, Miller DM. Compensatory ovarian hypertrophy occurs by a mechanism distinct from compensatory growth in the regenerating liver. 1989;161:1653-7
Amon E (*see* Sadovsky et al). 1989;161:613-7
Ananth U, Queenan JT. Does midtrimester ΔOD_{450} of amniotic fluid reflect severity of Rh disease? 1989;161:47-9
Andersen HF (*see* Elkins et al). 1989;161:150-4
Anderson CF (*see* Reed et al). 1989;161:1532-5
Anderson DJ (*see* Hill and Anderson). 1989;161:861-4
Anderson GD (*see* Ahokas et al). 1989;161:618-22
—— (*see* Elkins et al). 1989;161:150-4
Anderson JV (*see* Panos et al). 1989;161:357-61
Anderson RE (*see* Sauer et al). 1989;161:1673-6
Andreu A, Genover E, Coira A, Farrán I. Antepartum infection as a result of *Streptococcus pneumoniae* and sepsis in neonate. 1989;161:1424-5 (Letter)
Andrews L (*see* Moore et al). 1989;161:849-55 (Clin. opinion)
Andrianakis P, Walker DW, Ralph MM, Thorburn GD. Effect of inhibiting prostaglandin synthesis in pregnant sheep with 4-aminoantipyrine under normothermic and hyperthermic conditions. 1989;161:241-7
Angel JL (*see* Morales et al). 1989;161:568-72
Ansbacher R. Race and gender factors omitted from results. 1989;161:502 (Letter)
Anyaegbunam A, Billett HH, Langer O, Brustman L, Berger C, Wyse L, Nagel RL, Merkatz IR. Maternal hemoglobin F levels may have an adverse effect on neonatal birth weight in pregnancies with sickle cell disease. 1989;161:654-6
—— (*see* Langer et al). 1989;161:593-9
—— (*see* Langer et al). 1989;161:646-53
Aono T (*see* Mori et al). 1989;161:207-11
Apostolides AY (*see* Cohen et al). 1989;161:1013-4
Appelbaum PC (*see* Pankuch et al). 1989;161:802-7
Appleton CP (*see* Reed et al). 1989;161:1532-5
Arcaini L (*see* Fedele et al). 1989;161:871-6
Archer DF, Hofmann G, Brzyski R, Ross B, Scott RT, Philput CB, Oehninger S, Ware JC. Effects of clomiphene citrate on episodic luteinizing hormone secretion throughout the menstrual cycle. 1989;161:581-92
Arieli S (*see* Pansky et al). 1989;161:393-6
Ariet M (*see* Resnick et al). 1989;161:184-7
Arjomaa P (*see* Hallman et al). 1989;161:965-9
Arnoletti E (*see* Sideri et al). 1989;161:38-42
Arora CP (*see* Castro et al). 1989;161:623-7
—— (*see* Castro et al) (1989;161:623-7). 1989;161:1238 (Correction)
—— (*see* Castro et al). 1989;161:1620-3
Artal R, Masaki DI, Khodiguian N, Romem Y, Rutherford SE, Wiswell RA. Exercise prescription in pregnancy: weight-bearing versus non-weight-bearing exercise. 1989;161:1464-9
Assche AV (*see* Ballegeer et al). 1989;161:432-6
Athanassiadis AP (*see* Romero et al). 1989;161:817-24
Atlas SJ (*see* Miyazaki et al). 1989;161:1709-14
Ausbon WW (*see* Resnick et al). 1989;161:184-7
Avila C (*see* Romero et al). 1989;161:817-24

B

Bade ME (*see* Sadovsky et al). 1989;161:613-7
Baggish MS, Daniell JF. Death caused by air embolism associated with neodymium:yttrium-aluminum-garnet laser surgery and artificial sapphire tips. 1989;161:877-8
——, Dorsey JH, Adelson M. A ten-year experience treating cervical intraepithelial neoplasia with the CO_2 laser. 1989;161:60-8
Baill IC, Moore GW, Hedrick LA. Abscess of allantoic duct remnant. 1989;161:334-6
Ballard CA (*see* Bergman et al). 1989;161:97-101
Ballegeer V, Spitz B, Kieckens L, Moreau H, Assche AV, Collen D. Predictive value of increased plasma levels of fibronectin in gestational hypertension. 1989;161:432-6
Barclay M (*see* Elkins et al). 1989;161:150-4
Barnea ER (*see* Sanyal et al). 1989;161:446-53
Barrett JM (*see* Odom et al). 1989;161:1663-8
Barss VA (*see* Greene et al). 1989;161:106-11

*July, 1-260; August, 261-504; September, 505-842; October, 843-1094; November, 1095-1430; December, part 1, 1431-1824; part 2, 1825-1868.

Bartfai GS (*see* Kaali et al). 1989;161:406-8
Bast RC Jr (*see* Berchuck et al). 1989;161:1247-52
—— (*see* Soisson et al). 1989;161:1258-63
Basu J (*see* Palan et al). 1989;161:881-5
Bateman BG (*see* Nunley et al). 1989;161:573-6
Bauman J (*see* Berkowitz et al). 1989;161:146-9
Bawdon RE (*see* Little et al). 1989;161:732-4
Baylink DJ (*see* Genant et al). 1989;161:1842-6 (Symposium)
Beall M (*see* Small et al). 1989;161:323-9
Bear JL (*see* Smith et al). 1989;161:911-5
Beard RW (*see* Michel et al). 1989;161:409-14
Beckmann AM, Myerson D, McDougall JK. Presence of human papillomavirus deoxyribonucleic acid. 1989;161:1419-20 (Letter)
Behrens O (*see* Ivanisevic et al). 1989;161:1637-43
Behzadian A (*see* Jijon et al). 1989;161:1658-62
—— (*see* Johnson et al). 1989;161:1732-7
Beier S (*see* Garfield and Beier). 1989;161:454-61
Belanger K (*see* Copel et al). 1989;161:341-4
—— (*see* Copel et al). 1989;161:361-5
Belizán JM, Villar J. Calcium intake and pregnancy-induced hypertension. 1989;161:834-5 (Letter reply)
Bell RJ (*see* Eddie et al). 1989;161:970-3
Bellart J (*see* Quilez et al). 1989;161:1426-7 (Letter)
Bellinger J (*see* MacKenzie et al). 1989;161:920-3
Ben-Ami M (*see* Veille et al). 1989;161:1610-4
Ben-Chitrit A (*see* Palti et al). 1989;161:1147-8
Beneck D (*see* Bracero et al). 1989;161:388-93
Benedetti TJ (*see* Clark et al). 1989;161:1439-42
—— (*see* Easterling and Benedetti). 1989;161:1748-9 (Letter)
—— (*see* Easterling and Benedetti). 1989;161:1750 (Letter response)
Berchuck A, Soisson AP, Olt GJ, Soper JT, Clarke-Pearson DL, Bast RC Jr, McCarty KS Jr. Epidermal growth factor receptor expression in normal and malignant endometrium. 1989;161:1247-52
—— (*see* Soisson et al). 1989;161:1258-63
Berdanier CD (*see* Bue et al). 1989;161:234-40
Berg CJ, Druschel CM, McCarthy BJ, LaVoie M, Floyd RL. Neonatal mortality in normal birth weight babies: Does the level of hospital care make a difference? 1989;161:86-91
Berger C (*see* Anyaegbunam et al). 1989;161:654-6
Bergman A, Koonings PP, Ballard CA. Primary stress urinary incontinence and pelvic relaxation: prospective randomized comparison of three different operations. 1989;161:97-101
Berkowitz GS, Fiarman GS, Mojica MA, Bauman J, de Regt RH. Effect of physician characteristics on the cesarean birth rate. 1989;161:146-9
Bernardini I (*see* Economides et al). 1989;161:1004-8
—— (*see* Economides et al). 1989;161:1219-27
Bernaschek G, Rudelstorfer R. Vaginosonographic examination—Routine procedure? 1989;161:499 (Letter reply)
Besinger RE (*see* Thomas et al). 1989;161:141-5
Bewley S. Systolic/diastolic ratios and waveform shapes. 1989;161:1420-1 (Letter)
Beydoun S (*see* Burkett et al). 1989;161:540-7
Bianchi S (*see* Fedele et al). 1989;161:871-6
Biddinger PW, Stafford PA, Zumwalt RE. Fetomaternal hemorrhage—possible cause of fetal distress and death. 1989;161:1744 (Letter reply)
Biggar RJ, Pahwa S, Minkoff H, Mendes H, Willoughby A, Landesman S, Goedert JJ. Immunosuppression in pregnant women infected with human immunodeficiency virus. 1989;161:1239-44
Billett HH (*see* Anyaegbunam et al). 1989;161:654-6
Binienda Z (*see* Figueroa et al). 1989;161:481-6
Bischof P (*see* Sanyal et al). 1989;161:446-53
Bishop C (*see* Clark et al). 1989;161:1439-42
Bissonnette JM (*see* Chao et al). 1989;161:221-8
—— (*see* Clark et al). 1989;161:301-2
—— (*see* Hohimer and Bissonnette). 1989;161:1344-51
Biswas MK (*see* Smith et al). 1989;161:879-80
Bjornsson S (*see* Walker et al). 1989;161:676-9
Blackard WG (*see* Nestler et al). 1989;161:1095-7 (Clin. opinion)
Blackwell RE (*see* Humphreys-Beher and Blackwell) (1989;160:1160-5). 1989;161:816 (Correction)
Blair E (*see* Stiller et al). 1989;161:930-3
Blaschke T (*see* Ferguson et al). 1989;161:1485-90
Blendis L (*see* Hannah et al). 1989;161:322-3
Blessing JA (*see* Sutton et al). 1989;161:309-12
Blickstein I. Neurofibromatosis and pregnancy. 1989;161:501 (Letter reply)
Block BS. Understanding and eliminating errors in perinatal research. 1989;161:5-9 (Clin. opinion)
——, Schlafer DH, Wentworth RA, Kreitzer LA, Nathanielsz PW. Intrauterine growth retardation and the circulatory responses to acute hypoxemia in fetal sheep. 1989;161:1576-9
Block FE Jr (*see* Sellers et al). 1989;161:1001-3
Blombäck M (*see* Dahlman et al). 1989;161:420-5
Blot WJ (*see* Brinton et al). 1989;161:121-7
Blumenfeld Z (*see* Bronshtein et al). 1989;161:78-82
Blyakher L (*see* Abitbol et al). 1989;161:1324-31
Bocking AD, White S, Gagnon R, Hansford H. Effect of prolonged hypoxemia on fetal heart rate accelerations and decelerations in sheep. 1989;161:722-7
—— (*see* Gagnon et al). 1989;161:779-84
Bodurtha JN (*see* Hurt et al). 1989;161:530-1
Boehm FH (*see* Shah and Boehm). 1989;161:374-6
Boehnlein LM (*see* Haning et al). 1989;161:1549-53
Bolton V (*see* Hill et al). 1989;161:502 (Letter reply)
Bond AL (*see* Druzin et al). 1989;161:1476-8
Borazjani G, Twiggs LB, Leung BS, Prem KA, Adcock LL, Carson LF. Prognostic significance of steroid receptors measured in primary metastatic and recurrent endometrial carcinoma. 1989;161:1253-7
Bornstein J (*see* Kaufman et al). 1989;161:1420 (Letter reply)
Botti JJ (*see* Pankuch et al). 1989;161:802-7
Bottoms S (*see* Economides et al). 1989;161:1004-8
Bowdler N (*see* Elkins et al). 1989;161:150-4
Bowes WA Jr, Katta LR, Droegemueller W, Bright TG. Triphasic Randomized Clinical Trial: comparison of effects on carbohydrate metabolism. 1989;161:1402-7 (Symposium)
—— (*see* Droegemueller et al). 1989;161:1407-11 (Symposium)
—— (*see* Thorp et al). 1989;161:1245-6
Boylan P. Neonatal seizures and cesarean section. 1989;161:836-7 (Letter)
Boylan PC (*see* Thorp et al). 1989;161:670-5
Boys RJ (*see* Robson et al). 1989;161:1273-8
Brace RA. Fetal blood volume, urine flow, swallowing, and amniotic fluid volume responses to long-term intravascular infusions of saline. 1989;161:1049-54
——. Ovine fetal cardiovascular responses to packed red blood cell transfusions. 1989;161:1367-74
——, Wolf EJ. Normal amniotic fluid volume changes throughout pregnancy . 1989;161:382-8
Bracero LA, Beneck D, Kirshenbaum N, Peiffer M, Stalter P,

Schulman H. Doppler velocimetry and placental disease. 1989;161:388-93
Bracken MB (*see* Brinton et al). 1989;161:121-7
Bradley-Watson PJ. Pregnancy after aortofemoral bypass grafting in a diabetic patient. 1989;161:200-1
Brami CJ (*see* Sanyal et al). 1989;161:446-53
Brandes JM (*see* Urbach et al). 1989;161:953-9
Brar HS, Medearis AL, DeVore GR, Platt LD. Maternal and fetal blood flow velocity waveforms in patients with preterm labor: relationship to outcome. 1989;161:1519-22
—— (*see* Sarno et al). 1989;161:1508-14
Brawer J, Richard M, Farookhi R. Pattern of human chorionic gonadotropin binding in the polycystic ovary. 1989;161:474-80
Bray E (*see* Sibai et al). 1989;161:115-9
Breitenecker G (*see* Feichtinger et al). 1989;161:891-3
Brewer JI, Zuspan FP, Quilligan EJ, Gerbie AB. Criticism of publication of pharmaceutical-supported research results draws response. 1989;161:840 (Letter reply)
Bright TG (*see* Bowes et al). 1989;161:1402-7 (Symposium)
—— (*see* Droegemueller et al). 1989;161:1407-11 (Symposium)
Brinck-Johnsen T (*see* Conrad et al). 1989;161:1065-72
Brinton LA, Wu B-Z, Wang W, Ershow AG, Song H-Z, Li J-Y, Bracken MB, Blot WJ. Gestational trophoblastic disease: a case-control study from the People's Republic of China. 1989;161:121-7
Brondelli L (*see* Porcu et al). 1989;161:174-7
Bronshtein M, Rottem S, Yoffe N, Blumenfeld Z. First-trimester and early second-trimester diagnosis of nuchal cystic hygroma by transvaginal sonography: diverse prognosis of the septated from the nonseptated lesion. 1989;161:78-82
Bronsteen RA (*see* Sowers et al). 1989;161:441-5
Brossard Y (*see* Poissonnier et al). 1989;161:709-13
Brown MS. Does terbutaline cause uterine atony and increase intraoperative blood loss? 1989;161:259 (Letter)
Brown SA (*see* Patsch et al). 1989;161:1396-401 (Symposium)
Bruckner HW, Motwani BT. Treatment of advanced refractory ovarian carcinoma with a gonadotropin-releasing hormone analogue. 1989;161:1216-8
Bruell CL (*see* Milunsky et al). 1989;161:291-7
Brunham RC (*see* Paavonen et al). 1989;161:128-35
Brustman L (*see* Anyaegbunam et al). 1989;161:654-6
—— (*see* Langer et al). 1989;161:593-9
—— (*see* Langer et al). 1989;161:646-53
Bry K (*see* Hallman et al). 1989;161:313-8
Brzezinski A (*see* Wurtman et al). 1989;161:1228-34
Brzyski R (*see* Archer et al). 1989;161:581-92
Bucciarelli RL (*see* Resnick et al). 1989;161:184-7
Bue JM, Hausman DE, Berdanier CD. Gestational diabetes in the BHE rat: influence of dietary fat. 1989;161:234-40
Bukovsky I (*see* Pansky et al). 1989;161:393-6
—— (*see* Ron-El et al). 1989;161:211-2
Bull SB (*see* Morrow et al). 1989;161:1055-60
Bundy BN (*see* Stehman and Bundy). 1989;161:1426 (Letter reply)
Bürger RA, Riedmiller H, Knapstein PG, Friedberg V, Hohenfellner R. Ileocecal vaginal construction. 1989;161:162-7
Burk RD (*see* Goldberg et al). 1989;161:1669-72
Burkett G, Jensen LP, Lai A, O'Sullivan MJ, Yasin S, Beydoun S, McLeod AGW. Evaluation of surgical staples in cesarean section. 1989;161:540-7
Burris J (*see* Gilstrap et al). 1989;161:825-30
Burry KA, Patton PE, Illingworth DR (1989;160:1454-61). 1989;161:1755 (Correction)
Bushnell JM (*see* Hendricks et al). 1989;161:761-5

C

Cabinian AE (*see* Reyes et al). 1989;161:977-81
Calame JJ (*see* Helmerhorst et al). 1989;161:1191-4
Callahan R (*see* Romero et al). 1989;161:817-24
Calvert W (*see* Villar et al). 1989;161:1019-24
Cameron AD (*see* Walker et al). 1989;161:676-9
Cameron R (*see* Hannah et al). 1989;161:322-3
Campbell D (*see* Thorp et al). 1989;161:889-90
Campbell JM (*see* Taylor et al). 1989;161:377-9
Campbell S (*see* Jauniaux et al). 1989;161:1195-7
—— (*see* Rabinowitz et al). 1989;161:1264-6
—— (*see* Vyas et al). 1989;161:168-72
Campbell WA (*see* Vintzileos et al). 1989;161:606-12
Candiani GB (*see* Fedele et al). 1989;161:871-6
Capeless EL, Clapp JF. Cardiovascular changes in early pregnancy. 1989;161:1449-53
Cardwell MS. Detection of fetal blood. 1989;161:257-8 (Letter reply)
——, Snyder SW. Fetomaternal hemorrhage—possible cause of fetal distress and death. 1989;161:1744 (Letter)
—— (*see* Snyder and Cardwell). 1989;161:35-6
Caritis SN, Venkataramanan R, Cotroneo M, Chiao J-P. Pharmacokinetics of orally administered ritodrine. 1989;161:32-5
Carlson JR (*see* Hill et al). 1989;161:502 (Letter reply)
Carpenter RJ Jr (*see* Copeland et al). 1989;161:1289-94
—— (*see* Mari et al). 1989;161:1528-31
Carr BR (*see* Doody and Carr). 1989;161:1694-7
Carson LF (*see* Borazjani et al). 1989;161:1253-7
Carter LS (*see* Petersen et al). 1989;161:509-12
Carter RL (*see* Resnick et al). 1989;161:184-7
Caspi E (*see* Pansky et al). 1989;161:393-6
—— (*see* Ron-El et al). 1989;161:211-2
—— (*see* Schreyer et al). 1989;161:836-9
Castillo RA (*see* Devoe et al). 1989;161:524-9
Castle B (*see* MacKenzie et al). 1989;161:920-3
Castro LC, Arora CP, Davis JL, Hobel CJ, Parvez H. Atrial natriuretic factor: the effects of pregnancy and a high-salt diet on atrial levels. 1989;161:1620-3
——, ——, Roll KE. Perinatal factors influencing atrial natriuretic peptide levels in umbilical arterial plasma at the time of delivery (1989;161:623-7). 1989;161:1238 (Correction)
——, ——, ——, Sassoon DA, Hobel CJ. Perinatal factors influencing atrial natriuretic peptide levels in umbilical arterial plasma at the time of delivery. 1989;161:623-7
Castro R, Ervin MG, Ross MG, Sherman DJ, Leake RD, Fisher DA. Ovine fetal lung fluid response to atrial natriuretic factor. 1989;161:1337-43
Cecchetti G (*see* Sideri et al). 1989;161:38-42
Cefalo RC (*see* Petersen et al). 1989;161:509-12
—— (*see* Thorp et al). 1989;161:889-90
Cerami A (*see* Romero et al). 1989;161:336-41
Cetin E (*see* Nicolaides et al). 1989;161:351-6
Chaffin D (*see* Shan and Chaffin). 1989;161:1111-3
Challis JRG (*see* Cheung and Challis). 1989;161:1580-5
Chang H-HT (*see* Tipton et al). 1989;161:1546-8
Chao CR, Hohimer AR, Bissonnette JM. The effect of elevated blood glucose on the electroencephalogram and cerebral metabolism during short-term brain ischemia in fetal sheep. 1989;161:221-8
Chaudhry A (*see* Witkin and Chaudhry). 1989;161:900-3

Chavinie J (*see* Poissonnier et al). 1989;161:709-13
Cherouny PH (*see* Pankuch et al). 1989;161:802-7
Chervenak FA, McCullough LB, Chervenak JL. Prenatal informed consent for sonogram: an indication for obstetric ultrasonography. 1989;161:857-60 (Clin. opinion)
Chervenak JL, Divon MY, Hirsch J, Girz BA, Langer O. Macrosomia in the postdate pregnancy: Is routine ultrasonographic screening indicated? 1989;161:753-6
—— (*see* Chervenak et al). 1989;161:857-60 (Clin. opinion)
Chescheir NC (*see* Petersen et al). 1989;161:509-12
Chestnut DH, Pollack KL, Weiner CP, Robillard JE, Thompson CS, DeBruyn CS. Does furosemide alter the hemodynamic response to rapid intravascular transfusion of the anemic fetal lamb. 1989;161:1571-5
—— (*see* Weiner et al). 1989;161:1599-605
—— (*see* Weiner et al). 1989;161:1605-10
Cheung PYC, Challis JRG. Prostaglandin E_2 metabolism in the human fetal membranes. 1989;161:1580-5
Cheyovich DL (*see* Wassman et al). 1989;161:698-702
Chiao J-P (*see* Caritis et al). 1989;161:32-5
Chinn A (*see* FitzSimmons et al). 1989;161:1174-7
Chleborad J (*see* Rayburn et al). 1989;161:202-6
Christmas JT (*see* Little et al). 1989;161:732-4
Cibils LA (*see* Cucco et al). 1989;161:916-20
Clapp JF III. The effects of maternal exercise on early pregnancy outcome. 1989;161:1453-7
——. Oxygen consumption during treadmill exercise before, during, and after pregnancy. 1989;161:1458-64
—— (*see* Capeless and Clapp). 1989;161:1449-53
Clark BA, Bissonnette JM, Olson SB, Magenis RE. Pregnancy loss in a small chorionic villus sampling series. 1989;161:301-2
Clark DA (*see* Michel et al). 1989;161:409-14
Clark SL, Cotton DB. Is central venous pressure monitoring "contraindicated" in patients with severe preeclampsia? 1989;161:838-9 (Letter reply)
——, ——, Lee W, Bishop C, Hill T, Southwick J, Pivarnik J, Spillman T, DeVore GR, Phelan J, Hankins GDV, Benedetti TJ, Tolley D. Central hemodynamic assessment of normal term pregnancy. 1989;161:1439-42
Clarke-Pearson DL (*see* Berchuck et al). 1989;161:1247-52
—— (*see* Soisson et al). 1989;161:1258-63
Clore JN (*see* Nestler et al). 1989;161:1095-7 (Clin. opinion)
Clyne LP (*see* Lockwood et al). 1989;161:369-73
Coe AM (*see* Nicolini et al). 1989;161:924-7
Cohen A (*see* Leiberman et al). 1989;161:257 (Letter)
Cohen DL, Kessel RWI, McCullough LB, Apostolides AY, Heiderich KJ, Alden ER. Pelvic examinations by medical students. 1989;161:1013-4
Cohen I (*see* Veille et al). 1989;161:1610-4
Cohen M (*see* Petrikovsky et al). 1989;161:36-7
Coira A (*see* Andreu et al). 1989;161:1424-5 (Letter)
Collen D (*see* Ballegeer et al). 1989;161:432-6
Collins EM (*see* Morales et al). 1989;161:568-72
Collins JV (*see* Kingsland et al). 1989;161:381-2
Colpoys MC (*see* Conrad et al). 1989;161:1065-72
Conrad KP, Morganelli PM, Brinck-Johnsen T, Colpoys MC. The renin-angiotensin system during pregnancy in chronically instrumented, conscious rats. 1989;161:1065-72
Cook EJ (*see* Macri et al). 1989;161:254-5 (Letter reply)
Copel JA, Grannum PA, Green JJ, Belanger K, Hanna N, Jaffe CC, Hobbins JC, Kleinman CS. Fetal cardiac output in the isoimmunized pregnancy: a pulsed Doppler-echocardiographic study of patients undergoing intravascular intrauterine transfusion. 1989;161:361-5
——, ——, ——, ——, Hobbins JC. Pulsed Doppler flow-velocity waveforms in the prediction of fetal hematocrit of the severely isoimmunized pregnancy. 1989;161:341-4
Copeland KL, Carpenter RJ Jr, Fenolio KR, Ledbetter DH. Integration of the transabdominal technique into an ongoing chorionic villus sampling program. 1989;161:1289-94
Corsan GH, Shelden RM, Kemmann E. Multiple gestation with intrauterine insemination and menotropin therapy. 1989;161:1751-2 (Letter)
Coster B (*see* Lockwood et al). 1989;161:369-73
Cotroneo M (*see* Caritis et al). 1989;161:32-5
Cotton DB (*see* Clark and Cotton). 1989;161:838-9 (Letter reply)
—— (*see* Clark et al). 1989;161:1439-42
—— (*see* Lee et al). 1989;161:974-7
—— (*see* Lee et al). 1989;161:1012-3
—— (*see* Mari et al). 1989;161:1514-8
—— (*see* Mari et al). 1989;161:1528-31
Coulter S (*see* Walsh and Coulter). 1989;161:1586-92
Couser RJ (*see* Ferrara et al). 1989;161:1114-8
Cousins L (*see* Jacobson and Cousins). 1989;161:981-6
Cowchock FS (*see* Wapner et al). 1989;161:1271-2
Cox PJ (*see* Nicoll and Cox). 1989;161:177-8
Craddock GB Jr (*see* Nunley et al). 1989;161:573-6
Creasy RK (*see* Peaceman et al). 1989;161:771-4
—— (*see* Thorp et al). 1989;161:600-5
Cucco C, Osborne MA, Cibils LA. Maternal-fetal outcomes in prolonged pregnancy. 1989;161:916-20
Curet LB (*see* Haning et al). 1989;161:1549-53
Curran EM (*see* Nelson and Curran). 1989;161:1553-8
Curran JS (*see* Resnick et al). 1989;161:184-7
Cutter GR (*see* Goldenberg et al). 1989;161:271-7 (Clin. opinion)
Czajkowski K (*see* Wójcicka-Jagodzińska et al). 1989;161:959-64
Czeizel A, Lendvay A. Attempted suicide and pregnancy. 1989;161:497 (Letter)

D

Dabinett LN (*see* Nunley et al). 1989;161:573-6
d'Ablaing G III (*see* Steinleitner et al). 1989;161:1677-81
Dahlman TC, Hellgren MSE, Blombäck M. Thrombosis prophylaxis in pregnancy with use of subcutaneous heparin adjusted by monitoring heparin concentration in plasma. 1989;161:420-5
Daitoh T (*see* Mori et al). 1989;161:207-11
Dalton HP (*see* Soper et al). 1989;161:562-8
Damm P, Mølsted-Pedersen L. Significant decrease in congenital malformations in newborn infants of an unselected population of diabetic women. 1989;161:1163-7
Daniell JF (*see* Baggish and Daniell). 1989;161:877-8
Danskin FH, Neilson JP. Twin-to-twin transfusion syndrome: What are appropriate diagnostic criteria? 1989;161:365-9
Davey DA, MacGillivray I. Semantic changes suggested in hypertensive disorders. 1989;161:1422-3 (Letter reply)
Davidson SR (*see* Reid et al). 1989;161:229-33
Davis JL (*see* Castro et al). 1989;161:1620-3
Davis L (*see* Thurmond et al). 1989;161:83-5
Dawood MY (*see* Hecht et al). 1989;161:1688-93
Dawson MJ (*see* Noyszewski et al). 1989;161:282-8
Day L (*see* Ross et al). 1989;161:1559-65
Daya S. Optimal time in the menstrual cycle for serum progesterone measurement to diagnose luteal phase defects. 1989;161:1009-11

——, Gunby J, Kohut J. Semen predictors of in vitro fertilization and embryo cleavage. 1989;161:1284-9
DeBruyn CS (*see* Chestnut et al). 1989;161:1571-5
de Haan J (*see* van Huisseling et al). 1989;161:1061-4
de las Heras P (*see* Quilez et al). 1989;161:1426-7 (Letter)
Delgado C (*see* Matteri et al). 1989;161:1704-9
Delgado E (*see* Quilez et al). 1989;161:1426-7 (Letter)
Delgado G (*see* Smith et al). 1989;161:911-5
Delke I, Minkoff HL. Perinatal death as a result of social factors. 1989;161:1748 (Letter reply)
—— (*see* Feinkind et al). 1989;161:765-70
Demedeiros N (*see* Poissonnier et al). 1989;161:709-13
Dennis J, Johnson A, Mutch L, Yudkin P, Johnson P. Acid-base status at birth and neurodevelopmental outcome at four and one-half years. 1989;161:213-20
Denny ME (*see* Stumpf and Denny). 1989;161:1375-84 (Curr. dev.)
de Regt RH (*see* Berkowitz et al). 1989;161:146-9
—— (*see* Stiller et al). 1989;161:930-3
DeRosa R, Lenke RR, Kurczynski TW, Persutte WH, Nemes JM. In utero diagnosis of benign fetal macrocephaly. 1989;161:690-2
DeRouen T (*see* Paavonen et al). 1989;161:128-35
Deter RL (*see* Mari et al). 1989;161:1528-31
—— (*see* Stefos et al). 1989;161:179-83
Devoe LD, Searle NA, Ruedrich DA, Castillo RA, Metheny WP. The effects of vibroacoustic stimulation on baseline heart rate, breathing activity, and body movements of normal term fetuses. 1989;161:524-9
—— (*see* Ruedrich et al). 1989;161:188-92
DeVore GR (*see* Brar et al). 1989;161:1519-22
—— (*see* Clark et al). 1989;161:1439-42
De Wilde RL. Recurrence of functional ovarian cysts after laparoscopic fenestration. 1989;161:839 (Letter)
Dharmarajan AM (*see* Katz et al). 1989;161:1361-6
—— (*see* Miyazaki et al). 1989;161:1709-14
Diaz FG (*see* Rodriguez et al). 1989;161:666-9
Dickerson JK. Study design for the Triphasic Randomized Comparative Trial. 1989;161:1392-6 (Symposium)
Dijkhuizen HH (*see* Helmerhorst et al). 1989;161:1191-4
Dinh TV (*see* Nagamani et al). 1989;161:1738-43
Dirks D (*see* Small et al). 1989;161:323-9
DiSaia PJ (*see* Sutton et al). 1989;161:309-12
Divon MY, Girz BA, Lieblich R, Langer O. Clinical management of the fetus with markedly diminished umbilical artery end-diastolic flow. 1989;161:1523-7
——, ——, Sklar A, Guidetti DA, Langer O. Discordant twins—a prospective study of the diagnostic value of real-time ultrasonography combined with umbilical artery velocimetry. 1989;161:757-60
—— (*see* Chervenak et al). 1989;161:753-6
—— (*see* Guidetti et al). 1989;161:91-3
—— (*see* Langer et al). 1989;161:593-9
—— (*see* Langer et al). 1989;161:646-53
Dodson MG (*see* Minhas et al). 1989;161:1714-7
Dodson WC, Hughes CL, Haney AF. Multiple gestation in therapy with intrauterine insemination and menotropin. 1989;161:1752 (Letter reply)
Doody KJ, Carr BR. Aromatase in human fetal tissues. 1989;161:1694-7
Dorcester W (*see* Gocke et al). 1989;161:111-4
Dorsey JH (*see* Baggish et al). 1989;161:60-8
Douros TJ (*see* Macri et al). 1989;161:254-5 (Letter reply)
Droegemueller W, Katta LR, Bright TG, Bowes WA Jr. Triphasic Randomized Clinical Trial: comparative frequency of intermenstrual bleeding. 1989;161:1407-11 (Symposium)
—— (*see* Bowes et al). 1989;161:1402-7 (Symposium)
Droste S (*see* FitzSimmons et al). 1989;161:1174-7
Druschel CM (*see* Berg et al). 1989;161:86-91
Druzin ML, Edersheim TG, Hutson JM, Bond AL. The effect of vibroacoustic stimulation on the nonstress test at gestational ages of thirty-two weeks or less. 1989;161:1476-8
—— (*see* LaGuardia et al). 1989;161:303-6
Dubin NH (*see* Katz et al). 1989;161:1361-6
Duff P. Antibiotics for postcesarean endometritis. 1989;161:1087 (Letter)
Dunlop W (*see* Robson et al). 1989;161:1273-8
Durak EP (*see* Jovanovic-Peterson et al). 1989;161:415-9
Dusik JM (*see* Farrell et al). 1989;161:628-33
Dworetzky M. Allergic vaginitis. 1989;161:1752-3 (Letter)
Dwyer JM (*see* Sanyal et al). 1989;161:446-53
Dyson DC (*see* Ferguson et al). 1989;161:788-95

E

Eades C (*see* LaGuardia et al). 1989;161:303-6
Easterling TR, Benedetti TJ. Techniques differ in use of Doppler ultrasonography. 1989;161:1748-9 (Letter)
——, ——. Techniques differ in use of Doppler ultrasonography. 1989;161:1750 (Letter response)
Economides DL, Nicolaides KH, Gahl WA, Bernardini I, Bottoms S, Evans M. Cordocentesis in the diagnosis of intrauterine starvation. 1989;161:1004-8
——, ——, ——, ——, Evans MI. Plasma amino acids in appropriate- and small-for-gestational-age fetuses. 1989;161:1219-27
—— (*see* Nicolaides et al). 1989;161:996-1001
—— (*see* Panos et al). 1989;161:357-61
Eddie LW, Sutton B, Fitzgerald S, Bell RJ, Johnston PD, Tregear GW. Relaxin in paired samples of serum and milk from women after term and preterm delivery. 1989;161:970-3
Eder C (*see* Rando et al). 1989;161:50-5
—— (*see* Sedlacek et al). 1989;161:55-9
Edersheim TG (*see* Druzin et al). 1989;161:1476-8
Egarter C, Husslein P. First successful intrauterine pregnancy after treatment of tubal pregnancy with prostaglandin $F_{2\alpha}$. 1989;161:904
Egberts J, Wijnands JBG. Uselessness of the phosphatidylglycerol assay for prediction of lung maturity. 1989;161:1417-8 (Letter)
Eggleston MK (*see* Keith et al). 1989;161:1305-13
Egley C (*see* Winn and Egley). 1989;161:1419 (Letter)
Ehrlich CE (*see* Sutton et al). 1989;161:73-5
Elger WAG (*see* Puri et al). 1989;161:248-53
Elias S (*see* Tipton et al). 1989;161:1546-8
Elkins TE, Andersen HF, Barclay M, Mason T, Bowdler N, Anderson G. Court-ordered cesarean section: an analysis of ethical concerns in compelling cases. 1989;161:150-4
Elliott JP. *Candida* warrants concern. 1989;161:503 (Letter)
Ellrodt AG (*see* Madinger et al). 1989;161:657-62
Emancipator K. Analytical and clinical sensitivity and specificity in pregnancy testing. 1989;161:835-6 (Letter reply)
Ershow AG (*see* Brinton et al). 1989;161:121-7
Ervin MG (*see* Castro et al). 1989;161:1337-43
—— (*see* Ross et al). 1989;161:1559-65
Eschenbach DA (*see* Paavonen et al). 1989;161:128-35
Esteban-Altirriba J (*see* Quilez et al). 1989;161:1426-7 (Letter)
Evans JH (*see* Resnick et al). 1989;161:184-7
Evans MI (*see* Economides et al). 1989;161:1004-8

—— (*see* Economides et al). 1989;161:1219-27
Evans WS (*see* Veldhuis et al). 1989;161:1149-58

F

Fabbri R (*see* Porcu et al). 1989;161:174-7
Facchinetti F, Lanzani A, Genazzani AR. Fetal intermediate lobe is stimulated by parturition. 1989;161:1267-70
Fadel HE. Uselessness of the phosphatidylglycerol assay for prediction of lung maturity. 1989;161:1418 (Letter reply)
Fangman JJ (*see* Ferrara et al). 1989;161:1114-8
Farookhi R (*see* Brawer et al). 1989;161:474-80
Farrán I (*see* Andreu et al). 1989;161:1424-5 (Letter)
Farrell EE, Silver RK, Kimberlin LV, Wolf ES, Dusik JM. Impact of antenatal dexamethasone administration on respiratory distress syndrome in surfactant-treated infants. 1989;161:628-33
Fedele L, Bianchi S, Arcaini L, Vercellini P, Candiani GB. Buserelin versus danazol in the treatment of endometriosis-associated infertility. 1989;161:871-6
Feichtinger W, Breitenecker G, Fröhlich H. Prolongation of pregnancy and survival of twin B after loss of twin A at 21 weeks' gestation. 1989;161:891-3
Feinberg RF (*see* Lockwood et al). 1989;161:369-73
Feinkind L, Abulafia O, Delke I, Feldman J, Minkoff H. Screening with Doppler velocimetry in labor. 1989;161:765-70
Feldman J (*see* Feinkind et al). 1989;161:765-70
Fenolio KR (*see* Copeland et al). 1989;161:1289-94
Ferguson JE II, Dyson DC, Holbrook RH Jr, Schutz T, Stevenson DK. Cardiovascular and metabolic effects associated with nifedipine and ritodrine tocolysis. 1989;161:788-95
——, Schutz T, Pershe R, Stevenson DK, Blaschke T. Nifedipine pharmacokinetics during preterm labor tocolysis. 1989;161:1485-90
—— (*see* Hogge et al). 1989;161:520-4
Ferrara TB, Hoekstra RE, Gaziano E, Knox GE, Couser RJ, Fangman JJ. Changing outcome of extremely premature infants (≤26 weeks' gestation and ≤750 gm): survival and follow-up at a tertiary center. 1989;161:1114-8
Fiarman GS (*see* Berkowitz et al). 1989;161:146-9
Fields C (*see* Martin et al). 1989;161:1127-32
Figueroa JP, Honnebier MBOM, Binienda Z, Wimsatt J, Nathanielsz PW. Effect of a 48-hour Δ^4-androstenedione infusion on the pregnant rhesus monkey in the last third of gestation: changes in maternal plasma estradiol concentrations and myometrial contractility. 1989;161:481-6
—— (*see* Hsu et al). 1989;161:467-73
Firth KF, Pipkin FB. Human α- and β-calcitonin gene-related peptides are vasodilators in human chorionic plate vasculature. 1989;161:1318-9
Fisher DA (*see* Castro et al). 1989;161:1337-43
Fisk NM (*see* Hubinont et al). 1989;161:306-7
—— (*see* Nicolini et al). 1989;161:924-7
—— (*see* Nicolini et al). 1989;161:1098-101 (Clin. opinion)
Fitzgerald S (*see* Eddie et al). 1989;161:970-3
FitzSimmons J, Droste S, Shepard TH, Pascoe-Mason J, Chinn A, Mack LA. Long bone growth in fetuses with Down syndrome. 1989;161:1174-7
Flamigni C (*see* Porcu et al). 1989;161:174-7
Floyd RL (*see* Berg et al). 1989;161:86-91
Forbes HW (*see* Valenzuela et al). 1989;161:1566-70
Foreman J (*see* Gagnon et al). 1989;161:1470-6
—— (*see* Gagnon et al). 1989;161:1479-85
Fortunato SJ (*see* Minhas et al). 1989;161:1714-7
Foster JM (*see* Goldenberg et al). 1989;161:271-7 (Clin. opinion)
Fotheringham N (*see* Sacks et al). 1989;161:638-41
—— (*see* Sacks et al). 1989;161:642-5
Fowler LJ (*see* Thorp et al). 1989;161:1245-6
Fraser C (*see* Walker et al). 1989;161:676-9
Freedman S (*see* Schoenfeld et al). 1989;161:1204-5
Frenkel Y (*see* Lipitz et al). 1989;161:1279-84
Fribourg S. Perinatal death as a result of social factors. 1989;161:1748 (Letter)
Friedberg V (*see* Bürger et al). 1989;161:162-7
Fries JK (*see* Hill et al). 1989;161:193-9
Fröhlich H (*see* Feichtinger et al). 1989;161:891-3
Fuchs A-R (*see* Ivanisevic et al). 1989;161:1637-43
Fuith LC. Other routes charted after description of adventurous journey of CA 125. 1989;161:1747 (Letter)
—— (*see* Mitterschiffthaler and Fuith). 1989;161:501 (Letter)
Fujii S (*see* Nanbu et al). 1989;161:462-7
Fujimoto J-I (*see* Nozawa et al). 1989;161:1079-86
Furlough RR (*see* Resnick et al). 1989;161:184-7
Furness ME. Vaginosonographic examination—Routine procedure? 1989;161:499 (Letter)

G

Gable DS (*see* Gallup et al). 1989;161:533-9
Gabrielli S (*see* Winn et al). 1989;161:1540-2
Gagnon R, Foreman J, Hunse C, Patrick J. Effects of low-frequency vibration on human term fetuses. 1989;161:1479-85
——, Hunse C, Bocking AD. Fetal heart rate patterns in the small-for-gestational-age human fetus. 1989;161:779-84
——, ——, Foreman J. Human fetal behavioral states after vibratory stimulation. 1989;161:1470-6
—— (*see* Bocking et al). 1989;161:722-7
Gahl WA (*see* Economides et al). 1989;161:1004-8
—— (*see* Economides et al). 1989;161:1219-27
Gallagher JC (*see* Genant et al). 1989;161:1842-6 (Symposium)
—— (*see* Mazess et al). 1989;161:843-8 (Clin opinion)
Galland L. Allergic vaginitis. 1989;161:1753 (Letter reply)
Gallup DG, Gable DS, Talledo OE, Otken LB Jr. A clinicopathologic study of mixed müllerian tumors of the uterus over a 16-year period—The Medical College of Georgia experience. 1989;161:533-9
—— (*see* Jijon et al). 1989;161:1658-62
Garfield RE, Beier S. Increased myometrial responsiveness to oxytocin during term and preterm labor. 1989;161:454-61
Garite T (*see* Gocke et al). 1989;161:111-4
Garratty G (*see* Sacks and Garratty). 1989;161:928-9
Garsia S (*see* Sideri et al). 1989;161:38-42
Gast MJ, Sims HF, Murdock GL, Gast PM, Strauss AW. Isolation and sequencing of a complementary deoxyribonucleic acid clone encoding human placental 17β-estradiol dehydrogenase: identification of the putative cofactor binding site. 1989;161:1726-31
Gast PM (*see* Gast et al). 1989;161:1726-31
Gáti I (*see* Török and Gáti). 1989;161:1753-4 (Letter)
Gaziano E (*see* Ferrara et al). 1989;161:1114-8
Genant HK, Baylink DJ, Gallagher JC. Estrogens in the prevention of osteoporosis in postmenopausal women. 1989;161:1842-6 (Symposium)
Genazzani AR (*see* Facchinetti et al). 1989;161:1267-70
Genover E (*see* Andreu et al). 1989;161:1424-5 (Letter)
Gentzschein EE (*see* Matteri et al). 1989;161:1704-9
Gerbie AB (*see* Brewer et al). 1989;161:840 (Letter reply)
Ghodgaonkar RB (*see* Katz et al). 1989;161:1361-6

Ghodsi A (*see* Weiner et al). 1989;161:1599-605
—— (*see* Weiner et al). 1989;161:1605-10
Gibbs CP. Pulmonary artery catheterization in severe eclampsia. 1989;161:1089 (Letter)
Gibbs RS (*see* Silver et al). 1989;161:808-12
Gibson RN (*see* Holbrook et al). 1989;161:718-21
Gill TJ III (*see* Ho et al). 1989;161:942-8
Gilstrap LC III, Leveno KJ, Burris J, Williams ML, Little BB. Diagnosis of birth asphyxia on the basis of fetal pH, Apgar score, and newborn cerebral dysfunction. 1989;161:825-30
—— (*see* Little et al). 1989;161:732-4
Gimovsky ML, Nishiyama M. Relapsing polychondritis in pregnancy: a case report and review. 1989;161:332-4
Gingras JL (*see* Hume et al). 1989;161:685-90
Girton L (*see* Mortola et al). 1989;161:1682-7
Girz BA (*see* Chervenak et al). 1989;161:753-6
—— (*see* Divon et al). 1989;161:757-60
—— (*see* Divon et al). 1989;161:1523-7
Gleicher N. Breast disease programs in obstetrics and gynecology: a plea for training in mammography. 1989;161:267-70 (Clin. opinion)
Glezerman M, Piura B, Insler V. Cervical cancer in Jewish women. 1989;161:1186-90
Glynn M (*see* Hannah et al). 1989;161:322-3
Gocke SE, Nageotte MP, Garite T, Towers CV, Dorcester W. Management of the nonvertex second twin: primary cesarean section, external version, or primary breech extraction. 1989;161:111-4
Goedert JJ (*see* Biggar et al). 1989;161:1239-44
Golan A (*see* Pansky et al). 1989;161:393-6
Golbus MS (*see* Pinckert et al). 1989;161:693-8
Goldberg GL, Vermund SH, Schiffman MH, Ritter DB, Spitzer C, Burk RD. Comparison of Cytobrush and cervicovaginal lavage sampling methods for the detection of genital human papillomavirus. 1989;161:1669-72
Goldenberg RL, Cutter GR, Hoffman HJ, Foster JM, Nelson KG, Hauth JC. Intrauterine growth retardation: standards for diagnosis. 1989;161:271-7 (Clin. opinion)
Goldfien A (*see* Lee et al). 1989;161:1044-9
Goldsmith LT, Skurnick JH, Wojtczuk AS, Linden M, Kuhar MJ, Weiss G. The antagonistic effect of oxytocin and relaxin on rat uterine segment contractility. 1989;161:1644-9
Goldstein I. Race and gender factors omitted from results. 1989;161:502-3 (Letter reply)
——, Reece EA, O'Connor TZ, Hobbins JC. Estimating gestational age in the term pregnancy with a model based on multiple indices of fetal maturity. 1989;161:1235-8
Goldstein M (*see* Ogburn et al). 1989;161:728-32
—— (*see* Stonestreet et al). 1989;161:894-9
Gonen R (*see* Hannah et al). 1989;161:322-3
Gooden MD. Defining "primiparas." 1989;161:1417 (Letter)
Goodlin RC. Maternal hematocrit and premature labor. 1989;161:1750 (Letter)
——. Oxytocin may explain neonatal seizures. 1989;161:259 (Letter)
——. Role of ferritin supported in diagnosis of anemias of pregnancy. 1989;161:258 (Letter)
Goshen R (*see* Palti et al). 1989;161:1147-8
Gosset M (*see* Poissonnier et al). 1989;161:709-13
Gotto AM Jr (*see* Patsch et al). 1989;161:1396-401 (Symposium)
Grannum PA (*see* Copel et al). 1989;161:341-4
—— (*see* Copel et al). 1989;161:361-5
Grant S (*see* Weiner et al). 1989;161:706-8
—— (*see* Weiner et al). 1989;161:714-7
Greelish JP (*see* Hatjis et al). 1989;161:1015-9
Green JJ (*see* Copel et al). 1989;161:341-4
—— (*see* Copel et al). 1989;161:361-5
Greene CL (*see* Luder and Greene). 1989;161:1102-5 (Clin. opinion)
Greene MF, Hare JW, Krache M, Phillippe M, Barss VA, Saltzman DH, Nadel A, Younger MD, Heffner L, Scherl JE. Prematurity among insulin-requiring diabetic gravid women. 1989;161:106-11
Greenspoon JS, Settlage RH. Isolation of human immunodeficiency virus from the placenta or from maternal blood contaminating the placenta? 1989;161:501-2 (Letter)
—— (*see* Madinger et al). 1989;161:657-62
—— (*see* Masaki et al). 1989;161:680-4
—— (*see* Sacks et al). 1989;161:638-41
—— (*see* Sacks et al). 1989;161:642-5
Greiss FC Jr. The challenge of change revisited. 1989;161:505-9 (Pres. address)
—— (*see* May and Greiss). 1989;161:555-61
Griffin G (*see* Weaver et al). 1989;161:1331-7
Grimes DA. Evaluating triphasic oral contraceptives: the rationale for a randomized trial. 1989;161:1390-2 (Symposium)
Grizzle WE (*see* Alvarez et al). 1989;161:1653-7
Groff DE (*see* Smith et al). 1989;161:911-5
Grogan DM (*see* Hatjis and Grogan). 1989;161:493-6
—— (*see* Hatjis and Grogan). 1989;161:1633-6
—— (*see* Hatjis et al). 1989;161:1628-32
Guidetti DA, Divon My, Langer O. Postdate fetal surveillance: Is 41 weeks too early? 1989;161:91-3
—— (*see* Divon et al). 1989;161:757-60
Gunby J (*see* Daya et al). 1989;161:1284-9
Gurr DE (*see* Smith et al). 1989;161:879-80
Guzick D (*see* Hill et al). 1989;161:193-9
Guzick DS (*see* Parker et al). 1989;161:795-802

H

Haas J (*see* Petru et al). 1989;161:937-41
Hadi HA, Albazzaz SJ. Cardiac isoenzymes and electrocardiographic changes during ritodrine tocolysis. 1989;161:318-21
Hahlin M (*see* Lindblom et al). 1989;161:397-400
Hahn RG. Compliance considerations with estrogen replacement: withdrawal bleeding and other factors. 1989;161:1854-8 (Symposium)
Hallman M, Arjomaa P, Hoppu K, Teramo K, Akino T. Surfactant proteins in the diagnosis of fetal lung maturity. II. The 35 kd protein and phospholipids in complicated pregnancy. 1989;161:965-9
——, Bry K, Pitkänen O. Ceramide lactoside in amniotic fluid: high concentration in chorioamnionitis and in preterm labor. 1989;161:313-8
Halme J. Release of tumor necrosis factor-α by human peritoneal macrophages in vivo and in vitro. 1989;161:1718-25
Hamer C (*see* Newman et al). 1989;161:547-5
Hamilton CJCM, Hoogland HJ. Longitudinal ultrasonographic study of the ovarian suppressive activity of a low-dose triphasic oral contraceptive during correct and incorrect pill intake. 1989;161:1159-62
Hammond CB. Estrogen replacement therapy: what the future holds. 1989;161:1864-8 (Symposium)
Hammond MG. Dosage and duration differences lead to diverse conclusions. 1989;161:1745-6 (Letter reply)
Haney AF (*see* Dodson et al). 1989;161:1752 (Letter reply)
Haning RV Jr, Curet LB, Poole WK, Boehnlein LM, Kuzma

DL, Meier SM. Effects of fetal sex and dexamethasone on preterm maternal serum concentrations of human chorionic gonadotropin, progesterone, estrone, estradiol, and estriol. 1989;161:1549-53
Hankins GDV (*see* Clark et al). 1989;161:1439-42
Hanna N (*see* Copel et al). 1989;161:361-5
Hannah ME, Gonen R, Mocarski EJM, Cameron R, Blendis L, Glynn M. Elevated liver enzymes and thrombocytopenia in the third trimester of pregnancy: an unusual case report and a review of the literature. 1989;161:322-3
Hansford H (*see* Bocking et al). 1989;161:722-7
Harbert GM Jr (*see* Hogge et al). 1989;161:520-4
Hare JW (*see* Greene et al). 1989;161:106-11
Hargrove JT, Maxson WS, Wentz AC. Absorption of oral progesterone is influenced by vehicle and particle size. 1989;161:948-51
Hartge P, Schiffman MH, Hoover R, McGowan L, Lesher L, Norris HJ. A case-control study of epithelial ovarian cancer. 1989;161:10-6 (Clin. opinion)
Hasaart THM (*see* van Huisseling et al). 1989;161:1061-4
Hashimoto K (*see* Hatjis et al). 1989;161:1015-9
Hasty L (*see* Rando et al). 1989;161:50-5
—— (*see* Sedlacek et al). 1989;161:55-9
Hata J-I (*see* Nozawa et al). 1989;161:1079-86
Hatjis CG. Number clarification requested. 1989;161:1746 (Letter)
——, Greelish JP, Kofinas AD, Stroud A, Hashimoto K, Rose JC. Atrial natriuretic factor maternal and fetal concentrations in severe preeclampsia. 1989;161:1015-9
——, Grogan DM. Atrial natriuretic peptide receptors in guinea pig placentas. 1989;161:493-6
——, ——. Pregnancy-induced changes in the interaction of guinea pig myometrial β-adrenergic receptors with *l*-isoproterenol. 1989;161:1633-6
——, ——, Koritnik DR. Treatment of oophorectomized guinea pigs with intrauterine 17β-estradiol pellets may modulate myometrial β-adrenergic receptor binding properties. 1989;161:1628-32
——, Koritnik DR. Up-regulation of guinea pig myometrial adenylate cyclase activity by intrauterine estradiol and progesterone pellets. 1989;161:1073-8
—— (*see* Kofinas et al). 1989;161:1536-9
Hausman DB (*see* Bue et al). 1989;161:234-40
Hauth JC (*see* Goldenberg et al). 1989;161:271-7 (Clin. opinion)
—— (*see* Parker et al). 1989;161:795-802
Hazzard WR. Estrogen replacement and cardiovascular disease: serum lipids and blood pressure effects. 1989;161:1847-53 (Symposium)
Hecht BR, Khan-Dawood FS, Dawood MY. Peri-implantation phase endometrial estrogen and progesterone receptors: effect of ovulation induction with clomiphene citrate. 1989;161:1688-93
Hedrick LA (*see* Baill et al). 1989;161:334-6
Heffner L (*see* Greene et al). 1989;161:106-11
Heiderich KJ (*see* Cohen et al). 1989;161:1013-4
Heilskov J (*see* Weiner et al). 1989;161:714-7
—— (*see* Weiner et al). 1989;161:1498-501
Hellgren MSE (*see* Dahlman et al). 1989;161:420-5
Helmer H (*see* Ivanisevic et al). 1989;161:1637-43
Helmerhorst TJM, Wijnen HJA, Tjioe GSTD, Dijkhuizen HH, Calame JJ, Kenemans P, Stolk HG. Colposcopic findings and intraepithelial neoplasia in diethylstilbestrol-exposed offspring. 1989;161:1191-4
Henderson BE. The cancer question: an overview of recent epidemiologic and retrospective data. 1989;161:1859-64 (Symposium)
Henderson C (*see* Moore et al). 1989;161:849-55 (Clin. opinion)
Hendricks SK, Sorensen TK, Wang KY, Bushnell JM, Seguin EM, Zingheim RW. Doppler umbilical artery waveform indices—normal values from fourteen to forty-two weeks. 1989;161:761-5
Hertz RH (*see* Moore et al). 1989;161:849-55 (Clin. opinion)
Heyl PS (*see* Landy et al). 1989;161:308
Hill JA, Anderson DJ. Lymphocyte activity in the presence of peritoneal fluid from fertile women and infertile women with and without endometriosis. 1989;161:861-4
Hill LM, Guzick D, Thomas ML, Fries JK. Fetal radius length: a critical evaluation of race as a factor in gestational age assessment. 1989;161:193-9
Hill RM (*see* Stefos et al). 1989;161:179-83
Hill T (*see* Clark et al). 1989;161:1439-42
Hill WC, Carlson JR, Bolton V. Isolation of human immunodeficiency virus from the placenta or from maternal blood contaminating the placenta? 1989;161:502 (Letter reply)
Hillard PJA. The patient's reaction to side effects of oral contraceptives. 1989;161:1412-5 (Symposium)
Hillier S (*see* Paavonen et al). 1989;161:128-35
Hirsch J (*see* Chervenak et al). 1989;161:753-6
Ho H-N, Gill TJ III, Klionsky B, Ouyang P-C, Hsieh C-Y, Seski J, Kunschner A. Differences between white and Chinese populations in human leukocyte antigen sharing and gestational trophoblastic tumors. 1989;161:942-8
Hobart ED (*see* Silver et al). 1989;161:784-7
Hobbins JC (*see* Copel et al). 1989;161:341-4
—— (*see* Copel et al). 1989;161:361-5
—— (*see* Goldstein et al). 1989;161:1235-8
—— (*see* Lockwood et al). 1989;161:369-73
—— (*see* Romero et al). 1989;161:336-41
—— (*see* Romero et al). 1989;161:817-24
—— (*see* Winn et al). 1989;161:1540-2
Hobel CJ (*see* Castro et al). 1989;161:623-7
—— (*see* Castro et al). 1989;161:1620-3
Hochberg H (*see* Small et al). 1989;161:323-9
Hod M (*see* Schoenfeld et al). 1989;161:1204-5
Hoekstra RE (*see* Ferrara et al). 1989;161:1114-8
Hoffman HJ (*see* Goldenberg et al). 1989;161:271-7 (Clin. opinion)
Hofmann G (*see* Archer et al). 1989;161:581-92
Hogge WA, Thiagarajah S, Ferguson JE II, Schnatterly PT, Harbert GM Jr. The role of ultrasonography and amniocentesis in the evaluation of pregnancies at risk for neural tube defects. 1989;161:520-4
Hohenfellner R (*see* Bürger et al). 1989;161:162-7
Hohimer AR, Bissonnette JM. Effects of cephalic hypotension, hypertension, and barbiturates on fetal cerebral blood flow and metabolism. 1989;161:1344-51
—— (*see* Chao et al). 1989;161:221-8
Holbrook RH Jr, Voss EM, Gibson RN. Ovine fetal cardiorespiratory response to nicardipine. 1989;161:718-21
—— (*see* Ferguson et al). 1989;161:788-95
Hollister MC (*see* Reid et al). 1989;161:229-33
Holmes KK (*see* Paavonen et al). 1989;161:128-35
Honnebier MBOM (*see* Figueroa et al). 1989;161:481-6
—— (*see* Hsu et al). 1989;161:467-73
Hoogland HJ (*see* Hamilton and Hoogland). 1989;161:1159-62
Hoover R (*see* Hartge et al). 1989;161:10-6 (Clin. opinion)

Hoppu K (*see* Hallman et al). 1989;161:965-9
Horimoto N, Koyanagi T, Nagata S, Nakahara H, Nakano H. Concurrence of mouthing movement and rapid eye movement/non–rapid eye movement phases with advance in gestation of the human fetus. 1989;161:344-51
Hoskins IA, Katz J, Ordorica SA, Young BK. Esterase activity in second- and third-trimester amniotic fluid: an indicator of chorioamnionitis. 1989;161:1543-5
Howerton T (*see* Keith et al). 1989;161:1305-13
Hsieh C-Y (*see* Ho et al). 1989;161:942-8
Hsu HW, Figueroa JP, Honnebier MBOM, Wentworth R, Nathanielsz PW. Power spectrum analysis of myometrial electromyogram and intrauterine pressure changes in the pregnant rhesus monkey in late gestation. 1989;161:467-73
Hu MG (*see* Macri et al). 1989;161:254-5 (Letter reply)
Hubel CA, Roberts JM, Taylor RN, Musci TJ, Rogers GM, McLaughlin MK. Lipid peroxidation in pregnancy: new perspectives on preeclampsia. 1989;161:1025-34 (Curr. dev.)
—— (*see* Roberts et al). 1989;161:1200-4
Hubinont C, Fisk NM, Nicolini U, Rodeck CH. Fetal hyperinsulinism in anencephaly. 1989;161:306-7
—— (*see* Nicolini et al). 1989;161:924-7
Huchet J (*see* Poissonnier et al). 1989;161:709-13
Hudson J (*see* Weiner et al). 1989;161:706-8
Hughes CL (*see* Dodson et al). 1989;161:1752 (Letter reply)
Huhta JC (*see* Mari et al). 1989;161:1528-31
Hulka JF (*see* Wells and Hulka). 1989;161:1124-6
Hume RF Jr, O'Donnell KJ, Stanger CL, Killam AP, Gingras JL. In utero cocaine exposure: observations of fetal behavioral state may predict neonatal outcome. 1989;161:685-90
Humme J (*see* Ross et al). 1989;161:1559-65
Humphreys-Beher MG, Blackwell RE. Identification of a deoxyribonucleic acid allelic variant for β-4 galactosyltransferase expression associated with male sperm binding/penetration infertility (1989;160:1160-5). 1989;161:816 (Correction)
Hunse C (*see* Gagnon et al). 1989;161:779-84
—— (*see* Gagnon et al). 1989;161:1470-6
—— (*see* Gagnon et al). 1989;161:1479-85
Hunter DJS. Screening for gestational diabetes. 1989;161:1423-4 (Letter)
Hunter S (*see* Robson et al). 1989;161:1273-8
Hurt WG, Bodurtha JN, McCall JB, Ali MM. Seminoma in pubertal patient with androgen insensitivity syndrome. 1989;161:530-1
Husby H (*see* Jensen et al). 1989;161:951-2
Husslein P (*see* Egarter and Husslein). 1989;161:904
Hutson JM (*see* Druzin et al). 1989;161:1476-8

I

Iizuka R (*see* Nozawa et al). 1989;161:1079-86
Illingworth DR (*see* Burry et al) (1989;160:1454-61). 1989;161:1755 (Correction)
Imanaka M, Ogita S, Sugawa T. Saline solution amnioinfusion for oligohydramnios after premature rupture of the membranes. 1989;161:102-6
Inatomi K (*see* Kyodo et al). 1989;161:94-6
Insler V (*see* Glezerman et al). 1989;161:1186-90
Irahara M (*see* Mori et al). 1989;161:207-11
Ivanisevic M, Behrens O, Helmer H, Fuchs A-R. Vasopressin receptors in human pregnant myometrium and decidua: interactions with oxytocin and vasopressin agonists and antagonists. 1989;161:1637-43
Iwamori M (*see* Nozawa et al). 1989;161:1079-86

J

Jaakkola U-M (*see* Pulkkinen and Jaakkola). 1989;161:934-7
Jacobson JD, Cousins L. A population-based study of maternal and perinatal outcome in patients with gestational diabetes. 1989;161:981-6
Jaffe CC (*see* Copel et al). 1989;161:361-5
Jaffe R (*see* Abramowicz et al). 1989;161:1137-40
Jauniaux E, Campbell S, Vyas S. The use of color Doppler imaging for prenatal diagnosis of umbilical cord anomalies: report of three cases. 1989;161:1195-7
Jensen LK, Svanegaard J, Husby H. Atrial natriuretic peptide during the menstrual cycle. 1989;161:951-2
Jensen LP (*see* Burkett et al). 1989;161:540-7
Jick H (*see* Milunsky et al). 1989;161:291-7
Jick SS (*see* Milunsky et al). 1989;161:291-7
Jijon AJ, Gallup DG, Behzadian MA, Metheny WP. Assessment of epidermal growth factor in the healing process of clean full-thickness skin wounds. 1989;161:1658-62
Johnson A (*see* Dennis et al). 1989;161:213-20
Johnson MD, Tho SPT, Behzadian A, McDonough PG. Molecular scanning of Yq11 (interval 6) in men with Sertoli-cell-only syndrome. 1989;161:1732-7
Johnson MJ (*see* Russ et al). 1989;161:401-6
Johnson N, Johnson VA. Continuous fetal monitoring with a pulse oximeter: a case of cord compression. 1989;161:1295-6
——, Lilford RJ, Sharpe D. A new surgical technique to treat refractory labial fusion in the elderly. 1989;161:289-90
Johnson P (*see* Dennis et al). 1989;161:213-20
Johnson TRB (*see* Thomas et al). 1989;161:141-5
Johnson VA (*see* Johnson and Johnson). 1989;161:1295-6
Johnston DA (*see* Thorp et al). 1989;161:670-5
Johnston PD (*see* Eddie et al). 1989;161:970-3
Jouppila P (*see* Räsänen et al). 1989;161:136-40
Jovanovic-Peterson L, Durak EP, Peterson CM. Randomized trial of diet versus diet plus cardiovascular conditioning on glucose levels in gestational diabetes. 1989;161:415-9
Jovkovsky V (*see* Veille et al). 1989;161:1610-4

K

Kaali SG, Szigetvari IA, Bartfai GS. The frequency and management of uterine perforations during first-trimester abortions. 1989;161:406-8
Kadar N. Specifics of specificity and sensitivity. 1989;161:1416 (Letter)
Kalhan SC (*see* Rossi et al). 1989;161:1106-10
Källén B (*see* Mazze and Källén). 1989;161:1178-85
Kalo-Klein A, Witkin SS. *Candida albicans*: cellular immune system interactions during different stages of menstrual cycle. 1989;161:1132-6
Kalstone C. Methicillin-resistant staphylococcal mastitis. 1989;161:120
Kamada M (*see* Mori et al). 1989;161:207-11
Kambam J, Perry SM, Smith BE. Effect of magnesium and preeclampsia on plasma cholinesterase activity. 1989;161:1754-5 (Letter reply)
Kanaan C (*see* Veille and Kanaan). 1989;161:1502-7
Kanwar AJ, Kaur S, Abraham A, Nanda A. Pemphigus in pregnancy. 1989;161:995-6
Kassis AI (*see* Muto et al). 1989;161:1206-12
Kasturi RV (*see* Macri et al). 1989;161:254-5 (Letter reply)
Katta LR (*see* Bowes et al). 1989;161:1402-7 (Symposium)
—— (*see* Droegemueller et al). 1989;161:1407-11 (Symposium)

Katz E, Dharmarajan AM, Sueoka K, Ghodgaonkar RB, Dubin NH, Wallach EE. Effects of systemic administration of indomethacin on ovulation, luteinization, and steroidogenesis in the rabbit ovary. 1989;161:1361-6
Katz J (*see* Hoskins et al). 1989;161:1543-5
Katz VL, Seeds JW. Fetal and neonatal cardiovascular complications from β-sympathomimetic therapy for tocolysis. 1989;161:1-9 (Clin. opinion)
—— (*see* Petersen et al). 1989;161:509-12
—— (*see* Thorp et al). 1989;161:889-90
—— (*see* Thorp et al). 1989;161:1245-6
Kaufman RH, Bornstein J, Adam E, Adler-Storthz K. Presence of human papillomavirus deoxyribonucleic acid. 1989;161:1420 (Letter reply)
Kauppila A (*see* Telimaa et al). 1989;161:866-71
Kaur S (*see* Kanwar et al). 1989;161:995-6
Keith JC Jr, Miller K, Eggleston MK, Kutruff J, Howerton T, Konczal C, McDaniels C. Effects of thromboxane synthetase inhibition on maternal-fetal homeostasis in gravid ewes with ovine pregnancy-induced hypertension. 1989;161:1305-13
Kelly JC, Petrocik E, Wassman ER. Amniotic fluid acetylcholinesterase ratios in prenatal diagnosis of fetal abnormalities. 1989;161:703-5
—— (*see* Petrocik et al). 1989;161:1168-73
Kemmann E (*see* Corsan et al). 1989;161:1751-2 (Letter)
Kenemans P (*see* Helmerhorst et al). 1989;161:1191-4
Kessel RWI (*see* Cohen et al). 1989;161:1013-4
Khan-Dawood FS (*see* Hecht et al). 1989;161:1688-93
Khodiguian N (*see* Artal et al). 1989;161:1464-9
Kholkute SD (*see* Puri et al). 1989;161:248-53
Khoury AN (*see* Landy et al). 1989;161:308
Kieckens L (*see* Ballegeer et al). 1989;161:432-6
Killam AP (*see* Hume et al). 1989;161:685-90
Kimberlin LV (*see* Farrell et al). 1989;161:628-33
Kingsland CR, Collins JV, Rizk B, Mason BA. Ovarian hyperstimulation presenting as acute hydrothorax after in vitro fertilization. 1989;161:381-2
Kirkinen P (*see* Räsänen et al). 1989;161:136-40
Kirshenbaum N (*see* Bracero et al). 1989;161:388-93
Kirshon B (*see* Mari et al). 1989;161:1514-8
—— (*see* Mari et al). 1989;161:1528-31
Kirz DS (*see* Pircon et al). 1989;161:775-9
Kitayama S (*see* Nakano et al). 1989;161:905-10
Kitchin JD III (*see* Nunley et al). 1989;161:573-6
Kleinman CS (*see* Copel et al). 1989;161:361-5
Klionsky B (*see* Ho et al). 1989;161:942-8
Knapp RC (*see* Muto et al). 1989;161:1206-12
Knapstein PG (*see* Bürger et al). 1989;161:162-7
Knox GE (*see* Ferrara et al). 1989;161:1114-8
Knuppel RA (*see* Morales et al). 1989;161:568-72
—— (*see* Sims et al). 1989;161:532-3
Kofinas AD, Penry M, Swain M, Hatjis CG. Effect of placental laterality on uterine artery resistance and development of preeclampsia and intrauterine growth retardation. 1989;161:1536-9
—— (*see* Hatjis et al). 1989;161:1015-9
Kohut J (*see* Daya et al). 1989;161:1284-9
Kolins MD. Specifics of specificity and sensitivity. 1989;161:1416-7 (Letter reply)
Kolp LA (*see* Nunley et al). 1989;161:573-6
Konczal C (*see* Keith et al). 1989;161:1305-13
Konishi I (*see* Nanbu et al). 1989;161:462-7
Koonings PP (*see* Bergman et al). 1989;161:97-101
Koos BJ (*see* Salvador and Koos) (1989;160:1043-7). 1989;161:669 (Correction)
Koritnik DR (*see* Hatjis and Koritnik). 1989;161:1073-8
—— (*see* Hatjis et al). 1989;161:1628-32
Koutsilieris M. Human uterus-derived growth substances for rat bone cells and fibroblasts. 1989;161:1313-7
Koyanagi T (*see* Horimoto et al). 1989;161:344-51
Krache M (*see* Greene et al). 1989;161:106-11
Krantz DA (*see* Macri et al). 1989;161:254-5 (Letter reply)
Kreitzer LA (*see* Block et al). 1989;161:1576-9
Kuczyńska-Sicińska J (*see* Wójcicka-Jagodzińska et al). 1989;161:959-64
Kuhar MJ (*see* Goldsmith et al). 1989;161:1644-9
Kuhn M (*see* Lorenz and Kuhn). 1989;161:263-6 (Clin. opinion)
Kuhnert BR (*see* Lazebnik et al). 1989;161:437-40
Kuhnert PM (*see* Lazebnik et al). 1989;161:437-40
Kumar R (*see* Minhas et al). 1989;161:1714-7
Kunschner A (*see* Ho et al). 1989;161:942-8
Kuo C-C (*see* Paavonen et al). 1989;161:128-35
Kuo T-C (*see* Miyazaki et al). 1989;161:1709-14
Kurczynski TW (*see* DeRosa et al). 1989;161:690-2
Kurtzman JT (*see* Thorp et al). 1989;161:1245-6
Kutruff J (*see* Keith et al). 1989;161:1305-13
Kuzma DL (*see* Haning et al). 1989;161:1549-53
Kyodo Y, Inatomi K, Abe T. Sarcoma associated wtih pregnancy. 1989;161:94-6

L

Laferrere B (*see* Wurtman et al). 1989;161:1228-34
LaGuardia KD, Druzin ML, Eades C. Maternity shelter care for adolescents: its effect on incidence of low birth weight. 1989;161:303-6
Lahousen M (*see* Petru et al). 1989;161:937-41
Lai A (*see* Burkett et al). 1989;161:540-7
Landesman S (*see* Biggar et al). 1989;161:1239-44
Landy HJ, Khoury AN, Heyl PS. Antenatal ultrasonographic diagnosis of fetal seizure activity. 1989;161:308
Lange IR (*see* Ryan et al). 1989;161:1297-304 (Curr. invest.)
Langer O, Anyaegbunam A, Brustman L, Divon M. Management of women with one abnormal oral glucose tolerance test value reduces adverse outcome in pregnancy. 1989;161:593-9
——, Levy J, Brustman L, Anyaegbunam A, Merkatz R, Divon M. Glycemic control in gestational diabetes mellitus—How tight is tight enough: small for gestational age versus large for gestational age? 1989;161:646-53
—— (*see* Anyaegbunam et al). 1989;161:654-6
—— (*see* Chervenak et al). 1989;161:753-6
—— (*see* Divon et al). 1989;161:757-60
—— (*see* Divon et al). 1989;161:1523-7
—— (*see* Guidetti et al). 1989;161:91-3
Langer R (*see* Pansky et al). 1989;161:393-6
—— (*see* Ron-El et al). 1989;161:211-2
Lanzani A (*see* Facchinetti et al). 1989;161:1267-70
Larsen M (*see* Poissonnier et al). 1989;161:709-13
La Vecchia C (*see* Sideri et al). 1989;161:38-42
LaVoie M (*see* Berg et al). 1989;161:86-91
Lazebnik N, Kuhnert BR, Kuhnert PM. Zinc, cadmium, and hypertension in parturient women. 1989;161:437-40
Leake RD (*see* Castro et al). 1989;161:1337-43
Lebo RV (*see* Pinckert et al). 1989;161:693-8
Lechner W. Introduction of hexoprenaline into the treatment of preterm labor. 1989;161:1090-1 (Letter)
Ledbetter DH (*see* Copeland et al). 1989;161:1289-94
Ledger WJ. Criticism of publication of pharmaceutical-sup-

ported research results draws response. 1989;161:839-40 (Letter)
Leduc L (*see* Lee et al). 1989;161:1012-3
Lee JK, Goldfien A, Lykins DL, Roberts JM. Pulmonary β-adrenergic receptors and response do not mature precociously in growth-retarded rabbit fetuses. 1989;161:1044-9
Lee W, Leduc L, Cotton DB. Ultrasonographic guidance for central venous access during pregnancy. 1989;161:1012-3
——, Rokey R. Techniques differ in use of Doppler ultrasonography. 1989;161:1749-50 (Letter reply)
——, ——, Miller J, Cotton DB. Maternal hemodynamic effects of uterine contractions by M-mode and pulsed-Doppler echocardiography. 1989;161:974-7
—— (*see* Clark et al). 1989;161:1439-42
—— (*see* Mari et al). 1989;161:1514-8
Leiberman JR, Mazor M, Cohen A. Detection of fetal blood. 1989;161:257 (Letter)
Lendvay A (*see* Czeizel and Lendvay). 1989;161:497 (Letter)
Lenke RR. Data sought to link adverse pregnancy outcome with maternal serum α-fetoprotein levels in black gravid women. 1989;161:254 (Letter)
—— (*see* DeRosa et al). 1989;161:690-2
Lepisto EM (*see* Muto et al). 1989;161:1206-12
Lesher L (*see* Hartge et al). 1989;161:10-6 (Clin. opinion)
Lesser KB (*see* Timor-Tritsch et al). 1989;161:157-61
Lessey BA (*see* Soisson et al). 1989;161:1258-63
Leung BS (*see* Borazjani et al). 1989;161:1253-7
Leuschen P (*see* Rayburn et al). 1989;161:202-6
Leveno KJ (*see* Gilstrap et al). 1989;161:825-30
—— (*see* Lucas et al). 1989;161:426-31
Levin JH (*see* Steinleitner et al). 1989;161:1677-81
Levy J (*see* Langer et al). 1989;161:646-53
Li J-Y (*see* Brinton et al). 1989;161:121-7
Lieberman E, Ryan KJ, Monson RR, Schoenbaum SC. Maternal hematocrit and premature labor. 1989;161:1750-1 (Letter reply)
Lieblich R (*see* Divon et al). 1989;161:1523-7
Lilford RJ (*see* Johnson et al). 1989;161:289-90
—— (*see* Thornton and Lilford). 1989;161:256 (Letter)
Limaye NS, Tchabo J-G. Asymptomatic thrombocytopenia associated with chorioangioma of placenta. 1989;161:76-7
Lindblom B, Hahlin M, Sjöblom P. Serial human chorionic gonadotropin determinations by fluoroimmunoassay for differentiation between intrauterine and ectopic gestation. 1989;161:397-400
Linden M (*see* Goldsmith et al). 1989;161:1644-9
Lindheim S (*see* Rando et al). 1989;161:50-5
—— (*see* Sedlacek et al). 1989;161:55-9
Lipitz S, Reichman B, Paret G, Modan M, Shalev J, Serr DM, Mashiach S, Frenkel Y. The improving outcome of triplet pregnancies. 1989;161:1279-84
Little BB, Bawdon RE, Christmas JT, Sobhi S, Gilstrap LC III. Pharmacokinetics of azidothymidine during late pregnancy in Long-Evans rats. 1989;161:732-4
—— (*see* Gilstrap et al). 1989;161:825-30
Llauradó A (*see* Quilez et al). 1989;161:1426-7 (Letter)
Lobo RA (*see* Matteri et al). 1989;161:1704-9
—— (*see* Sauer et al). 1989;161:380-1
—— (*see* Steinleitner et al). 1989;161:1677-81
Lockwood CJ, Romero R, Feinberg RF, Clyne LP, Coster B, Hobbins JC. The prevalence and biologic significance of lupus anticoagulant and anticardiolipin antibodies in a general obstetric population. 1989;161:369-73
—— (*see* Peters et al). 1989;161:297-300
Longo LD (*see* Valenzuela and Longo). 1989;161:1615-9
Lorenz RP, Kuhn M. Multidisciplinary team counseling for fetal anomalies. 1989;161:263-6 (Clin. opinion)
Lovell G (*see* McGregor et al). 1989;161:987-95
Lowensohn RI (*see* Thurmond et al). 1989;161:83-5
Lucas MJ, Leveno KJ, Williams ML, Raskin P, Whalley PJ. Early pregnancy glycosylated hemoglobin, severity of diabetes, and fetal malformations. 1989;161:426-31
Luder AS, Greene CL. Maternal phenylketonuria and hyperphenylalaninemia: implications for medical practice in the United States. 1989;161:1102-5 (Clin. opinion)
Ludomirsky A (*see* Sedlacek et al). 1989;161:55-9
Lund P (*see* Smith et al). 1989;161:879-80
Lykins DL (*see* Lee et al). 1989;161:1044-9
Lynch L (*see* Wilkins and Lynch). 1989;161:1746 (Letter reply)

M

Mabie WC, Ratts TE, Sibai BM. The central hemodynamics of severe preeclampsia. 1989;161:1443-8
MacDonald PC (*see* Parker et al). 1989;161:795-802
MacGillivray I (*see* Davey and MacGillivray). 1989;161:1422-3 (Letter reply)
MacGregor SN, Socol ML, Pielet BW, Sholl JS, Silver RK. Prediction of hematocrit decline after intravascular fetal transfusion. 1989;161:1491-3
—— (*see* Neerhof et al). 1989;161:633-8
—— (*see* Silver et al). 1989;161:784-7
—— (*see* Socol et al). 1989;161:255-6 (Letter reply)
Mack LA (*see* FitzSimmons et al). 1989;161:1174-7
MacKenzie IZ, Castle B, Bellinger J. Transfer of prostaglandins to the fetus after prostaglandin E_2 vaginal pessary administration. 1989;161:920-3
MacLaughlin DS (*see* Milunsky et al). 1989;161:291-7
Macri JN, Kasturi RV, Hu MG, Krantz DA, Douros TJ, Sajda P, Cook EJ. Data sought to link adverse pregnancy outcome with maternal serum α-fetoprotein levels in black gravid women. 1989;161:254-5 (Letter reply)
Madinger NE, Greenspoon JS, Ellrodt AG. Pneumonia during pregnancy: Has modern technology improved maternal and fetal outcome? 1989;161:657-62
Magenis RE (*see* Clark et al). 1989;161:301-2
Manogue KR (*see* Romero et al). 1989;161:336-41
Margulies M (*see* Voto and Margulies). 1989;161:255 (Letter)
Mari G, Kirshon B, Moise KJ Jr, Lee W, Cotton DB. Doppler assessment of the fetal and uteroplacental circulation during nifedipine therapy for preterm labor. 1989;161:1514-8
——, Moise KJ Jr, Deter RL, Kirshon B, Huhta JC, Carpenter RJ Jr, Cotton DB. Doppler assessment of the pulsatility index of the middle cerebral artery during constriction of the fetal ductus arteriosus after indomethacin therapy. 1989;161:1528-31
Markovits Y (*see* Ophir et al). 1989;161:25-8
Markush L (*see* Villar et al). 1989;161:1019-24
Marmelstein L (*see* Tuttle et al). 1989;161:1319-23
Martin AD, Notelovitz M, Fields C, O'kroy J. Predicting maximal oxygen uptake from treadmill testing in trained and untrained women. 1989;161:1127-32
Martinez E (*see* Weiner et al). 1989;161:1599-605
—— (*see* Weiner et al). 1989;161:1605-10
Marx GF. Effect of magnesium and preeclampsia on plasma cholinesterase activity. 1989;161:1754 (Letter)
Masaki DI, Greenspoon JS, Ouzounian JG. Measurement of cardiac output in pregnancy by thoracic electrical bioimpedance and thermodilution. 1989;161:680-4
—— (*see* Artal et al). 1989;161:1464-9
—— (*see* Rodriguez et al). 1989;161:666-9

Mashiach S (*see* Lipitz et al). 1989;161:1279-84
Mason BA (*see* Kingsland et al). 1989;161:381-2
Mason T (*see* Elkins et al). 1989;161:150-4
Matt DW (*see* Van Voorhis et al). 1989;161:1698-703
Matteri RK, Stanczyk FZ, Gentzschein EE, Delgado C, Lobo RA. Androgen sulfate and glucuronide conjugates in nonhirsute and hirsute women with polycystic ovarian syndrome. 1989;161:1704-9
Maxson WS (*see* Hargrove et al). 1989;161:948-51
May WJ, Greiss FC Jr. Maternal mortality in North Carolina: a forty-year experience. 1989;161:555-61
Mayhall CG (*see* Soper et al). 1989;161:562-8
Mazess RB, Gallagher JC, Notelovitz M, Schiff I, Utian W. Monitoring skeletal response to estrogen. 1989;161:843-8 (Clin opinion)
Mazor M (*see* Leiberman et al). 1989;161:257 (Letter)
—— (*see* Romero et al). 1989;161:817-24
Mazze RI, Källén B. Reproductive outcome after anesthesia and operation during pregnancy: registry study of 5405 cases. 1989;161:1178-85
McCall JB (*see* Hurt et al). 1989;161:530-1
McCarthy BJ (*see* Berg et al). 1989;161:86-91
McCarty KS Jr (*see* Berchuck et al). 1989;161:1247-52
—— (*see* Soisson et al). 1989;161:1258-63
McCullough LB (*see* Chervenak et al). 1989;161:857-60 (Clin. opinion)
—— (*see* Cohen et al). 1989;161:1013-4
McDaniels C (*see* Keith et al). 1989;161:1305-13
McDonald JS (*see* Sellers et al). 1989;161:1001-3
McDonough PG (*see* Johnson et al). 1989;161:1732-7
—— (*see* Odom et al). 1989;161:1663-8
McDougall JK (*see* Beckmann et al). 1989;161:1419-20 (Letter)
McFarlin BL (*see* Noyszewski et al). 1989;161:282-8
McGowan L (*see* Hartge et al). 1989;161:10-6 (Clin. opinion)
McGregor JA, Soper DE, Lovell G, Todd JK. Maternal deaths associated with *Clostridium sordellii* infection. 1989;161:987-95
McLaughlin MK (*see* Hubel et al). 1989;161:1025-34 (Curr. dev.)
——(*see* Roberts et al). 1989;161:1200-4
McLeod AGW (*see* Burkett et al). 1989;161:540-7
McSwain HF (*see* Mitchell et al). 1989;161:576-80
Medearis AL (*see* Brar et al). 1989;161:1519-22
Meier SM (*see* Haning et al). 1989;161:1549-53
Mendenhall HW (*see* Spinnato et al). 1989;161:742-7
Mendes H (*see* Biggar et al). 1989;161:1239-44
Merkatz IR (*see* Anyaegbunam et al). 1989;161:654-6
Merkatz R (*see* Langer et al). 1989;161:646-53
Merlob P. Fetal death: diagnosis and management. 1989;161:254 (Letter)
Merrell CL (*see* Weaver et al). 1989;161:1331-7
Metheny WP (*see* Devoe et al). 1989;161:524-9
—— (*see* Jijon et al). 1989;161:1658-62
Meyer BA (*see* Peaceman et al). 1989;161:771-4
Mibashan RS (*see* Nicolaides et al). 1989;161:1197-200
Michel M, Underwood J, Clark DA, Mowbray JF, Beard RW. Histologic and immunologic study of uterine biopsy tissue of women with incipient abortion. 1989;161:409-14
Mikhail MG (*see* Palan et al). 1989;161:881-5
—— (*see* Palan et al). 1989;161:1649-52
Milet T. Pregnancy in obstetrics residents. 1989;161:1422 (Letter)
Miller DM (*see* Alvarez et al). 1989;161:1653-7
Miller J (*see* Lee et al). 1989;161:974-7
Miller K (*see* Keith et al). 1989;161:1305-13
Miller MC (*see* Newman et al). 1989;161:547-5
Miller WA (*see* Peters et al). 1989;161:297-300
Milunsky A, Jick SS, Bruell CL, MacLaughlin DS, Tsung Y-K, Jick H, Rothman KJ, Willett W. Predictive values, relative risks, and overall benefits of high and low maternal serum α-fetoprotein screening in singleton pregnancies: new epidemiologic data. 1989;161:291-7
Minhas BS, Kumar R, Ricker DD, Roudebush WE, Dodson MG, Fortunato SJ. Effects of platelet activating factor on mouse oocyte fertilization in vitro. 1989;161:1714-7
Minkoff HL (*see* Biggar et al). 1989;161:1239-44
—— (*see* Delke and Minkoff). 1989;161:1748 (Letter reply)
—— (*see* Feinkind et al). 1989;161:765-70
Minogue J (*see* Silver and Minogue). 1989;161:257 (Letter reply)
Mishell DR Jr. Correcting misconceptions about oral contraceptives. 1989;161:1385-9 (Symposium)
——. Estrogen replacement therapy: an overview. 1989;161:1825-7 (Symposium)
Mitchell DE, McSwain HF, Peterson HB. Fertility after ectopic pregnancy. 1989;161:576-80
Mitchell JM (*see* Roberts et al). 1989;161:42-6
Mitchell MD (*see* Romero et al). 1989;161:336-41
Mitterschiffthaler G, Fuith LC. Neurofibromatosis and pregnancy. 1989;161:501 (Letter)
Miyazaki T, Kuo T-C, Dharmarajan AM, Atlas SJ, Wallach EE. In vivo administration of allopurinol affects ovulation and early embryonic development in rabbits. 1989;161:1709-14
Mølsted-Pedersen L (*see* Damm and Mølsted-Pedersen). 1989;161:1163-7
Moawad AH (*see* Pridjian and Moawad). 1989;161:261-2 (Clin. opinion)
Mocarski EJM (*see* Hannah et al). 1989;161:322-3
Mochizuki H (*see* Nozawa et al). 1989;161:1079-86
Modan M (*see* Lipitz et al). 1989;161:1279-84
Moise KJ Jr (*see* Mari et al). 1989;161:1514-8
—— (*see* Mari et al). 1989;161:1528-31
Mojica MA (*see* Berkowitz et al). 1989;161:146-9
Monheit AG (*see* Abitbol et al). 1989;161:1324-31
Monson RR (*see* Lieberman et al). 1989;161:1750-1 (Letter reply)
Moore GW (*see* Baill et al). 1989;161:334-6
Moore JJ, Andrews L, Henderson C, Zuspan KJ, Hertz RH. Neonatal resuscitation in community hospitals: a regional-based, team-oriented training program coordinated by the tertiary center. 1989;161:849-55 (Clin. opinion)
Mor L (*see* Urbach et al). 1989;161:953-9
Morales WJ, Collins EM, Angel JL, Knuppel RA. Short course of antibiotic therapy in treatment of postpartum endomyometritis. 1989;161:568-72
Moreau H (*see* Ballegeer et al). 1989;161:432-6
Moretti ML (*see* Skoll et al). 1989;161:813-6
Morganelli PM (*see* Conrad et al). 1989;161:1065-72
Mori K, Daitoh T, Irahara M, Kamada M, Aono T. Significance of D-mannose as a sperm receptor site on the zona pellucida in human fertilization. 1989;161:207-11
Mori T (*see* Nanbu et al). 1989;161:462-7
Morrow RJ, Adamson SL, Bull SB, Ritchie JWK. Effect of placental embolization on the umbilical arterial velocity waveform in fetal sheep. 1989;161:1055-60
Mortola JF, Girton L, Yen SSC. Depressive episodes in premenstrual syndrome. 1989;161:1682-7
Motwani BT (*see* Bruckner and Motwani). 1989;161:1216-8
Mowbray JF (*see* Michel et al). 1989;161:409-14

Muechler EK (*see* Small and Muechler). 1989;161:17-24 (Clin. opinion)
Muijsers GJJM (*see* van Huisseling et al). 1989;161:1061-4
Muram D. Anal and perianal abnormalities in prepubertal victims of sexual abuse. 1989;161:278-81
Murdock GL (*see* Gast et al). 1989;161:1726-31
Musci TJ (*see* Hubel et al). 1989;161:1025-34 (Curr. dev.)
—— (*see* Roberts et al). 1989;161:1200-4
Mutch L (*see* Dennis et al). 1989;161:213-20
Muto MG, Lepisto EM, Van den Abbeele AD, Knapp RC, Kassis AI. The influence of human antimurine antibody on CA 125 levels in patients with ovarian cancer undergoing radioimmunotherapy or immunoscintigraphy with murine monoclonal antibody OC 125. 1989;161:1206-12
Myerson D (*see* Beckmann et al). 1989;161:1419-20 (Letter)

N

Nadel A (*see* Greene et al). 1989;161:106-11
Naftolin F (*see* Sanyal et al). 1989;161:446-53
Nagai Y (*see* Nozawa et al). 1989;161:1079-86
Nagamani M, Stuart CA, Dinh TV. Steroid biosynthesis in the Sertoli-Leydig cell tumor: effects of insulin and luteinizing hormone. 1989;161:1738-43
Nagata S (*see* Horimoto et al). 1989;161:344-51
Nagel RL (*see* Anyaegbunam et al). 1989;161:654-6
Nageotte MP. Results of uterine activity studies in agreement, but methods differ. 1989;161:1754 (Letter reply)
—— (*see* Gocke et al). 1989;161:111-4
Nagy PS. Placenta percreta induced uterine rupture and resulted in intraabdominal abortion. 1989;161:1185-6
Nakahara H (*see* Horimoto et al). 1989;161:344-51
Nakahara Y (*see* Wassman et al). 1989;161:698-702
Nakano H (*see* Horimoto et al). 1989;161:344-51
Nakano R, Kitayama S, Yamoto M, Shima K, Ooshima A. Localization of gonadotropin binding sites in human ovarian neoplasms. 1989;161:905-10
Nanbu Y, Fujii S, Konishi I, Nonogaki H, Mori T. CA 125 in the epithelium closely related to the embryonic ectoderm: the periderm and the amnion. 1989;161:462-7
Nanda A (*see* Kanwar et al). 1989;161:995-6
Natan NB (*see* Schreyer et al). 1989;161:886-9
Nathanielsz PW (*see* Block et al). 1989;161:1576-9
—— (*see* Figueroa et al). 1989;161:481-6
—— (*see* Hsu et al). 1989;161:467-73
Naugler MA (*see* Ryan et al). 1989;161:1297-304 (Curr. invest.)
Neerhof MG, MacGregor SN, Retzky SS, Sullivan TP. Cocaine abuse during pregnancy: peripartum prevalence and perinatal outcome. 1989;161:633-8
Negri E (*see* Sideri et al). 1989;161:38-42
Neilson JP (*see* Danskin and Neilson). 1989;161:365-9
Nelson DM, Curran EM. High glucose level decreases proliferation of cultured human fetal cells from placenta. 1989;161:1553-8
——, Walsh SW. Aspirin differentially affects thromboxane and prostacyclin production by trophoblast and villous core compartments of human placental villi. 1989;161:1593-8
Nelson DW (*see* Thurmond et al). 1989;161:83-5
Nelson KG (*see* Goldenberg et al). 1989;161:271-7 (Clin. opinion)
Nemes JM (*see* DeRosa et al). 1989;161:690-2
Nestler JE, Clore JN, Blackard WG. The central role of obesity (hyperinsulinemia) in the pathogenesis of the polycystic ovary syndrome. 1989;161:1095-7 (Clin. opinion)
Newman RB, Hamer C, Miller MC. Outpatient triplet management: a contemporary review. 1989;161:547-5
Nicolaides KH, Economides DL, Soothill PW. Blood gases, pH, and lactate in appropriate- and small-for-gestational-age fetuses. 1989;161:996-1001
——, Sadovsky G, Cetin E. Fetal heart rate patterns in red blood cell isoimmunized pregnancies. 1989;161:351-6
——, Thilaganathan B, Mibashan RS. Cordocentesis in the investigation of fetal erythropoiesis. 1989;161:1197-200
—— (*see* Economides et al). 1989;161:1004-8
—— (*see* Economides et al). 1989;161:1219-27
—— (*see* Panos et al). 1989;161:357-61
—— (*see* Rabinowitz et al). 1989;161:1264-6
—— (*see* Vyas et al). 1989;161:168-72
Nicolini U, Fisk NM, Rodeck CH, Talbert DG, Wigglesworth JS. Low amniotic pressure in oligohydramnios—Is this the cause of pulmonary hypoplasia? 1989;161:1098-101 (Clin. opinion)
——, Hubinont C, Santolaya J, Fisk NM, Coe AM, Rodeck CH. Maternal-fetal glucose gradient in normal pregnancies and in pregnancies complicated by alloimmiunization and fetal growth retardation. 1989;161:924-7
—— (*see* Hubinont et al). 1989;161:306-7
Nicoll JJ, Cox PJ. Leiomyoma of the ovary with ascites and hydrothorax. 1989;161:177-8
Nielsen MD (*see* Wilken-Jensen et al). 1989;161:155-6
Nikliński W. Which is better, being small or thin? 1989;161:1089-90 (Letter)
Nishiyama M (*see* Gimovsky and Nishiyama). 1989;161:332-4
Nochimson DJ (*see* Vintzileos et al). 1989;161:606-12
Nolan TE (*see* Yetman and Nolan). 1989;161:1119-23
Nonogaki H (*see* Nanbu et al). 1989;161:462-7
Norman RJ. Analytical and clinical sensitivity and specificity in pregnancy testing. 1989;161:835 (Letter)
Norris HJ (*see* Hartge et al). 1989;161:10-6 (Clin. opinion)
Notelovitz M. Estrogen replacement therapy: indications, contraindications, and agent selection. 1989;161:1832-41 (Symposium)
—— (*see* Martin et al). 1989;161:1127-32
—— (*see* Mazess et al). 1989;161:843-8 (Clin opinion)
Noyszewski EA, Raman J, Trupin SR, McFarlin BL, Dawson MJ. Phosphorus 31 nuclear magnetic resonance examination of female reproductive tissues. 1989;161:282-8
Nozawa S, Sakayori M, Ohta K, Iizuka R, Mochizuki H, Soma M, Fujimoto J-I, Hata J-I, Iwamori M, Nagai Y. A monoclonal antibody (MSN-1) against a newly established uterine endometrial cancer cell line (SNG-II) and its application to immunohistochemistry and flow cytometry. 1989;161:1079-86
Nunley WC Jr, Kolp LA, Dabinett LN, Kitchin JD III, Bateman BG, Craddock GB Jr. Subsequent fertility in women who undergo cardiac surgery. 1989;161:573-6

O

O'Brien TJ (*see* Quirk and O'Brien). 1989;161:1747-8 (Letter reply)
O'Connor TZ (*see* Goldstein et al). 1989;161:1235-8
Odom LD, Barrett JM, Pantazis CG, Stoddard LD, McDonough PG. Immunocytochemical study of *ras* and *myc* proto-oncogene polypeptide expression in the human menstrual cycle. 1989;161:1663-8
O'Donnell KJ (*see* Hume et al). 1989;161:685-90
Oehninger S (*see* Archer et al). 1989;161:581-92
Oettinger M (*see* Ophir et al). 1989;161:25-8
Ogburn PL Jr, Goldstein M, Walker J, Stonestreet BS. Prolonged hyperinsulinemia reduces plasma fatty acid levels in the major lipid groups in fetal sheep. 1989;161:728-32

—— (*see* Stonestreet et al). 1989;161:894-9
Ogita S (*see* Imanaka et al). 1989;161:102-6
Oh W (*see* Stonestreet et al). 1989;161:894-9
Ohta K (*see* Nozawa et al). 1989;161:1079-86
O'kroy J (*see* Martin et al). 1989;161:1127-32
Olson SB (*see* Clark et al). 1989;161:301-2
Olt GJ (*see* Berchuck et al). 1989;161:1247-52
Ooshima A (*see* Nakano et al). 1989;161:905-10
Oosting H (*see* Wolf et al). 1989;161:1140-5
Ophir E, Oettinger M, Yagoda A, Markovits Y, Rojansky N, Shapiro H. Breech presentation after cesarean section: always a section. 1989;161:25-8
Ordorica SA (*see* Hoskins et al). 1989;161:1543-5
Orsini LF (*see* Porcu et al). 1989;161:174-7
Osborne MA (*see* Cucco et al). 1989;161:916-20
Ostrea EM Jr (*see* Reyes et al). 1989;161:977-81
O'Sullivan MJ (*see* Burkett et al). 1989;161:540-7
Otken LB Jr (*see* Gallup et al). 1989;161:533-9
Ouyang P-C (*see* Ho et al). 1989;161:942-8
Ouzounian JG (*see* Masaki et al). 1989;161:680-4
Ovadia Y (*see* Schoenfeld et al). 1989;161:1204-5
Owen J, Stedman CM, Tucker TL. Comparison of predelivery versus postdelivery Kleihauer–Betke stains in cases of fetal death. 1989;161:663-6
Oyarzun E (*see* Romero et al). 1989;161:336-41
—— (*see* Romero et al). 1989;161:817-24

P

Paavonen J, Roberts PL, Stevens CE, Wølner-Hanssen P, Brunham RC, Hillier S, Stamm WE, Kuo C-C, DeRouen T, Holmes KK, Eschenbach DA. Randomized treatment of mucopurulent cervicitis with doxycycline or amoxicillin. 1989;161:128-35
Pahwa S (*see* Biggar et al). 1989;161:1239-44
Palan PR, Mikhail M, Romney SL. Decreased β-carotene tissue levels in uterine leiomyomas and cancers of reproductive and nonreproductive organs. 1989;161:1649-52
——, Romney SL, Vermund SH, Mikhail MG, Basu J. Effects of smoking and oral contraception on plasma β-carotene levels in healthy women. 1989;161:881-5
Palmer JR (*see* Rosenberg et al). 1989;161:1087-8 (Letter)
Palti Z, Rosenn B, Goshen R, Ben-Chitrit A, Yagel S. Successful treatment of a viable cervical pregnancy with methotrexate. 1989;161:1147-8
Pankuch GA, Cherouny PH, Botti JJ, Appelbaum PC. Amniotic fluid leukotaxis assay as an early indicator of chorioamnionitis. 1989;161:802-7
Panos MZ, Nicolaides KH, Anderson JV, Economides DL, Rees L, Williams R. Plasma atrial natriuretic peptide in human fetus: response to intravascular blood transfusion. 1989;161:357-61
Pansky M, Bukovsky I, Golan A, Langer R, Schneider D, Arieli S, Caspi E. Local methotrexate injection: a nonsurgical treatment of ectopic pregnancy. 1989;161:393-6
Pantazis CG (*see* Odom et al). 1989;161:1663-8
Paradisi R (*see* Porcu et al). 1989;161:174-7
Parazzini F (*see* Sideri et al). 1989;161:38-42
Paret G (*see* Lipitz et al). 1989;161:1279-84
Parisi VM, Salinas J, Stockmar EJ. Fetal vascular responses to maternal nicardipine administration in the hypertensive ewe. 1989;161:1035-9
——, ——, ——. Placental vascular responses to nicardipine in the hypertensive ewe. 1989;161:1039-43
—— (*see* Peaceman et al). 1989;161:771-4
—— (*see* Thorp et al). 1989;161:600-5
—— (*see* Thorp et al). 1989;161:670-5
Parker CR Jr, MacDonald PC, Guzick DS, Porter JC, Rosenfeld CR, Hauth JC. Prolactin levels in umbilical cord blood of human infants: relation to gestational age, maternal complications, and neonatal lung function. 1989;161:795-802
Parnet F (*see* Poissonnier et al). 1989;161:709-13
Parra J (*see* Quilez et al). 1989;161:1426-7 (Letter)
Parvez H (*see* Castro et al). 1989;161:1620-3
Pascoe-Mason J (*see* FitzSimmons et al). 1989;161:1174-7
Patil RK (*see* Puri et al). 1989;161:248-53
Patriarco MS. Does terbutaline cause uterine atony and increase intraoperative blood loss? 1989;161:259 (Letter reply)
Patrick J (*see* Gagnon et al). 1989;161:1479-85
Patsch W, Brown SA, Gotto AM Jr, Young RL. The effect of triphasic oral contraceptives on plasma lipids and lipoproteins. 1989;161:1396-401 (Symposium)
Patterson RM. Which is better, being small or thin? 1989;161:1090 (Letter reply)
Pattison NS (*see* Roberts et al). 1989;161:42-6
Patton PE (*see* Burry et al) (1989;160:1454-61). 1989;161:1755 (Correction)
Paulson RJ (*see* Sauer et al). 1989;161:380-1
—— (*see* Sauer et al). 1989;161:1673-6
Peaceman AM, Meyer BA, Thorp JA, Parisi VM, Creasy RK. The effect of magnesium sulfate tocolysis on the fetal biophysical profile. 1989;161:771-4
Peiffer M (*see* Bracero et al). 1989;161:388-93
Peisner DB (*see* Timor-Tritsch et al). 1989;161:157-61
—— (*see* Timor-Tritsch et al). 1989;161:831-3
—— (*see* Warren et al). 1989;161:747-53
Pelzer GD (*see* Weiner et al). 1989;161:714-7
—— (*see* Weiner et al). 1989;161:1498-501
Penry M (*see* Kofinas et al). 1989;161:1536-9
Pernoll ML (*see* Smith et al). 1989;161:879-80
Perry SM (*see* Kambam et al). 1989;161:1754-5 (Letter reply)
Pershe R (*see* Ferguson et al). 1989;161:1485-90
Persutte WH (*see* DeRosa et al). 1989;161:690-2
Peters MT, Lockwood CJ, Miller WA. The efficacy of fetal sonographic biometry in Down syndrome screening. 1989;161:297-300
—— (*see* Rabinowitz et al). 1989;161:1264-6
Petersen R, Carter LS, Chescheir NC, Katz VL, Cefalo RC. Effects of terbutaline sulfate on fetal cardiac function. 1989;161:509-12
Peterson CM (*see* Jovanovic-Peterson et al). 1989;161:415-9
Peterson HB (*see* Mitchell et al). 1989;161:576-80
Petrie RH (*see* Sadovsky et al). 1989;161:613-7
Petrikovsky BM, Cohen M, Tancer ML. Usefulness of continuous fetal heart rate monitoring during cesarean section. 1989;161:36-7
—— (*see* Vintzileos et al). 1989;161:606-12
Petrocik E, Wassman R, Kelly JC. Prenatal screening for Down syndrome with maternal serum human chorionic gonadotropin levels. 1989;161:1168-73
—— (*see* Kelly et al). 1989;161:703-5
Petru E, Tamussino K, Lahousen M, Winter R, Pickel H, Haas J. Pelvic and paraaortic lymphocysts after radical surgery because of cervical and ovarian cancer. 1989;161:937-41
Phelan JP (*see* Clark et al). 1989;161:1439-42
—— (*see* Rodriguez et al). 1989;161:666-9
—— (*see* Sarno et al). 1989;161:1508-14
—— (*see* Strong et al). 1989;161:29-32
Phernetton TM (*see* Reid et al). 1989;161:229-33
Philipson EH (*see* Rossi et al). 1989;161:1106-10

Phillippe M (*see* Greene et al). 1989;161:106-11
—— (*see* Rotmensch et al). 1989;161:855-6 (Clin. opinion)
Philput CB (*see* Archer et al). 1989;161:581-92
Photopulos G (*see* Sutton et al). 1989;161:309-12
Pickel H (*see* Petru et al). 1989;161:937-41
Pielet BW (*see* MacGregor et al). 1989;161:1491-3
—— (*see* Socol et al). 1989;161:255-6 (Letter reply)
Pijpers L, Reuss A, Stewart PA, Wladimiroff JW. Noninvasive management of isolated bilateral fetal hydrothorax. 1989;161:330-2
Pinckert TL, Lebo RV, Golbus MS. Rapid determination of fetal sex by deoxyribonucleic acid amplification of Y chromosome–specific sequences. 1989;161:693-8
Pinette MG (*see* Vintzileos et al). 1989;161:606-12
Pipkin FB (*see* Firth and Pipkin). 1989;161:1318-9
Pircon RA, Strassner HT, Kirz DS, Towers CV. Controlled trial of hydration and bed rest versus bed rest alone in the evaluation of preterm uterine contractions. 1989;161:775-9
Pirrone E (*see* Timor-Tritsch et al). 1989;161:831-3
Pitkänen O (*see* Hallman et al). 1989;161:313-8
Piura B. Nerve entrapment after Pfannenstiel incision. 1989;161:499-500 (Letter)
—— (*see* Glezerman et al). 1989;161:1186-90
Pivarnik J (*see* Clark et al). 1989;161:1439-42
Piver MS. Standard administration of hydroxyurea recommended. 1989;161:1425-6 (Letter)
Platt LD (*see* Brar et al). 1989;161:1519-22
—— (*see* Sarno et al). 1989;161:1508-14
—— (*see* Small et al). 1989;161:323-9
Poissonnier M-H, Brossard Y, Demedeiros N, Vassileva J, Parnet F, Larsen M, Gosset M, Chavinie J, Huchet J. Two hundred intrauterine exchange transfusions in severe blood incompatibilities. 1989;161:709-13
Pokul RK (*see* Smith et al). 1989;161:911-5
Pollack KL (*see* Chestnut et al). 1989;161:1571-5
Poole WK (*see* Haning et al). 1989;161:1549-53
Porcu E, Venturoli S, Fabbri R, Orsini LF, Sganga E, Brondelli L, Paradisi R, Flamigni C. Uterine development and endocrine relationships after menarche. 1989;161:174-7
Porter JC (*see* Parker et al). 1989;161:795-802
Porter KB (*see* Sims et al). 1989;161:532-3
Prem KA (*see* Borazjani et al). 1989;161:1253-7
Pretorius DH (*see* Russ et al). 1989;161:401-6
Pridjian G, Moawad AH. Missed abortion: Still appropriate terminology? 1989;161:261-2 (Clin. opinion)
Pulkkinen MO, Jaakkola U-M. Low serum progesterone levels and tubal dysfunction—a possible cause of ectopic pregnancy. 1989;161:934-7
Puri CP, Patil RK, Kholkute SD, Elger WAG, Swamy XR. Progesterone antagonist lilopristone: a potent abortifacient in the common marmoset. 1989;161:248-53

Q

Queenan JT (*see* Ananth and Queenan). 1989;161:47-9
Quilez M, de las Heras P, Delgado E, Vaz M, Parra J, Bellart J, Salamero F, Llauradó A, Esteban-Altirriba J. Double endometrial aspirative biopsy. 1989;161:1426-7 (Letter)
Quilligan EJ (*see* Brewer et al). 1989;161:840 (Letter reply)
Quirk JG Jr, O'Brien TJ. Other routes charted after description of adventurous journey of CA 125. 1989;161:1747-8 (Letter reply)
Quirke P (*see* Thornton et al). 1989;161:487-92

R

Rabinowitz R, Peters MT, Vyas S, Campbell S, Nicolaides KH. Measurement of fetal urine production in normal pregnancy by real-time ultrasonography. 1989;161:1264-6
Radley T (*see* Tuttle et al). 1989;161:1319-23
Rafkin D (*see* Thomas et al). 1989;161:141-5
Raju S (*see* Warren et al). 1989;161:747-53
Ralph MM (*see* Andrianakis et al). 1989;161:241-7
Raman J (*see* Noyszewski et al). 1989;161:282-8
Rando RF, Lindheim S, Hasty L, Sedlacek TV, Woodland M, Eder C. Increased frequency of detection of human papillomavirus deoxyribonucleic acid in exfoliated cervical cells during pregnancy. 1989;161:50-5
—— (*see* Sedlacek et al). 1989;161:55-9
Rankin JHG (*see* Reid et al). 1989;161:229-33
Räsänen J, Kirkinen P, Jouppila P. Right ventricular dysfunction in human fetal compromise. 1989;161:136-40
Raskin P (*see* Lucas et al). 1989;161:426-31
Rathke A (*see* Rayburn et al). 1989;161:202-6
Ratts TE (*see* Mabie et al). 1989;161:1443-8
Rayburn W, Rathke A, Leuschen P, Chleborad J, Weidner W. Fentanyl citrate analgesia during labor. 1989;161:202-6
Reamy KJ. Semantic changes suggested in hypertensive disorders. 1989;161:1422 (Letter)
Reddy S (*see* Tuttle et al). 1989;161:1319-23
Redwine DB. Peritoneal blood painting: an aid in the diagnosis of endometriosis. 1989;161:865-6
Reece EA (*see* Goldstein et al). 1989;161:1235-8
—— (*see* Winn et al). 1989;161:1540-2
Reed KL, Appleton CP, Sahn DJ, Anderson CF. Human fetal tricuspid and mitral deceleration time: changes with normal pregnancy and intrauterine growth retardation. 1989;161:1532-5
Rees L (*see* Panos et al). 1989;161:357-61
Reichman B (*see* Lipitz et al). 1989;161:1279-84
Reid DL, Hollister MC, Davidson SR, Phernetton TM, Rankin JHG. Uncoupling of excitation from contraction in uterine smooth muscle in near-term ewes. 1989;161:229-33
Repke J (*see* Villar et al). 1989;161:1019-24
Resnick MB, Carter RL, Ariet M, Bucciarelli RL, Evans JH, Furlough RR, Ausbon WW, Curran JS. Effect of birth weight, race, and sex on survival of low-birth-weight infants in neonatal intensive care. 1989;161:184-7
Retzky SS (*see* Neerhof et al). 1989;161:633-8
Reuss A (*see* Pijpers et al). 1989;161:330-2
Reyes MP, Ostrea EM Jr, Cabinian AE, Schmitt C, Rintelmann W. Vancomycin during pregnancy: Does it cause hearing loss or nephrotoxicity in the infant? 1989;161:977-81
Rhoads G (*see* Villar et al). 1989;161:1019-24
Richard M (*see* Brawer et al). 1989;161:474-80
Ricker DD (*see* Minhas et al). 1989;161:1714-7
Riedmiller H (*see* Bürger et al). 1989;161:162-7
Rintelmann W (*see* Reyes et al). 1989;161:977-81
Ritchie JWK (*see* Morrow et al). 1989;161:1055-60
Ritter DB (*see* Goldberg et al). 1989;161:1669-72
Rizk B (*see* Kingsland et al). 1989;161:381-2
Roberts AB, Mitchell JM, Pattison NS. Fetal liver length in normal and isoimmunized pregnancies. 1989;161:42-6
Roberts JA (*see* Winn et al). 1989;161:1540-2
Roberts JM, Taylor RN, Musci TJ, Rodgers GM, Hubel CA, McLaughlin MK. Preeclampsia: an endothelial cell disorder. 1989;161:1200-4
—— (*see* Hubel et al). 1989;161:1025-34 (Curr. dev.)
—— (*see* Lee et al). 1989;161:1044-9

Roberts PL (*see* Paavonen et al). 1989;161:128-35
Robillard JE (*see* Chestnut et al). 1989;161:1571-5
—— (*see* Weiner and Robillard). 1989;161:1494-7
Robson SC, Hunter S, Boys RJ, Dunlop W. Hemodynamic changes during twin pregnancy: a Doppler and M-mode echocardiographic study. 1989;161:1273-8
Rochelson BL (*see* Abitbol et al). 1989;161:1324-31
Rodeck CH (*see* Hubinont et al). 1989;161:306-7
—— (*see* Nicolini et al). 1989;161:924-7
—— (*see* Nicolini et al). 1989;161:1098-101 (Clin. opinion)
Rodgers GM (*see* Roberts et al). 1989;161:1200-4
Rodis JF (*see* Vintzileos et al). 1989;161:606-12
Rodriguez MH, Masaki DI, Phelan JP, Diaz FG. Uterine rupture: Are intrauterine pressure catheters useful in the diagnosis? 1989;161:666-9
Rogers GM (*see* Hubel et al). 1989;161:1025-34 (Curr. dev.)
Rogers RE (*see* Sutton et al). 1989;161:73-5
Rognoni MT (*see* Sideri et al). 1989;161:38-42
Rojansky N (*see* Ophir et al). 1989;161:25-8
Rokey R (*see* Lee and Rokey). 1989;161:1749-50 (Letter reply)
—— (*see* Lee et al). 1989;161:974-7
Roll KE (*see* Castro et al). 1989;161:623-7
—— (*see* Castro et al) (1989;161:623-7). 1989;161:1238 (Correction)
Romanoff ME (*see* Woodward and Romanoff). 1989;161:837-8 (Letter)
Romem Y (*see* Artal et al). 1989;161:1464-9
Romero R, Manogue KR, Mitchell MD, Wu YK, Oyarzun E, Hobbins JC, Cerami A. Infection and labor. IV. Cachectin–tumor necrosis factor in the amniotic fluid of women with intraamniotic infection and preterm labor. 1989;161:336-41
——, Sirtori M, Oyarzun E, Avila C, Mazor M, Callahan R, Sabo V, Athanassiadis AP, Hobbins JC. Infection and labor. V. Prevalence, microbiology, and clinical significance of intraamniotic infection in women with preterm labor and intact membranes. 1989;161:817-24
—— (*see* Lockwood et al). 1989;161:369-73
Romney SL (*see* Palan et al). 1989;161:881-5
—— (*see* Palan et al). 1989;161:1649-52
Ron-El R, Weinraub Z, Langer R, Bukovsky I, Caspi E. The importance of ultrasonography in infertile women with "forgotten" intrauterine contraceptive devices. 1989;161:211-2
Ronen N (*see* Urbach et al). 1989;161:953-9
Rönnberg L (*see* Telimaa et al). 1989;161:866-71
Rose JC (*see* Hatjis et al). 1989;161:1015-9
Rosen MG (*see* Warren et al). 1989;161:747-53
Rosenberg L, Palmer JR, Shapiro S. Gestational trophoblastic disease and use of oral contraceptives. 1989;161:1087-8 (Letter)
Rosenfeld CR (*see* Parker et al). 1989;161:795-802
Rosenkilde-Gram B (*see* Wilken-Jensen et al). 1989;161:155-6
Rosenn B (*see* Palti et al). 1989;161:1147-8
Rosenshein N (*see* Sutton et al). 1989;161:309-12
Rosenzweig BA (*see* Rotmensch et al). 1989;161:855-6 (Clin. opinion)
Ross B (*see* Archer et al). 1989;161:581-92
Ross MG, Sherman DJ, Ervin MG, Day L, Humme J. Stimuli for fetal swallowing: systemic factors. 1989;161:1559-65
—— (*see* Castro et al). 1989;161:1337-43
Rossi EM, Philipson EH, Williams TG, Kalhan SC. Meconium aspiration syndrome: intrapartum and neonatal attributes. 1989;161:1106-10
Roth LM (*see* Sutton et al). 1989;161:73-5
Rothman KJ (*see* Milunsky et al). 1989;161:291-7
Rotmensch S, Rosenzweig BA, Phillippe M. The impact of the acquired immunodeficiency syndrome epidemic on the philosophy of childbirth. 1989;161:855-6 (Clin. opinion)
Rottem S (*see* Bronshtein et al). 1989;161:78-82
Roudebush WE (*see* Minhas et al). 1989;161:1714-7
Rudelstorfer R (*see* Bernaschek and Rudelstorfer). 1989;161:499 (Letter reply)
Ruedrich DA, Devoe LD, Searle N. Effects of maternal hyperoxia on the biophysical assessment of fetuses with suspected intrauterine growth retardation. 1989;161:188-92
—— (*see* Devoe et al). 1989;161:524-9
Ruissen CJ (*see* van Huisseling et al). 1989;161:1061-4
Russ PD, Pretorius DH, Johnson MJ. Dandy-Walker syndrome: a review of fifteen cases evaluated by prenatal sonography. 1989;161:401-6
Rutherford SE (*see* Artal et al). 1989;161:1464-9
Ryan G, Lange IR, Naugler MA. Clinical experience with phenytoin prophylaxis in severe preeclampsia. 1989;161:1297-304 (Curr. invest.)
Ryan KJ (*see* Lieberman et al). 1989;161:1750-1 (Letter reply)

S

Sabbagha RE (*see* Socol et al). 1989;161:255-6 (Letter reply)
Sabo V (*see* Romero et al). 1989;161:817-24
Sacks DA, Abu-Fadil S, Greenspoon JS, Fotheringham N. Do the current standards for glucose tolerance testing in pregnancy represent a valid conversion of O'Sullivan's original criteria? 1989;161:638-41
——, ——, ——, ——. How reliable is the fifty-gram, one-hour glucose screening test? 1989;161:642-5
——, Garratty G. Isoimmunization to Cromer antigen in pregnancy. 1989;161:928-9
Sadovsky G (*see* Nicolaides et al). 1989;161:351-6
Sadovsky Y, Amon E, Bade ME, Petrie RH. Prophylactic amnioinfusion during labor complicated by meconium: a preliminary report. 1989;161:613-7
Sahn DJ (*see* Reed et al). 1989;161:1532-5
St. Clair PJ (*see* Silver et al). 1989;161:808-12
Sajda P (*see* Macri et al). 1989;161:254-5 (Letter reply)
Sakayori M (*see* Nozawa et al). 1989;161:1079-86
Salafia C (*see* Winn et al). 1989;161:1540-2
Salamero F (*see* Quilez et al). 1989;161:1426-7 (Letter)
Salinas J (*see* Parisi et al). 1989;161:1035-9
—— (*see* Parisi et al). 1989;161:1039-43
Saltzman DH (*see* Greene et al). 1989;161:106-11
Salvador HS, Koos BJ. Effects of regular and decaffeinated coffee on fetal breathing and heart rate (1989;160:1043-7). 1989;161:669 (Correction)
Sampson JE (*see* Thorp et al). 1989;161:600-5
Santolaya J (*see* Nicolini et al). 1989;161:924-7
Sanyal MK, Brami CJ, Bischof P, Simmons E, Barnea ER, Dwyer JM, Naftolin F. Immunoregulatory activity in supernatants from cultures of normal human trophoblast cells of the first trimester. 1989;161:446-53
Saraf V (*see* Abitbol et al). 1989;161:1324-31
Sarno AP Jr, Ahn MO, Brar HS, Phelan JP, Platt LD. Intrapartum Doppler velocimetry, amniotic fluid volume, and fetal heart rate as predictors of subsequent fetal distress. I. An initial report. 1989;161:1508-14
—— (*see* Strong et al). 1989;161:29-32
Sassoon DA (*see* Castro et al). 1989;161:623-7
Sauer MV, Anderson RE, Vermesh M, Stone BA, Paulson RJ. Spontaneously resorbing ectopic pregnancy: preservation

of human chorionic gonadotropin bioactivity despite declining steroid hormone levels. 1989;161:1673-6
——, Lobo RA, Paulson RJ. Successful twin pregnancy after embryo donation to a patient with XY gonadal dysgenesis. 1989;161:380-1
Sawers RS (*see* Tang and Sawers). 1989;161:172-3
Schemper M (*see* Sevelda et al). 1989;161:1213-6
Scherl JE (*see* Greene et al). 1989;161:106-11
Schiff I (*see* Mazess et al). 1989;161:843-8 (Clin opinion)
Schiffman MH (*see* Goldberg et al). 1989;161:1669-72
—— (*see* Hartge et al). 1989;161:10-6 (Clin. opinion)
Schifman RB. Role of ferritin supported in diagnosis of anemias of pregnancy. 1989;161:258-9 (Letter reply)
Schlafer DH (*see* Block et al). 1989;161:1576-9
Schmitt C (*see* Reyes et al). 1989;161:977-81
Schnatterly PT (*see* Hogge et al). 1989;161:520-4
Schneider D (*see* Pansky et al). 1989;161:393-6
Schoenbaum SC (*see* Lieberman et al). 1989;161:1750-1 (Letter reply)
Schoenfeld A, Freedman S, Hod M, Ovadia Y. Antagonism of antihypertensive drug therapy in pregnancy by indomethacin? 1989;161:1204-5
Schreyer P, Caspi E, Natan NB, Tal E, Weinraub Z. The predictive value of fetal breathing movement and Bishop score in the diagnosis of "true" preterm labor. 1989;161:886-9
Schulman H. Systolic/diastolic ratios and waveform shapes. 1989;161:1421 (Letter reply)
—— (*see* Bracero et al). 1989;161:388-93
Schutz T (*see* Ferguson et al). 1989;161:788-95
—— (*see* Ferguson et al). 1989;161:1485-90
Scott JS (*see* Taylor et al). 1989;161:377-9
Scott RT (*see* Archer et al). 1989;161:581-92
Searle NA (*see* Devoe et al). 1989;161:524-9
—— (*see* Ruedrich et al). 1989;161:188-92
Sedlacek TV, Lindheim S, Eder C, Hasty L, Woodland M, Ludomirsky A, Rando RF. Mechanism for human papillomavirus transmission at birth. 1989;161:55-9
—— (*see* Rando et al). 1989;161:50-5
Seeds JW (*see* Katz and Seeds). 1989;161:1-9 (Clin. opinion)
Seguin EM (*see* Hendricks et al). 1989;161:761-5
Sellers JD, Block FE Jr, McDonald JS. Anesthetic management of labor in a patient with dextrocardia, congenitally corrected transposition, Wolff-Parkinson-White syndrome, and congestive heart failure. 1989;161:1001-3
Semchyshyn S. Calcium intake and pregnancy-induced hypertension. 1989;161:834 (Letter)
Seppälä M (*see* Telimaa et al). 1989;161:866-71
Serr DM (*see* Lipitz et al). 1989;161:1279-84
Seski J (*see* Ho et al). 1989;161:942-8
Settlage RH (*see* Greenspoon and Settlage). 1989;161:501-2 (Letter)
Sevelda P, Schemper M, Spona J. CA 125 as an independent prognostic factor for survival in patients with epithelial ovarian cancer. 1989;161:1213-6
Sganga E (*see* Porcu et al). 1989;161:174-7
Shafik A. Anal submucosal injection: a new route for drug administration in pelvic malignancies. IV. Submucosal anal injection in the treatment of cancer of uterine cervix—preliminary study. 1989;161:69-72
Shah DM, Boehm FH. Fetal blood gas analysis from cordocentesis for abnormal fetal heart rate patterns. 1989;161:374-6
Shahbazian VL (*see* Steinleitner et al). 1989;161:1677-81
Shalev J (*see* Lipitz et al). 1989;161:1279-84
Shan DM, Chaffin D. Perinatal outcome in very preterm births with twin-twin transfusion syndrome. 1989;161:1111-3
Shanklin DR, Sibai BM. Ultrastructural aspects of preeclampsia. I. Placental bed and uterine boundary vessels. 1989;161:735-41
Shapiro H (*see* Ophir et al). 1989;161:25-8
Shapiro S (*see* Rosenberg et al). 1989;161:1087-8 (Letter)
Shapiro SS (*see* Wapner et al). 1989;161:1271-2
Sharpe D (*see* Johnson et al). 1989;161:289-90
Shelden RM (*see* Corsan et al). 1989;161:1751-2 (Letter)
Shepard TH (*see* FitzSimmons et al). 1989;161:1174-7
Sherman DJ (*see* Castro et al). 1989;161:1337-43
—— (*see* Ross et al). 1989;161:1559-65
Shima K (*see* Nakano et al). 1989;161:905-10
Sholl JS (*see* MacGregor et al). 1989;161:1491-3
Shulman S. Dosage and duration differences lead to diverse conclusions. 1989;161:1744-5 (Letter)
——. Sperm antibodies and infertility. 1989;161:497-8 (Letter)
Sibai BM, Villar L MA, Bray E. Magnesium supplementation during pregnancy: a double-blind randomized controlled clinical trial. 1989;161:115-9
—— (*see* Ahokas et al). 1989;161:618-22
—— (*see* Mabie et al). 1989;161:1443-8
—— (*see* Shanklin and Sibai). 1989;161:735-41
—— (*see* Skoll et al). 1989;161:813-6
Sideri M, Parazzini F, Rognoni MT, La Vecchia C, Negri E, Garsia S, Arnoletti E, Cecchetti G. Risk factors for vulvar lichen sclerosus. 1989;161:38-42
Silver HM, Sperling RS, St. Clair PJ, Gibbs RS. Evidence relating bacterial vaginosis to intraamniotic infection. 1989;161:808-12
Silver RK, MacGregor SN, Hobart ED. Impact of residual amniotic fluid volume in patients receiving parenteral tocolysis after premature rupture of the membranes. 1989;161:784-7
——, Minogue J. Basic reference gambles recommended for utility assessment. 1989;161:257 (Letter reply)
—— (*see* Farrell et al). 1989;161:628-33
—— (*see* MacGregor et al). 1989;161:1491-3
Simmons E (*see* Sanyal et al). 1989;161:446-53
Simon NV (*see* Stefos et al). 1989;161:179-83
Simpson JL (*see* Tipton et al). 1989;161:1546-8
Sims CJ, Porter KB, Knuppel RA. Successful pregnancy after a liver transplant. 1989;161:532-3
Sims HF (*see* Gast et al). 1989;161:1726-31
Singer CRJ (*see* Walker et al). 1989;161:676-9
Sippo WC. Nerve entrapment after Pfannenstiel incision. 1989;161:500 (Letter reply)
Sirtori M (*see* Romero et al). 1989;161:817-24
Sivakoff M (*see* Veille et al). 1989;161:1610-4
Sjöblom P (*see* Lindblom et al). 1989;161:397-400
Sklar A (*see* Divon et al). 1989;161:757-60
Skoll MA, Moretti ML, Sibai BM. The incidence of positive amniotic fluid cultures in patients in preterm labor with intact membranes. 1989;161:813-6
Skurnick JH (*see* Goldsmith et al). 1989;161:1644-9
Slavik TA (*see* Timor-Tritsch et al). 1989;161:157-61
Small ML, Beall M, Platt LD, Dirks D, Hochberg H. Continuous tissue pH monitoring in the term fetus. 1989;161:323-9
Small SL, Muechler EK. Heuristic determination of relevant diagnostic procedures in a medical expert system for gynecology. 1989;161:17-24 (Clin. opinion)
Smith BE (*see* Kambam et al). 1989;161:1754-5 (Letter reply)

Smith CV. *Candida* warrants concern. 1989;161:503 (Letter reply)
Smith DM, Groff DE, Pokul RK, Bear JL, Delgado G. Determination of cellular oncogene rearrangement or amplification in ovarian adenocarcinomas. 1989;161:911-5
Smith LG Jr, Summers PR, Biswas MK, Gurr DE, Lund P, Pernoll ML. High hepatitis B carrier rate among non-Oriental patients with acute salpingitis. 1989;161:879-80
Smith LJ (*see* Alvarez et al). 1989;161:1653-7
Smolarczyk R (*see* Wójcicka-Jagodzińska et al). 1989;161:959-64
Snyder SW, Cardwell MS. Neuromuscular blockade with magnesium sulfate and nifedipine. 1989;161:35-6
—— (*see* Cardwell and Snyder). 1989;161:1744 (Letter)
Sobhi S (*see* Little et al). 1989;161:732-4
Socol ML, MacGregor SN, Pielet BW, Tamura RK, Sabbagha RE. Frequency and timing of intravascular intrauterine transfusions reconsidered. 1989;161:255-6 (Letter reply)
—— (*see* MacGregor et al). 1989;161:1491-3
Soisson AP, Berchuck A, Lessey BA, Soper JT, Clarke-Pearson DL, McCarty KS Jr, Bast RC Jr. Immunohistochemical expression of TAG-72 in normal and malignant endometrium: correlation of antigen expression with estrogen receptor and progesterone receptor levels. 1989;161:1258-63
—— (*see* Berchuck et al). 1989;161:1247-52
Sokol RJ (*see* Sowers et al). 1989;161:441-5
Soma M (*see* Nozawa et al). 1989;161:1079-86
Song H-Z (*see* Brinton et al). 1989;161:121-7
Soothill PW (*see* Nicolaides et al). 1989;161:996-1001
Soper DE, Mayhall CG, Dalton HP. Risk factors for intraamniotic infection: a prospective epidemiologic study. 1989;161:562-8
—— (*see* McGregor et al). 1989;161:987-95
Soper JT (*see* Berchuck et al). 1989;161:1247-52
—— (*see* Soisson et al). 1989;161:1258-63
Sorensen TK (*see* Hendricks et al). 1989;161:761-5
Southwick J (*see* Clark et al). 1989;161:1439-42
Sowers JR, Zemel MB, Bronsteen RA, Zemel PC, Walsh MF, Standley PR, Sokol RJ. Erythrocyte cation metabolism in preeclampsia. 1989;161:441-5
Spellacy WN. Neonatal seizures and cesarean section. 1989;161:837 (Letter reply)
Sperling RS (*see* Silver et al). 1989;161:808-12
Spillman T (*see* Clark et al). 1989;161:1439-42
Spinnato JA, Allen RD, Mendenhall HW. Birth weight prediction from remote ultrasonographic examination. 1989;161:742-7
Spitz B (*see* Ballegeer et al). 1989;161:432-6
Spitzer C (*see* Goldberg et al). 1989;161:1669-72
Spona J (*see* Sevelda et al). 1989;161:1213-6
Stafford PA (*see* Biddinger et al). 1989;161:1744 (Letter reply)
Stalter P (*see* Bracero et al). 1989;161:388-93
Stamm WE (*see* Paavonen et al). 1989;161:128-35
Stanczyk FZ (*see* Matteri et al). 1989;161:1704-9
—— (*see* Steinleitner et al). 1989;161:1677-81
Standley PR (*see* Sowers et al). 1989;161:441-5
Stanger CL (*see* Hume et al). 1989;161:685-90
Stedman CM (*see* Owen et al). 1989;161:663-6
Stefos T, Deter RL, Hill RM, Simon NV. Individual growth curve standards in twins: prediction of third-trimester growth and birth characteristics. 1989;161:179-83
Stehman FB, Bundy BN. Standard administration of hydroxyurea recommended. 1989;161:1426 (Letter reply)
Steingold KA (*see* Van Voorhis et al). 1989;161:1698-703
Steinleitner A, Stanczyk FZ, Levin JH, d'Ablaing G III, Vijod MA, Shahbazian VL, Lobo RA. Decreased in vitro production of 6-keto-prostaglandin $F_{1\alpha}$ by uterine arteries from postmenopausal women. 1989;161:1677-81
Stephens JD. Empty bladder recommended in initial ultrasonographic examinations. 1989;161:1088-9 (Letter)
——. Unexplained elevated maternal serum α-fetoprotein level associated with third-trimester problems. 1989;161:500 (Letter)
Stern W (*see* Abitbol et al). 1989;161:1324-31
Stevens CE (*see* Paavonen et al). 1989;161:128-35
Stevenson DK (*see* Ferguson et al). 1989;161:788-95
—— (*see* Ferguson et al). 1989;161:1485-90
Stewart PA (*see* Pijpers et al). 1989;161:330-2
Stiller RJ, de Regt RH, Blair E. Transvaginal ultrasonography in patients at risk for ectopic pregnancy. 1989;161:930-3
Stockmar EJ (*see* Parisi et al). 1989;161:1035-9
—— (*see* Parisi et al). 1989;161:1039-43
Stoddard LD (*see* Odom et al). 1989;161:1663-8
Stolk HG (*see* Helmerhorst et al). 1989;161:1191-4
Stone BA (*see* Sauer et al). 1989;161:1673-6
Stonestreet BS, Ogburn PL Jr, Goldstein M, Oh W, Widness JA. Effects of chronic fetal hyperinsulinemia on plasma arachidonic acid and prostaglandin concentrations. 1989;161:894-9
—— (*see* Ogburn et al). 1989;161:728-32
Strassner HT (*see* Pircon et al). 1989;161:775-9
Strauss AW (*see* Gast et al). 1989;161:1726-31
Strøm V (*see* Wilken-Jensen et al). 1989;161:155-6
Strobino D (*see* Thomas et al). 1989;161:141-5
Strong TH Jr, Phelan JP, Ahn MO, Sarno AP Jr. Vaginal birth after cesarean delivery in the twin gestation. 1989;161:29-32
Stroud A (*see* Hatjis et al). 1989;161:1015-9
Stuart CA (*see* Nagamani et al). 1989;161:1738-43
Stumpf PG (*see* Veldhuis et al). 1989;161:1149-58
Stumpf WE, Denny ME. Vitamin D (soltriol), light, and reproduction. 1989;161:1375-84 (Curr. dev.)
Sueoka K (*see* Katz et al). 1989;161:1361-6
Sugawa T (*see* Imanaka et al). 1989;161:102-6
Suikkari A-M (*see* Telimaa et al). 1989;161:866-71
Sullivan TP (*see* Neerhof et al). 1989;161:633-8
Summers PR (*see* Smith et al). 1989;161:879-80
Sutton B (*see* Eddie et al). 1989;161:970-3
Sutton GP, Blessing JA, Rosenshein N, Photopulos G, DiSaia PJ. Phase II trial of ifosfamide and mesna in mixed mesodermal tumors of the uterus (A Gynecologic Oncology Group Study). 1989;161:309-12
——, Rogers RE, Roth LM, Ehrlich CE. Aggressive angiomyxoma first diagnosed as levator hernia. 1989;161:73-5
Svanegaard J (*see* Jensen et al). 1989;161:951-2
Swain M (*see* Kofinas et al). 1989;161:1536-9
Swamy XR (*see* Puri et al). 1989;161:248-53
Szigetvari IA (*see* Kaali et al). 1989;161:406-8

T

Tal E (*see* Schreyer et al). 1989;161:886-9
Talbert DG (*see* Nicolini et al). 1989;161:1098-101 (Clin. opinion)
Talledo OE (*see* Gallup et al). 1989;161:533-9
Tamura RK (*see* Socol et al). 1989;161:255-6 (Letter reply)
Tamussino K (*see* Petru et al). 1989;161:937-41
Tancer ML (*see* Petrikovsky et al). 1989;161:36-7
Tang L, Sawers RS. Twin pregnancy in premature ovarian failure after estrogen treatment: a case report. 1989;161:172-3

Tavill M (*see* Veille et al). 1989;161:1610-4
Taylor PV, Campbell JM, Scott JS. Presence of autoantibodies in women with unexplained infertility. 1989;161:377-9
Taylor RN (*see* Hubel et al). 1989;161:1025-34 (Curr. dev.)
—— (*see* Roberts et al). 1989;161:1200-4
Tchabo J-G (*see* Limaye and Tchabo). 1989;161:76-7
Tedeschi ML (*see* Van Voorhis et al). 1989;161:1698-703
Telimaa S, Kauppila A, Rönnberg L, Suikkari A-M, Seppälä M. Elevated serum levels of endometrial secretory protein PP14 in patients with advanced endometriosis: suppression by treatment with danazol and high-dose medroxyprogesterone acetate. 1989;161:866-71
Teramo K (*see* Hallman et al). 1989;161:965-9
Tharapel AT (*see* Tipton et al). 1989;161:1546-8
Thiagarajah S (*see* Hogge et al). 1989;161:520-4
Thilaganathan B (*see* Nicolaides et al). 1989;161:1197-200
Tho SPT (*see* Johnson et al). 1989;161:1732-7
Thomas ML (*see* Hill et al). 1989;161:193-9
Thomas RL, Johnson TRB, Besinger RE, Rafkin D, Treanor C, Strobino D. Preterm and term fetal cardiac and movement responses to vibratory acoustic stimulation. 1989;161:141-5
Thompson CS (*see* Chestnut et al). 1989;161:1571-5
Thomson M. Defining "primiparas." 1989;161:1417 (Letter reply)
Thorburn GD (*see* Andrianakis et al). 1989;161:241-7
Thornton JG, Lilford RJ. Basic reference gambles recommended for utility assessment. 1989;161:256 (Letter)
——, Quirke P, Wells M. Flow cytometry of normal, hyperplastic, and malignant human endometrium. 1989;161:487-92
Thorp JA, Parisi VM, Boylan PC, Johnston DA. The effect of continuous epidural analgesia on cesarean section for dystocia in nulliparous women. 1989;161:670-5
——, Sampson JE, Parisi VM, Creasy RK. Routine umbilical cord blood gas determinations? 1989;161:600-5
—— (*see* Peaceman et al). 1989;161:771-4
Thorp JM Jr, Katz VL, Campbell D, Cefalo RC. Hypersensitivity to magnesium sulfate. 1989;161:889-90
——, ——, Fowler LJ, Kurtzman JT, Bowes WA Jr. Fetal death from chlamydial infection across intact amniotic membranes. 1989;161:1245-6
Thurmond AS, Nelson DW, Lowensohn RI, Young WP, Davis L. Enlarged cisterna magna in trisomy 18: prenatal ultrasonographic diagnosis. 1989;161:83-5
Timor-Tritsch IE, Warren WB, Peisner DB, Pirrone E. First-trimester midgut herniation: a high-frequency transvaginal sonographic study. 1989;161:831-3
——, Yeh MN, Peisner DB, Lesser KB, Slavik TA. The use of transvaginal ultrasonography in the diagnosis of ectopic pregnancy. 1989;161:157-61
—— (*see* Warren et al). 1989;161:747-53
Tipton RE, Tharapel AT, Chang H-HT, Simpson JL, Elias S. Rapid chromosome analysis with spontaneously dividing cells derived from umbilical cord blood (fetal and neonatal). 1989;161:1546-8
Tjioe GSTD (*see* Helmerhorst et al). 1989;161:1191-4
Todd JK (*see* McGregor et al). 1989;161:987-95
Tolley D (*see* Clark et al). 1989;161:1439-42
Török M, Gáti I. Results of uterine activity studies in agreement, but methods differ. 1989;161:1753-4 (Letter)
Towers CV (*see* Gocke et al). 1989;161:111-4
—— (*see* Pircon et al). 1989;161:775-9
Trad T (*see* Tuttle et al). 1989;161:1319-23
Treanor C (*see* Thomas et al). 1989;161:141-5
Treffers PE (*see* Wolf et al). 1989;161:1140-5
Tregear GW (*see* Eddie et al). 1989;161:970-3
Trupin SR (*see* Noyszewski et al). 1989;161:282-8
Tsung Y-K (*see* Milunsky et al). 1989;161:291-7
Tucker TL (*see* Owen et al). 1989;161:663-6
Tuttle RS, Marmelstein L, Trad T, Reddy S, Radley T. In vitro uterine response to tetramethylpyrazine, the active constituent of *Chung Chong* (a traditional Chinese medicine). 1989;161:1319-23
Twiggs LB (*see* Borazjani et al). 1989;161:1253-7

U

Underwood J (*see* Michel et al). 1989;161:409-14
Urbach J, Mor L, Ronen N, Brandes JM. Does insulin affect placental glucose metabolism and transfer? 1989;161:953-9
Utian WH. Biosynthesis and physiologic effects of estrogen and pathophysiologic effects of estrogen deficiency: a review. 1989;161:1828-31 (Symposium)
—— (*see* Mazess et al). 1989;161:843-8 (Clin opinion)

V

Valenzuela GJ. Is a decrease in plasma oncotic pressure enough to explain the edema of pregnancy? 1989;161:1624-7
——, Forbes HW, Alonso JG. Effect of whole-body pH changes on thoracic duct lymph flow in anesthetized sheep. 1989;161:1566-70
——, Longo LD. Lymph flow rate response to angiotensin II is decreased in pregnant sheep. 1989;161:1615-9
Van den Abbeele AD (*see* Muto et al). 1989;161:1206-12
van Huisseling H, Hasaart THM, Ruissen CJ, Muijsers GJJM, de Haan J. Umbilical artery flow velocity waveforms during acute hypoxemia and the relationship with hemodynamic changes in the fetal lamb. 1989;161:1061-4
Van Voorhis B, Matt DW, Tedeschi ML, Steingold KA. Comparison of in vivo bioavailability of progestational agents into the rat uterus and liver and the effect of plasma protein binding. 1989;161:1698-703
Vassileva J (*see* Poissonnier et al). 1989;161:709-13
Vaz M (*see* Quilez et al). 1989;161:1426-7 (Letter)
Veille JC, Kanaan C. Duplex Doppler ultrasonographic evaluation of the fetal renal artery in normal and abnormal fetuses. 1989;161:1502-7
——, Tavill M, Sivakoff M, Cohen I, Ben-Ami M, Yang Y-C, Jovkovsky V. Evaluation of pulsed Doppler echocardiography for measurement of aortic blood flow in the fetal lamb. 1989;161:1610-4
Veldhuis JD, Evans WS, Stumpf PG. Mechanisms that subserve estradiol's induction of increased prolactin concentrations: evidence of amplitude modulation of spontaneous prolactin secretory bursts. 1989;161:1149-58
Venkataramanan R (*see* Caritis et al). 1989;161:32-5
Venturoli S (*see* Porcu et al). 1989;161:174-7
Vercellini P (*see* Fedele et al). 1989;161:871-6
Verhoeven ATM (*see* Weyerman et al). 1989;161:1145-6
Vermesh M (*see* Sauer et al). 1989;161:1673-6
Vermund SH (*see* Goldberg et al). 1989;161:1669-72
—— *see* Palan et al). 1989;161:881-5
Verp MS. Pulmonary artery agenesis. 1989;161:1746-7 (Letter)
Vijod MA (*see* Steinleitner et al). 1989;161:1677-81
Villar J, Repke J, Markush L, Calvert W, Rhoads G. The measuring of blood pressure during pregnancy. 1989;161:1019-24

—— (*see* Belizán and Villar). 1989;161:834-5 (Letter reply)
Villar L MA (*see* Sibai et al). 1989;161:115-9
Vintzileos AM, Campbell WA, Rodis JF, Nochimson DJ, Pinette MG, Petrikovsky BM. Comparison of six different ultrasonographic methods for predicting lethal fetal pulmonary hypoplasia. 1989;161:606-12
Voss EM (*see* Holbrook et al). 1989;161:718-21
Voto LS, Margulies M. Frequency and timing of intravascular intrauterine transfusions reconsidered. 1989;161:255 (Letter)
Vyas S, Nicolaides KH, Campbell S. Renal artery flow-velocity waveforms in normal and hypoxemic fetuses. 1989;161:168-72
—— (*see* Jauniaux et al). 1989;161:1195-7
—— (*see* Rabinowitz et al). 1989;161:1264-6

W

Wade RV, Young SR. Analysis of fetal loss after transcervical chorionic villus sampling—a review of 719 patients. 1989;161:513-9
Walker DW (*see* Andrianakis et al). 1989;161:241-7
Walker J (*see* Ogburn et al). 1989;161:728-32
Walker JJ, Cameron AD, Bjornsson S, Singer CRJ, Fraser C. Can platelet volume predict progressive hypertensive disease in pregnancy? 1989;161:676-9
Wallach EE (*see* Katz et al). 1989;161:1361-6
—— (*see* Miyazaki et al). 1989;161:1709-14
Walsh MF (*see* Sowers et al). 1989;161:441-5
Walsh SW. 5-Hydroxyeicosatetraenoic acid, leukotriene C_4, and prostaglandin $F_{2\alpha}$ in amniotic fluid before and during term and preterm labor. 1989;161:1352-60
——, Coulter S. Increased placental progesterone may cause decreased placental prostacyclin production in preeclampsia. 1989;161:1586-92
—— (*see* Nelson and Walsh). 1989;161:1593-8
Wang KY (*see* Hendricks et al). 1989;161:761-5
Wang W (*see* Brinton et al). 1989;161:121-7
Wapner RJ, Cowchock FS, Shapiro SS. Successful treatment of two women with antiphospholipid antibodies and refractory pregnancy losses with intravenous immunoglobulin infusions. 1989;161:1271-2
Ware JC (*see* Archer et al). 1989;161:581-92
Warren WB, Timor-Tritsch I, Peisner DB, Raju S, Rosen MG. Dating the early pregnancy by sequential appearance of embryonic structures. 1989;161:747-53
—— (*see* Timor-Tritsch et al). 1989;161:831-3
Warsof SL (*see* Abramowicz et al). 1989;161:1137-40
Wassman ER, Cheyovich DL, Nakahara Y. "Possibly" de novo translocations: prenatal risk counseling. 1989;161:698-702
—— (*see* Kelly et al). 1989;161:703-5
—— (*see* Petrocik et al). 1989;161:1168-73
Weaver K, Merrell CL, Griffin G. Effect of magnesium on cocaine-induced, catecholamine-mediated platelet and vascular response in term pregnant ewes. 1989;161:1331-7
Weidner W (*see* Rayburn et al). 1989;161:202-6
Weiner CP. Delivery of fetuses with gastroschisis. 1989;161:1089 (Letter)
——. Screening for gestational diabetes. 1989;161:1424 (Letter reply)
——. Thrombocytopenia and cordocentesis clarifications requested. 1989;161:1091-2 (Letter)
——, Grant S, Hudson J, Williamson R, Wenstrom K. Effect of diagnostic and therapeutic cordocentesis on maternal serum α-fetoprotein concentration. 1989;161:706-8
——, Heilskov J, Pelzer G, Grant S, Wenstrom K, Williamson RA. Normal values for human umbilical venous and amniotic fluid pressures and their alteration by fetal disease. 1989;161:714-7
——, Martinez E, Chestnut DH, Ghodsi A. Effect of pregnancy on uterine and carotid artery response to norepinephrine, epinephrine, and phenylephrine in vessels with documented functional endothelium. 1989;161:1605-10
——, ——, Zhu LK, Ghodsi A, Chestnut D. In vitro release of endothelium-derived relaxing factor by acetylcholine is increased during the guinea pig pregnancy. 1989;161:1599-605
——, Pelzer GD, Heilskov J, Wenstrom KD, Williamson RA. The effect of intravascular transfusion on umbilical venous pressure in anemic fetuses with and without hydrops. 1989;161:1498-501
——, Robillard JE. Effect of acute intravascular volume expansion on human fetal prostaglandin concentrations. 1989;161:1494-7
—— (*see* Chestnut et al). 1989;161:1571-5
Weinraub Z (*see* Ron-El et al). 1989;161:211-2
—— (*see* Schreyer et al). 1989;161:886-9
Weiss G (*see* Goldsmith et al). 1989;161:1644-9
Wells EC, Hulka JF. Cervical dilation: a comparison of Lamicel and Dilapan. 1989;161:1124-6
Wells M (*see* Thornton et al). 1989;161:487-92
Wenstrom KD (*see* Weiner et al). 1989;161:706-8
—— (*see* Weiner et al). 1989;161:714-7
—— (*see* Weiner et al). 1989;161:1498-501
Wentworth RA (*see* Block et al). 1989;161:1576-9
—— (*see* Hsu et al). 1989;161:467-73
Wentz AC (*see* Hargrove et al). 1989;161:948-51
Weyerman PC, Verhoeven ATM, Alberda AT. Cervical pregnancy after in vitro fertilization and embryo transfer. 1989;161:1145-6
Whalley PJ (*see* Lucas et al). 1989;161:426-31
White S (*see* Bocking et al). 1989;161:722-7
Widness JA (*see* Stonestreet et al). 1989;161:894-9
Wigglesworth JS (*see* Nicolini et al). 1989;161:1098-101 (Clin. opinion)
Wijnands JBG (*see* Egberts and Wijnands). 1989;161:1417-8 (Letter)
Wijnen HJA (*see* Helmerhorst et al). 1989;161:1191-4
Wilken-Jensen C, Strøm V, Nielsen MD, Rosenkilde-Gram B. Removing a retained placenta by oxytocin—a controlled study. 1989;161:155-6
Wilkins I, Lynch L. Number clarification requested. 1989;161:1746 (Letter reply)
Willett W (*see* Milunsky et al). 1989;161:291-7
Williams ML (*see* Gilstrap et al). 1989;161:825-30
—— (*see* Lucas et al). 1989;161:426-31
Williams R (*see* Panos et al). 1989;161:357-61
Williams TG (*see* Rossi et al). 1989;161:1106-10
Williamson RA (*see* Weiner et al). 1989;161:706-8
—— (*see* Weiner et al). 1989;161:714-7
—— (*see* Weiner et al). 1989;161:1498-501
Willoughby A (*see* Biggar et al). 1989;161:1239-44
Wimsatt J (*see* Figueroa et al). 1989;161:481-6
Winn HN, Egley C. *Haemophilus influenzae* culture. 1989;161:1419 (Letter)
——, Gabrielli S, Reece EA, Roberts JA, Salafia C, Hobbins JC. Ultrasonographic criteria for the prenatal diagnosis of placental chorionicity in twin gestations. 1989;161:1540-2
Winter R (*see* Petru et al). 1989;161:937-41

Wiswell RA (*see* Artal et al). 1989;161:1464-9
Witkin SS. Sperm antibodies and infertility. 1989;161:498-9 (Letter reply)
——, Chaudhry A. Relationship between circulating antisperm antibodies in women and autoantibodies on the ejaculated sperm of their partners. 1989;161:900-3
—— (*see* Kalo-Klein and Witkin). 1989;161:1132-6
Wladimiroff JW (*see* Pijpers et al). 1989;161:330-2
Wølner-Hanssen P (*see* Paavonen et al). 1989;161:128-35
Wójcicka-Jagodzińska J, Kuczyńska-Sicińska J, Czajkowski K, Smolarczyk R. Carbohydrate metabolism in the course of intrahepatic cholestasis in pregnancy. 1989;161:959-64
Wojtczuk AS (*see* Goldsmith et al). 1989;161:1644-9
Wolf EJ (*see* Brace and Wolf). 1989;161:382-8
Wolf ES (*see* Farrell et al). 1989;161:628-33
Wolf H, Oosting H, Treffers PE. A longitudinal study of the relationship between placental and fetal growth as measured by ultrasonography. 1989;161:1140-5
Woodland M (*see* Rando et al). 1989;161:50-5
—— (*see* Sedlacek et al). 1989;161:55-9
Woodward DG, Romanoff ME. Is central venous pressure monitoring "contraindicated" in patients with severe preeclampsia? 1989;161:837-8 (Letter)
Wu B-Z (*see* Brinton et al). 1989;161:121-7
Wu YK (*see* Romero et al). 1989;161:336-41
Wurtman JJ, Brzezinski A, Wurtman RJ, Laferrere B. Effect of nutrient intake on premenstrual depression. 1989;161:1228-34
Wurtman RJ (*see* Wurtman et al). 1989;161:1228-34
Wyse L (*see* Anyaegbunam et al). 1989;161:654-6

Y

Yagel S (*see* Palti et al). 1989;161:1147-8
Yagoda A (*see* Ophir et al). 1989;161:25-8
Yamoto M (*see* Nakano et al). 1989;161:905-10
Yang Y-C (*see* Veille et al). 1989;161:1610-4
Yasin S (*see* Burkett et al). 1989;161:540-7
Yeh MN (*see* Timor-Tritsch et al). 1989;161:157-61
Yen SSC (*see* Mortola et al). 1989;161:1682-7
Yetman TJ, Nolan TE. Vaginal birth after cesarean section: a reappraisal of risk. 1989;161:1119-23
Yoffe N (*see* Bronshtein et al). 1989;161:78-82
Young BK (*see* Hoskins et al). 1989;161:1543-5
Young RL (*see* Patsch et al). 1989;161:1396-401 (Symposium)
Young SR (*see* Wade and Young). 1989;161:513-9
Young WP (*see* Thurmond et al). 1989;161:83-5
Younger MD (*see* Greene et al). 1989;161:106-11
Yudkin P (*see* Dennis et al). 1989;161:213-20

Z

Zemel MB (*see* Sowers et al). 1989;161:441-5
Zemel PC (*see* Sowers et al). 1989;161:441-5
Zhu LK (*see* Weiner et al). 1989;161:1599-605
Zingheim RW (*see* Hendricks et al). 1989;161:761-5
Zumwalt RE (*see* Biddinger et al). 1989;161:1744 (Letter reply)
Zuspan FP (*see* Brewer et al). 1989;161:840 (Letter reply)
Zuspan KJ (*see* Moore et al). 1989;161:849-55 (Clin. opinion)

Subject index*

A

Abdominal wall, pathology
Delivery of fetuses with gastroschisis (Weiner). 1989;161:1089 (Letter)

Abnormalities
Amniotic fluid acetylcholinesterase ratios in prenatal diagnosis of fetal abnormalities (Kelly et al). 1989;161:703-5
Comparison of six different ultrasonographic methods for predicting lethal fetal pulmonary hypoplasia (Vintzileos et al). 1989;161:606-12
Dandy-Walker syndrome: a review of fifteen cases evaluated by prenatal sonography (Russ et al). 1989;161:401-6
Delivery of fetuses with gastroschisis (Weiner). 1989;161:1089 (Letter)
The effects of maternal exercise on early pregnancy outcome (Clapp). 1989;161:1453-7
Enlarged cisterna magna in trisomy 18: prenatal ultrasonographic diagnosis (Thurmond et al). 1989;161:83-5
First-trimester midgut herniation: a high-frequency transvaginal sonographic study (Timor-Tritsch et al). 1989;161:831-3
Maternal phenylketonuria and hyperphenylalaninemia: implications for medical practice in the United States (Luder and Greene). 1989;161:1102-5 (Clin. opinion)
Multidisciplinary team counseling for fetal anomalies (Lorenz and Kuhn). 1989;161:263-6 (Clin. opinion)
Significant decrease in congenital malformations in newborn infants of an unselected population of diabetic women (Damm and Mølsted-Pedersen). 1989;161:1163-7

Abnormalities, drug-induced
Attempted suicide and pregnancy (Czeizel and Lendvay). 1989;161:497 (Letter)
Fetal and neonatal cardiovascular complications from β-sympathomimetic therapy for tocolysis (Katz and Seeds). 1989;161:1-9 (Clin. opinion)

Abortifacient agents
Progesterone antagonist lilopristone: a potent abortifacient in the common marmoset (Puri et al). 1989;161:248-53

Abortion
Analysis of fetal loss after transcervical chorionic villus sampling a review of 719 patients (Wade and Young). 1989;161:513-9
The effects of maternal exercise on early pregnancy outcome (Clapp). 1989;161:1453-7
Histologic and immunologic study of uterine biopsy tissue of women with incipient abortion (Michel et al). 1989;161:409-14
Placenta percreta induced uterine rupture and resulted in intraabdominal abortion (Nagy). 1989;161:1185-6
Successful treatment of two women with antiphospholipid antibodies and refractory pregnancy losses with intravenous immunoglobulin infusions (Wapner et al). 1989;161:1271-2

Abortion, induced
Cervical dilation: a comparison of Lamicel and Dilapan (Wells and Hulka). 1989;161:1124-6
The frequency and management of uterine perforations during first-trimester abortions (Kaali et al). 1989;161:406-8

Abortion, missed
Missed abortion: Still appropriate terminology? (Pridjian and Moawad). 1989;161:261-2 (Clin. opinion)

Acetylcholine
In vitro release of endothelium-derived relaxing factor by acetylcholine is increased during the guinea pig pregnancy (Weiner et al). 1989;161:1599-605

Acetylcholinesterase
Amniotic fluid acetylcholinesterase ratios in prenatal diagnosis of fetal abnormalities (Kelly et al). 1989;161:703-5

Acid-base equilibrium
Acid-base status at birth and neurodevelopmental outcome at four and one-half years (Dennis et al). 1989;161:213-20

Acidosis
Acid-base status at birth and neurodevelopmental outcome at four and one-half years (Dennis et al). 1989;161:213-20

Acoustic stimulation
The effect of vibroacoustic stimulation on the nonstress test at gestational ages of thirty-two weeks or less (Druzin et al). 1989;161:1476-8
Effects of low-frequency vibration on human term fetuses (Gagnon et al). 1989;161:1479-85
The effects of vibroacoustic stimulation on baseline heart rate, breathing activity, and body movements of normal term fetuses (Dévoe et al). 1989;161:524-9
Human fetal behavioral states after vibratory stimulation (Gagnon et al). 1989;161:1470-6
Preterm and term fetal cardiac and movement responses to vibratory acoustic stimulation (Thomas et al). 1989;161:141-5

Acquired immunodeficiency syndrome
Immunosuppression in pregnant women infected with human immunodeficiency virus (Biggar et al). 1989;161:1239-44
The impact of the acquired immunodeficiency syndrome epidemic on the philosophy of childbirth (Rotmensch et al). 1989;161:855-6 (Clin. opinion)

Adenocarcinoma
Determination of cellular oncogene rearrangement or amplification in ovarian adenocarcinomas (Smith et al). 1989;161:911-5

Adenosine monophosphate
Uncoupling of excitation from contraction in uterine

*July, 1-260; August, 261-504; September, 505-842; October, 843-1094; November, 1095-1430; December, part 1, 1431-1824; part 2, 1825-1868.

smooth muscle in near-term ewes (Reid et al). 1989;161:229-33

Adenyl cyclase
Up-regulation of guinea pig myometrial adenylate cyclase activity by intrauterine estradiol and progesterone pellets (Hatjis and Koritnik). 1989;161:1073-8

Adenylate cyclase; *see* **Adenyl cyclase**

Administration, rectal
Anal submucosal injection: a new route for drug administration in pelvic malignancies. IV. Submucosal anal injection in the treatment of cancer of uterine cervix preliminary study (Shafik). 1989;161:69-72

Adolescence
Seminoma in pubertal patient with androgen insensitivity syndrome (Hurt et al). 1989;161:530-1
Uterine development and endocrine relationships after menarche (Porcu et al). 1989;161:174-7

Adolescent pregnancy; *see* **Pregnancy in adolescence**

Adrenal cortex hormones
Depressive episodes in premenstrual syndrome (Mortola et al). 1989;161:1682-7

Adrenal glands
Effects of fetal sex and dexamethasone on preterm maternal serum concentrations of human chorionic gonadotropin, progesterone, estrone, estradiol, and estriol (Haning et al). 1989;161:1549-53

Aerobic exercise; *see* **Exercise**

Aged
A new surgical technique to treat refractory labial fusion in the elderly (Johnson et al). 1989;161:289-90

AIDS virus; *see* **HIV**

Albumin
Comparison of in vivo bioavailability of progestational agents into the rat uterus and liver and the effect of plasma protein binding (Van Voorhis et al). 1989;161:1698-703

Allergy; *see* **Hypersensitivity**

Alloimmunization; *see* **Fetal blood, immunology**

Allopurinol
In vivo administration of allopurinol affects ovulation and early embryonic development in rabbits (Miyazaki et al). 1989;161:1709-14

Alpha fetoproteins
Data sought to link adverse pregnancy outcome with maternal serum α-fetoprotein levels in black gravid women (Lenke) (Letter); (Macri et al) (Reply). 1989;161:254-5
Detection of fetal blood (Leiberman et al) (Letter); (Cardwell) (Reply). 1989;161:257-8
Effect of diagnostic and therapeutic cordocentesis on maternal serum α-fetoprotein concentration (Weiner et al). 1989;161:706-8
Predictive values, relative risks, and overall benefits of high and low maternal serum α-fetoprotein screening in singleton pregnancies: new epidemiologic data (Milunsky et al). 1989;161:291-7
The role of ultrasonography and amniocentesis in the evaluation of pregnancies at risk for neural tube defects (Hogge et al). 1989;161:520-4
Unexplained elevated maternal serum α-fetoprotein level associated with third-trimester problems (Stephens). 1989;161:500 (Letter)

Ambulatory care
Outpatient triplet management: a contemporary review (Newman et al). 1989;161:547-5

Amenorrhea
The patient's reaction to side effects of oral contraceptives (Hillard). 1989;161:1412-5 (Symposium)

The American Board of Obstetrics and Gynecology
Board status. 1989;161:1428

American Journal of Obstetrics and Gynecology
Consultants for the American Journal of Obstetrics and Gynecology. 1989;161:1431-8

Amino acids
Plasma amino acids in appropriate- and small-for-gestational-age fetuses (Economides et al). 1989;161:1219-27

Amniocentesis
Amniotic fluid acetylcholinesterase ratios in prenatal diagnosis of fetal abnormalities (Kelly et al). 1989;161:703-5
Amniotic fluid leukotaxis assay as an early indicator of chorioamnionitis (Pankuch et al). 1989;161:802-7
Candida warrants concern (Elliott) (Letter); (Smith) (Reply). 1989;161:503
Ceramide lactoside in amniotic fluid: high concentration in chorioamnionitis and in preterm labor (Hallman et al). 1989;161:313-8
Does midtrimester ΔOD_{450} of amniotic fluid reflect severity of Rh disease? (Ananth and Queenan). 1989;161:47-9
Esterase activity in second- and third-trimester amniotic fluid: an indicator of chorioamnionitis (Hoskins et al). 1989;161:1543-5
5-Hydroxyeicosatetraenoic acid, leukotriene C_4, and prostaglandin $F_{2\alpha}$ in amniotic fluid before and during term and preterm labor (Walsh). 1989;161:1352-60
The incidence of positive amniotic fluid cultures in patients in preterm labor with intact membranes (Skoll et al). 1989;161:813-6
Infection and labor. V. Prevalence, microbiology, and clinical significance of intraamniotic infection in women with preterm labor and intact membranes (Romero et al). 1989;161:817-24
Integration of the transabdominal technique into an ongoing chorionic villus sampling program (Copeland et al). 1989;161:1289-94
The role of ultrasonography and amniocentesis in the evaluation of pregnancies at risk for neural tube defects (Hogge et al). 1989;161:520-4
Surfactant proteins in the diagnosis of fetal lung maturity. II. The 35 kd protein and phospholipids in complicated pregnancy (Hallman et al). 1989;161:965-9
Uselessness of the phosphatidylglycerol assay for prediction of lung maturity (Egberts and Wijnands) (Letter); (Fadel) (Reply). 1989;161:1417-8

Amnion
CA 125 in the epithelium closely related to the embryonic ectoderm: the periderm and the amnion (Nanbu et al). 1989;161:462-7
Ultrasonographic criteria for the prenatal diagnosis of placental chorionicity in twin gestations (Winn et al). 1989;161:1540-2

Amniotic fluid
Antepartum infection as a result of *Streptococcus pneumoniae* and sepsis in neonate (Andreu et al). 1989;161:1424-5 (Letter)
Duplex Doppler ultrasonographic evaluation of the fetal renal artery in normal and abnormal fetuses (Veille and Kanaan). 1989;161:1502-7

Amniotic fluid—cont'd
Evidence relating bacterial vaginosis to intraamniotic infection (Silver et al). 1989;161:808-12
Fetal blood volume, urine flow, swallowing, and amniotic fluid volume responses to long-term intravascular infusions of saline (Brace). 1989;161:1049-54
Impact of residual amniotic fluid volume in patients receiving parenteral tocolysis after premature rupture of the membranes (Silver et al). 1989;161:784-7
Infection and labor. IV. Cachectin–tumor necrosis factor in the amniotic fluid of women with intraamniotic infection and preterm labor (Romero et al). 1989;161:336-41
Intrapartum Doppler velocimetry, amniotic fluid volume, and fetal heart rate as predictors of subsequent fetal distress. I. An initial report (Sarno et al). 1989;161:1508-14
Low amniotic pressure in oligohydramnios Is this the cause of pulmonary hypoplasia? (Nicolini et al). 1989;161:1098-101 (Clin. opinion)
Normal amniotic fluid volume changes throughout pregnancy (Brace and Wolf). 1989;161:382-8
Normal values for human umbilical venous and amniotic fluid pressures and their alteration by fetal disease (Weiner et al). 1989;161:714-7
Saline solution amnioinfusion for oligohydramnios after premature rupture of the membranes (Imanaka et al). 1989;161:102-6
Amniotic fluid, microbiology
Evidence relating bacterial vaginosis to intraamniotic infection (Silver et al). 1989;161:808-12
The incidence of positive amniotic fluid cultures in patients in preterm labor with intact membranes (Skoll et al). 1989;161:813-6
Infection and labor. V. Prevalence, microbiology, and clinical significance of intraamniotic infection in women with preterm labor and intact membranes (Romero et al). 1989;161:817-24
Amoxicillin
Randomized treatment of mucopurulent cervicitis with doxycycline or amoxicillin (Paavonen et al). 1989;161:128-35
Anal drug administration; *see* **Administration, rectal**
Anal sphincter; *see* **Anus**
Analgesia
Fentanyl citrate analgesia during labor (Rayburn et al). 1989;161:202-6
Analgesia, epidural
The effect of continuous epidural analgesia on cesarean section for dystocia in nulliparous women (Thorp et al). 1989;161:670-5
Androgen insensitivity; *see* **Receptors, androgen, deficiency**
Androgens
Androgen sulfate and glucuronide conjugates in nonhirsute and hirsute women with polycystic ovarian syndrome (Matteri et al). 1989;161:1704-9
Androstenedione
Effect of a 48-hour Δ^4-androstenedione infusion on the pregnant rhesus monkey in the last third of gestation: changes in maternal plasma estradiol concentrations and myometrial contractility (Figueroa et al). 1989;161:481-6
Androsterone
Androgen sulfate and glucuronide conjugates in nonhirsute and hirsute women with polycystic ovarian syndrome (Matteri et al). 1989;161:1704-9
Anemia
Does furosemide alter the hemodynamic response to rapid intravascular transfusion of the anemic fetal lamb (Chestnut et al). 1989;161:1571-5
Effect of acute intravascular volume expansion on human fetal prostaglandin concentrations (Weiner and Robillard). 1989;161:1494-7
The effect of intravascular transfusion on umbilical venous pressure in anemic fetuses with and without hydrops (Weiner et al). 1989;161:1498-501
Anemia, fetal; *see* **Fetal hemoglobin**
Anemia, hypochromic
Role of ferritin supported in diagnosis of anemias of pregnancy (Goodlin) (Letter); (Schifman) (Reply). 1989;161:258-9
Anemia, sickle cell
Maternal hemoglobin F levels may have an adverse effect on neonatal birth weight in pregnancies with sickle cell disease (Anyaegbunam et al). 1989;161:654-6
Anencephaly
Fetal hyperinsulinism in anencephaly (Hubinont et al). 1989;161:306-7
Anesthesia, conduction
Pulmonary artery catheterization in severe eclampsia (Gibbs). 1989;161:1089 (Letter)
Anesthesia, obstetrical
Anesthetic management of labor in a patient with dextrocardia, congenitally corrected transposition, Wolff-Parkinson-White syndrome, and congestive heart failure (Sellers et al). 1989;161:1001-3
Reproductive outcome after anesthesia and operation during pregnancy: registry study of 5405 cases (Mazze and Källén). 1989;161:1178-85
Angiomyxoma; *see* **Pelvic neoplasms**
Angiotensin II
Human α- and β-calcitonin gene-related peptides are vasodilators in human chorionic plate vasculature (Firth and Pipkin). 1989;161:1318-9
Lack of evidence of a vasodepressor role for relaxin in spontaneously hypertensive and normotensive pregnant rats (Ahokas et al). 1989;161:618-22
Lymph flow rate response to angiotensin II is decreased in pregnant sheep (Valenzuela and Longo). 1989;161:1615-9
The renin-angiotensin system during pregnancy in chronically instrumented, conscious rats (Conrad et al). 1989;161:1065-72
Angiotensins
Effects of cephalic hypotension, hypertension, and barbiturates on fetal cerebral blood flow and metabolism (Hohimer and Bissonnette). 1989;161:1344-51
Announcements
Board status. 1989;161:1428
Anoxemia
Fetal heart rate patterns in red blood cell isoimmunized pregnancies (Nicolaides et al). 1989;161:351-6
Anoxemia, animal
Effect of prolonged hypoxemia on fetal heart rate accelerations and decelerations in sheep (Bocking et al). 1989;161:722-7
Intrauterine growth retardation and the circulatory responses to acute hypoxemia in fetal sheep (Block et al). 1989;161:1576-9
Umbilical artery flow velocity waveforms during acute hypoxemia and the relationship with hemodynamic changes in the fetal lamb (van Huisseling et al). 1989;161:1061-4

Antibiotics
Antibiotics for postcesarean endometritis (Duff). 1989;161:1087 (Letter)
Short course of antibiotic therapy in treatment of postpartum endomyometritis (Morales et al). 1989;161:568-72
Antibodies
Presence of autoantibodies in women with unexplained infertility (Taylor et al). 1989;161:377-9
The prevalence and biologic significance of lupus anticoagulant and anticardiolipin antibodies in a general obstetric population (Lockwood et al). 1989;161:369-73
Relationship between circulating antisperm antibodies in women and autoantibodies on the ejaculated sperm of their partners (Witkin and Chaudhry). 1989;161:900-3
Sperm antibodies and infertility (Shulman) (Letter); (Witkin) (Reply). 1989;161:497-9
Successful treatment of two women with antiphospholipid antibodies and refractory pregnancy losses with intravenous immunoglobulin infusions (Wapner et al). 1989;161:1271-2
Antibodies, monoclonal
The influence of human antimurine antibody on CA 125 levels in patients with ovarian cancer undergoing radioimmunotherapy or immunoscintigraphy with murine monoclonal antibody OC 125 (Muto et al). 1989;161:1206-12
A monoclonal antibody (MSN-1) against a newly established uterine endometrial cancer cell line (SNG-II) and its application to immunohistochemistry and flow cytometry (Nozawa et al). 1989;161:1079-86
Antibodies, neoplasm
CA 125 in the epithelium closely related to the embryonic ectoderm: the periderm and the amnion (Nanbu et al). 1989;161:462-7
Anticoagulants
Thrombosis prophylaxis in pregnancy with use of subcutaneous heparin adjusted by monitoring heparin concentration in plasma (Dahlman et al). 1989;161:420-5
Antigens
Isoimmunization to Cromer antigen in pregnancy (Sacks and Garratty). 1989;161:928-9
Antigens, neoplasm
Immunohistochemical expression of TAG-72 in normal and malignant endometrium: correlation of antigen expression with estrogen receptor and progesterone receptor levels (Soisson et al). 1989;161:1258-63
Antigens, tumor-associated, carbohydrate
CA 125 in the epithelium closely related to the embryonic ectoderm: the periderm and the amnion (Nanbu et al). 1989;161:462-7
CA 125 as an independent prognostic factor for survival in patients with epithelial ovarian cancer (Sevelda et al). 1989;161:1213-6
The influence of human antimurine antibody on CA 125 levels in patients with ovarian cancer undergoing radioimmunotherapy or immunoscintigraphy with murine monoclonal antibody OC 125 (Muto et al). 1989;161:1206-12
Other routes charted after description of adventurous journey of CA 125 (Fuith) (Letter); (Quirk and O'Brien) (Reply). 1989;161:1747-8
Antihypertensive agents, antagonists
Antagonism of antihypertensive drug therapy in pregnancy by indomethacin? (Schoenfeld et al). 1989;161:1204-5
Antineoplastic agents
Local methotrexate injection: a nonsurgical treatment of ectopic pregnancy (Pansky et al). 1989;161:393-6
Antineoplastic agents, combined
Phase II trial of ifosfamide and mesna in mixed mesodermal tumors of the uterus (A Gynecologic Oncology Group Study) (Sutton et al). 1989;161:309-12
Antiphospholipid antibodies; *see* **Antibodies**
Antispermatogenic agents
Relationship between circulating antisperm antibodies in women and autoantibodies on the ejaculated sperm of their partners (Witkin and Chaudhry). 1989;161:900-3
Anus
Anal and perianal abnormalities in prepubertal victims of sexual abuse (Muram). 1989;161:278-81
Aorta, descending, animal; *see* **Aorta, thoracic, animal**
Aorta, thoracic, animal
Evaluation of pulsed Doppler echocardiography for measurement of aortic blood flow in the fetal lamb (Veille et al). 1989;161:1610-4
Apgar score
Acid-base status at birth and neurodevelopmental outcome at four and one-half years (Dennis et al). 1989;161:213-20
Diagnosis of birth asphyxia on the basis of fetal pH, Apgar score, and newborn cerebral dysfunction (Gilstrap et al). 1989;161:825-30
Arachidonic acids
Effects of chronic fetal hyperinsulinemia on plasma arachidonic acid and prostaglandin concentrations (Stonestreet et al). 1989;161:894-9
Prolonged hyperinsulinemia reduces plasma fatty acid levels in the major lipid groups in fetal sheep (Ogburn et al). 1989;161:728-32
Arginine
Vasopressin receptors in human pregnant myometrium and decidua: interactions with oxytocin and vasopressin agonists and antagonists (Ivanisevic et al). 1989;161:1637-43
Aromatase
Aromatase in human fetal tissues (Doody and Carr). 1989;161:1694-7
Arrhenoblastoma
Steroid biosynthesis in the Sertoli-Leydig cell tumor: effects of insulin and luteinizing hormone (Nagamani et al). 1989;161:1738-43
Arterial lines; *see* **Catheters, indwelling**
Arterial occlusive diseases
Pregnancy after aortofemoral bypass grafting in a diabetic patient (Bradley-Watson). 1989;161:200-1
Artificial intelligence
Heuristic determination of relevant diagnostic procedures in a medical expert system for gynecology (Small and Muechler). 1989;161:17-24 (Clin. opinion)
Ascites
Leiomyoma of the ovary with ascites and hydrothorax (Nicoll and Cox). 1989;161:177-8
Asphyxia neonatorum
Acid-base status at birth and neurodevelopmental outcome at four and one-half years (Dennis et al). 1989;161:213-20
Diagnosis of birth asphyxia on the basis of fetal pH, Apgar score, and newborn cerebral dysfunction (Gilstrap et al). 1989;161:825-30
Aspirin
Aspirin differentially affects thromboxane and prostacyclin production by trophoblast and villous core compartments

Aspirin—cont'd
of human placental villi (Nelson and Walsh). 1989;161:1593-8
Atrial natriuretic peptides; *see* **Natriuretic peptides, atrial**
Autoantibodies
Presence of autoantibodies in women with unexplained infertility (Taylor et al). 1989;161:377-9
Relationship between circulating antisperm antibodies in women and autoantibodies on the ejaculated sperm of their partners (Witkin and Chaudhry). 1989;161:900-3
Autoimmune diseases
Relapsing polychondritis in pregnancy: a case report and review (Gimovsky and Nishiyama). 1989;161:332-4
Azidothymidine
Pharmacokinetics of azidothymidine during late pregnancy in Long-Evans rats (Little et al). 1989;161:732-4

B

Bacteriological techniques
Haemophilus influenzae culture (Winn and Egley). 1989;161:1419 (Letter)
Bed rest
Controlled trial of hydration and bed rest versus bed rest alone in the evaluation of preterm uterine contractions (Pircon et al). 1989;161:775-9
Beta-adrenergic receptors, animals; *see* **Receptors, adrenergic, beta, animal**
Bicycle ergometry test; *see* **Exercise test**
Binding sites
Isolation and sequencing of a complementary deoxyribonucleic acid clone encoding human placental 17β-estradiol dehydrogenase: identification of the putative cofactor binding site (Gast et al). 1989;161:1726-31
Localization of gonadotropin binding sites in human ovarian neoplasms (Nakano et al). 1989;161:905-10
Biophysics
The effect of magnesium sulfate tocolysis on the fetal biophysical profile (Peaceman et al). 1989;161:771-4
Biopsy
Double endometrial aspirative biopsy (Quilez et al). 1989;161:1426-7 (Letter)
Histologic and immunologic study of uterine biopsy tissue of women with incipient abortion (Michel et al). 1989;161:409-14
Birth defects; *see* **Abnormalities**
Birth weight
Birth weight prediction from remote ultrasonographic examination (Spinnato et al). 1989;161:742-7
Effect of birth weight, race, and sex on survival of low-birth-weight infants in neonatal intensive care (Resnick et al). 1989;161:184-7
Intrauterine growth retardation: standards for diagnosis (Goldenberg et al). 1989;161:271-7 (Clin. opinion)
Macrosomia in the postdate pregnancy: Is routine ultrasonographic screening indicated? (Chervenak et al). 1989;161:753-6
Maternal hemoglobin F levels may have an adverse effect on neonatal birth weight in pregnancies with sickle cell disease (Anyaegbunam et al). 1989;161:654-6
Neonatal mortality in normal birth weight babies: Does the level of hospital care make a difference? (Berg et al). 1989;161:86-91
Role of ferritin supported in diagnosis of anemias of pregnancy (Goodlin) (Letter); (Schifman) (Reply). 1989;161:258-9
Bladder
Empty bladder recommended in initial ultrasonographic examinations (Stephens). 1989;161:1088-9 (Letter)
Measurement of fetal urine production in normal pregnancy by real-time ultrasonography (Rabinowitz et al). 1989;161:1264-6
Blood circulation, animal
Intrauterine growth retardation and the circulatory responses to acute hypoxemia in fetal sheep (Block et al). 1989;161:1576-9
Blood flow velocity
Clinical management of the fetus with markedly diminished umbilical artery end-diastolic flow (Divon et al). 1989;161:1523-7
Doppler assessment of the fetal and uteroplacental circulation during nifedipine therapy for preterm labor (Mari et al). 1989;161:1514-8
Doppler assessment of the pulsatility index of the middle cerebral artery during constriction of the fetal ductus arteriosus after indomethacin therapy (Mari et al). 1989;161:1528-31
Duplex Doppler ultrasonographic evaluation of the fetal renal artery in normal and abnormal fetuses (Veille and Kanaan). 1989;161:1502-7
Effect of placental embolization on the umbilical arterial velocity waveform in fetal sheep (Morrow et al). 1989;161:1055-60
Effect of placental laterality on uterine artery resistance and development of preeclampsia and intrauterine growth retardation (Kofinas et al). 1989;161:1536-9
Human fetal tricuspid and mitral deceleration time: changes with normal pregnancy and intrauterine growth retardation (Reed et al). 1989;161:1532-5
Intrapartum Doppler velocimetry, amniotic fluid volume, and fetal heart rate as predictors of subsequent fetal distress. I. An initial report (Sarno et al). 1989;161:1508-14
Maternal and fetal blood flow velocity waveforms in patients with preterm labor: relationship to outcome (Brar et al). 1989;161:1519-22
Umbilical artery flow velocity waveforms during acute hypoxemia and the relationship with hemodynamic changes in the fetal lamb (van Huisseling et al). 1989;161:1061-4
Blood gas analysis
Blood gases, pH, and lactate in appropriate- and small-for-gestational-age fetuses (Nicolaides et al). 1989;161:996-1001
Fetal blood gas analysis from cordocentesis for abnormal fetal heart rate patterns (Shah and Boehm). 1989;161:374-6
Routine umbilical cord blood gas determinations? (Thorp et al). 1989;161:600-5
Blood glucose
Does insulin affect placental glucose metabolism and transfer? (Urbach et al). 1989;161:953-9
The effect of elevated blood glucose on the electroencephalogram and cerebral metabolism during short-term brain ischemia in fetal sheep (Chao et al). 1989;161:221-8
High glucose level decreases proliferation of cultured human fetal cells from placenta (Nelson and Curran). 1989;161:1553-8
Maternal-fetal glucose gradient in normal pregnancies and in pregnancies complicated by alloimmunization and fetal growth retardation (Nicolini et al). 1989;161:924-7
Randomized trial of diet versus diet plus cardiovascular con-

ditioning on glucose levels in gestational diabetes (Jovanovic-Peterson et al). 1989;161:415-9

Blood platelets
Can platelet volume predict progressive hypertensive disease in pregnancy? (Walker et al). 1989;161:676-9
Effect of magnesium on cocaine-induced, catecholamine-mediated platelet and vascular response in term pregnant ewes (Weaver et al). 1989;161:1331-7

Blood pressure
Lack of evidence of a vasodepressor role for relaxin in spontaneously hypertensive and normotensive pregnant rats (Ahokas et al). 1989;161:618-22
The measuring of blood pressure during pregnancy (Villar et al). 1989;161:1019-24
Systolic/diastolic ratios and waveform shapes (Bewley) (Letter); (Schulman) (Reply). 1989;161:1420-1

Blood pressure, high; *see* **Hypertension**

Blood transfusion, intrauterine
Does furosemide alter the hemodynamic response to rapid intravascular transfusion of the anemic fetal lamb (Chestnut et al). 1989;161:1571-5
Does midtrimester ΔOD_{450} of amniotic fluid reflect severity of Rh disease? (Ananth and Queenan). 1989;161:47-9
Effect of acute intravascular volume expansion on human fetal prostaglandin concentrations (Weiner and Robillard). 1989;161:1494-7
The effect of intravascular transfusion on umbilical venous pressure in anemic fetuses with and without hydrops (Weiner et al). 1989;161:1498-501
Fetal cardiac output in the isoimmunized pregnancy: a pulsed Doppler-echocardiographic study of patients undergoing intravascular intrauterine transfusion (Copel et al). 1989;161:361-5
Fetal liver length in normal and isoimmunized pregnancies (Roberts et al). 1989;161:42-6
Frequency and timing of intravascular intrauterine transfusions reconsidered (Voto and Margulies) (Letter); (Socol et al) (Reply). 1989;161:255-6
Ovine fetal cardiovascular responses to packed red blood cell transfusions (Brace). 1989;161:1367-74
Plasma atrial natriuretic peptide in human fetus: response to intravascular blood transfusion (Panos et al). 1989;161:357-61
Prediction of hematocrit decline after intravascular fetal transfusion (MacGregor et al). 1989;161:1491-3
Pulsed Doppler flow-velocity waveforms in the prediction of fetal hematocrit of the severely isoimmunized pregnancy (Copel et al). 1989;161:341-4
Two hundred intrauterine exchange transfusions in severe blood incompatibilities (Poissonnier et al). 1989;161:709-13

Blood volume
Atrial natriuretic factor: the effects of pregnancy and a high-salt diet on atrial levels (Castro et al). 1989;161:1620-3
Fetal blood volume, urine flow, swallowing, and amniotic fluid volume responses to long-term intravascular infusions of saline (Brace). 1989;161:1049-54
Ovine fetal cardiovascular responses to packed red blood cell transfusions (Brace). 1989;161:1367-74
Plasma atrial natriuretic peptide in human fetus: response to intravascular blood transfusion (Panos et al). 1989;161:357-61

Bone development
Long bone growth in fetuses with Down syndrome (Fitz-Simmons et al). 1989;161:1174-7

Books
Books received. 1989; 161:841, 1093, 1429

Brain, blood supply, animal
The effect of elevated blood glucose on the electroencephalogram and cerebral metabolism during short-term brain ischemia in fetal sheep (Chao et al). 1989;161:221-8
Effects of cephalic hypotension, hypertension, and barbiturates on fetal cerebral blood flow and metabolism (Hohimer and Bissonnette). 1989;161:1344-51

Breast diseases
Breast disease programs in obstetrics and gynecology: a plea for training in mammography (Gleicher). 1989;161:267-70 (Clin. opinion)

Breast feeding
Methicillin-resistant staphylococcal mastitis (Kalstone). 1989;161:120

Breast neoplasms
Breast disease programs in obstetrics and gynecology: a plea for training in mammography (Gleicher). 1989;161:267-70 (Clin. opinion)
A case-control study of epithelial ovarian cancer (Hartge et al). 1989;161:10-6 (Clin. opinion)

Breast neoplasms, epidemiology
The cancer question: an overview of recent epidemiologic and retrospective data (Henderson). 1989;161:1859-64 (Symposium)

Breech presentation
Breech presentation after cesarean section: always a section (Ophir et al). 1989;161:25-8
Management of the nonvertex second twin: primary cesarean section, external version, or primary breech extraction (Gocke et al). 1989;161:111-4

Buserelin
Buserelin versus danazol in the treatment of endometriosis-associated infertility (Fedele et al). 1989;161:371-6

C

CA 125; *see* **Antigens, tumor-associated, carbohydrate**

Cadmium poisoning
Zinc, cadmium, and hypertension in parturient women (Lazebnik et al). 1989;161:437-40

Calcitonin
Human α- and β-calcitonin gene-related peptides are vasodilators in human chorionic plate vasculature (Firth and Pipkin). 1989;161:1318-9

Calcium
Calcium intake and pregnancy-induced hypertension (Semchyshyn) (Letter); (Belizán and Villar) (Reply). 1989;161:834-5
Erythrocyte cation metabolism in preeclampsia (Sowers et al). 1989;161:441-5

Calcium channel blockers
Doppler assessment of the fetal and uteroplacental circulation during nifedipine therapy for preterm labor (Mari et al). 1989;161:1514-8
Fetal vascular responses to maternal nicardipine administration in the hypertensive ewe (Parisi et al). 1989;161:1035-9
Nifedipine pharmacokinetics during preterm labor tocolysis (Ferguson et al). 1989;161:1485-90

Calcium channel blockers—cont'd
Ovine fetal cardiorespiratory response to nicardipine (Holbrook et al). 1989;161:718-21
Placental vascular responses to nicardipine in the hypertensive ewe (Parisi et al). 1989;161:1039-43
Calcium entry blockers; *see* **Calcium channel blockers**
Callithricidae
Progesterone antagonist lilopristone: a potent abortifacient in the common marmoset (Puri et al). 1989;161:248-53
Cancer; *see* **Neoplasms**
Cancer syndromes, hereditary
Cervical cancer in Jewish women (Glezerman et al). 1989;161:1186-90
Candida albicans
Candida albicans: cellular immune system interactions during different stages of menstrual cycle (Kalo-Klein and Witkin). 1989;161:1132-6
Candida warrants concern (Elliott) (Letter); (Smith) (Reply). 1989;161:503
Carbohydrates
Effect of nutrient intake on premenstrual depression (Wurtman et al). 1989;161:1228-34
Carbohydrates, metabolism
Carbohydrate metabolism in the course of intrahepatic cholestasis in pregnancy (Wójcicka-Jagodzińska et al). 1989;161:959-64
Triphasic Randomized Clinical Trial: comparison of effects on carbohydrate metabolism (Bowes et al). 1989;161:1402-7 (Symposium)
Cardiac output
Cardiovascular changes in early pregnancy (Capeless and Clapp). 1989;161:1449-53
Measurement of cardiac output in pregnancy by thoracic electrical bioimpedance and thermodilution (Masaki et al). 1989;161:680-4
Cardiography, impedance
Measurement of cardiac output in pregnancy by thoracic electrical bioimpedance and thermodilution (Masaki et al). 1989;161:680-4
Cardiolipin antibodies; *see* **Antibodies**
Cardiopulmonary bypass
Subsequent fertility in women who undergo cardiac surgery (Nunley et al). 1989;161:573-6
Cardiovascular diseases
Estrogen replacement and cardiovascular disease: serum lipids and blood pressure effects (Hazzard). 1989;161:1847-53 (Symposium)
Cardiovascular system
Cardiovascular changes in early pregnancy (Capeless and Clapp). 1989;161:1449-53
Carotene
Decreased β-carotene tissue levels in uterine leiomyomas and cancers of reproductive and nonreproductive organs (Palan et al). 1989;161:1649-52
Effects of smoking and oral contraception on plasma β-carotene levels in healthy women (Palan et al). 1989;161:881-5
Carotenoids
Risk factors for vulvar lichen sclerosus (Sideri et al). 1989;161:38-42
Carotid arteries, animal
Effect of pregnancy on uterine and carotid artery response to norepinephrine, epinephrine, and phenylephrine in vessels with documented functional endothelium (Weiner et al). 1989;161:1605-10
In vitro release of endothelium-derived relaxing factor by acetylcholine is increased during the guinea pig pregnancy (Weiner et al). 1989;161:1599-605
Catecholamines
Effect of magnesium on cocaine-induced, catecholamine-mediated platelet and vascular response in term pregnant ewes (Weaver et al). 1989;161:1331-7
Effect of pregnancy on uterine and carotid artery response to norepinephrine, epinephrine, and phenylephrine in vessels with documented functional endothelium (Weiner et al). 1989;161:1605-10
Catheterization, central venous
Central hemodynamic assessment of normal term pregnancy (Clark et al). 1989;161:1439-42
The central hemodynamics of severe preeclampsia (Mabie et al). 1989;161:1443-8
Is central venous pressure monitoring "contraindicated" in patients with severe preeclampsia? (Woodward and Romanoff) (Letter); (Clark and Cotton) (Reply). 1989;161:837-9
Pulmonary artery catheterization in severe eclampsia (Gibbs). 1989;161:1089 (Letter)
Ultrasonographic guidance for central venous access during pregnancy (Lee et al). 1989;161:1012-3
Catheterization, Swan-Ganz; *see also* **Pulmonary artery**
The central hemodynamics of severe preeclampsia (Mabie et al). 1989;161:1443-8
Catheters, indwelling
Central hemodynamic assessment of normal term pregnancy (Clark et al). 1989;161:1439-42
Caucasoid race
Fetal radius length: a critical evaluation of race as a factor in gestational age assessment (Hill et al). 1989;161:193-9
Cell membrane permeability
Is a decrease in plasma oncotic pressure enough to explain the edema of pregnancy? (Valenzuela). 1989;161:1624-7
Cells, cultured
High glucose level decreases proliferation of cultured human fetal cells from placenta (Nelson and Curran). 1989;161:1553-8
Human uterus-derived growth substances for rat bone cells and fibroblasts (Koutsilieris). 1989;161:1313-7
Central nervous system
Dandy-Walker syndrome: a review of fifteen cases evaluated by prenatal sonography (Russ et al). 1989;161:401-6
Central venous pressure
Central hemodynamic assessment of normal term pregnancy (Clark et al). 1989;161:1439-42
The central hemodynamics of severe preeclampsia (Mabie et al). 1989;161:1443-8
Is central venous pressure monitoring "contraindicated" in patients with severe preeclampsia? (Woodward and Romanoff) (Letter); (Clark and Cotton) (Reply). 1989;161:837-9
Pulmonary artery catheterization in severe eclampsia (Gibbs). 1989;161:1089 (Letter)
Ultrasonographic guidance for central venous access during pregnancy (Lee et al). 1989;161:1012-3
Ceramide lactoside
Ceramide lactoside in amniotic fluid: high concentration in chorioamnionitis and in preterm labor (Hallman et al). 1989;161:313-8
Cerebellum
Enlarged cisterna magna in trisomy 18: prenatal ultrasonographic diagnosis (Thurmond et al). 1989;161:83-5

Cerebral arteries
Doppler assessment of the pulsatility index of the middle cerebral artery during constriction of the fetal ductus arteriosus after indomethacin therapy (Mari et al). 1989;161:1528-31
Cerebral ischemia, animal
The effect of elevated blood glucose on the electroencephalogram and cerebral metabolism during short-term brain ischemia in fetal sheep (Chao et al). 1989;161:221-8
Effects of cephalic hypotension, hypertension, and barbiturates on fetal cerebral blood flow and metabolism (Hohimer and Bissonnette). 1989;161:1344-51
Cervicitis
Randomized treatment of mucopurulent cervicitis with doxycycline or amoxicillin (Paavonen et al). 1989;161:128-35
Cervicovaginal lavage; *see* **Cervix uteri, cytology**
Cervix diseases
Comparison of Cytobrush and cervicovaginal lavage sampling methods for the detection of genital human papillomavirus (Goldberg et al). 1989;161:1669-72
Mechanism for human papillomavirus transmission at birth (Sedlacek et al). 1989;161:55-9
Cervix dysplasia
Colposcopic findings and intraepithelial neoplasia in diethylstilbestrol-exposed offspring (Helmerhorst et al). 1989;161:1191-4
A ten-year experience treating cervical intraepithelial neoplasia with the CO_2 laser (Baggish et al). 1989;161:60-8
Cervix neoplasms
Anal submucosal injection: a new route for drug administration in pelvic malignancies. IV. Submucosal anal injection in the treatment of cancer of uterine cervix—preliminary study (Shafik). 1989;161:69-72
Cervical cancer in Jewish women (Glezerman et al). 1989;161:1186-90
Pelvic and paraaortic lymphocysts after radical surgery because of cervical and ovarian cancer (Petru et al). 1989;161:937-41
A ten-year experience treating cervical intraepithelial neoplasia with the CO_2 laser (Baggish et al). 1989;161:60-8
Cervix uteri, cytology
Comparison of Cytobrush and cervicovaginal lavage sampling methods for the detection of genital human papillomavirus (Goldberg et al). 1989;161:1669-72
Cesarean section
Antibiotics for postcesarean endometritis (Duff). 1989;161:1087 (Letter)
Basic reference gambles recommended for utility assessment (Thornton and Lilford) (Letter); (Silver and Minogue) (Reply). 1989;161:256-7
Breech presentation after cesarean section: always a section (Ophir et al). 1989;161:25-8
Court-ordered cesarean section: an analysis of ethical concerns in compelling cases (Elkins et al). 1989;161:150-4
The effect of continuous epidural analgesia on cesarean section for dystocia in nulliparous women (Thorp et al). 1989;161:670-5
Effect of physician characteristics on the cesarean birth rate (Berkowitz et al). 1989;161:146-9
Evaluation of surgical staples in cesarean section (Burkett et al). 1989;161:540-7
Management of the nonvertex second twin: primary cesarean section, external version, or primary breech extraction (Gocke et al). 1989;161:111-4
Neonatal seizures and cesarean section (Boylan) (Letter); (Spellacy) (Reply). 1989;161:836-7
Usefulness of continuous fetal heart rate monitoring during cesarean section (Petrikovsky et al). 1989;161:36-7
Vaginal birth after cesarean delivery in the twin gestation (Strong et al). 1989;161:29-32
Vaginal birth after cesarean section: a reappraisal of risk (Yetman and Nolan). 1989;161:1119-23
Chemotaxis, leukocyte
Amniotic fluid leukotaxis assay as an early indicator of chorioamnionitis (Pankuch et al). 1989;161:802-7
Child abuse, sexual
Anal and perianal abnormalities in prepubertal victims of sexual abuse (Muram). 1989;161:278-81
Chlamydia trachomatis
Fetal death from chlamydial infection across intact amniotic membranes (Thorp et al). 1989;161:1245-6
Randomized treatment of mucopurulent cervicitis with doxycycline or amoxicillin (Paavonen et al). 1989;161:128-35
Cholestasis, intrahepatic
Carbohydrate metabolism in the course of intrahepatic cholestasis in pregnancy (Wójcicka-Jagodzińska et al). 1989;161:959-64
Cholesterol
The effect of triphasic oral contraceptives on plasma lipids and lipoproteins (Patsch et al). 1989;161:1396-401 (Symposium)
Cholinesterases
Effect of magnesium and preeclampsia on plasma cholinesterase activity (Marx) (Letter); (Kambam et al) (Reply). 1989;161:1754-5
Chorioamnionitis
Amniotic fluid leukotaxis assay as an early indicator of chorioamnionitis (Pankuch et al). 1989;161:802-7
Candida warrants concern (Elliott) (Letter); (Smith) (Reply). 1989;161:503
Ceramide lactoside in amniotic fluid: high concentration in chorioamnionitis and in preterm labor (Hallman et al). 1989;161:313-8
Esterase activity in second- and third-trimester amniotic fluid: an indicator of chorioamnionitis (Hoskins et al). 1989;161:1543-5
Haemophilus influenzae culture (Winn and Egley). 1989;161:1419 (Letter)
Choriocarcinoma
Gestational trophoblastic disease and use of oral contraceptives (Rosenberg et al). 1989;161:1087-8 (Letter)
Chorion
Human α- and β-calcitonin gene-related peptides are vasodilators in human chorionic plate vasculature (Firth and Pipkin). 1989;161:1318-9
Ultrasonographic criteria for the prenatal diagnosis of placental chorionicity in twin gestations (Winn et al). 1989;161:1540-2
Chorionic gonadotropins; *see* **Gonadotropins, chorionic**
Chorionic villi
Analysis of fetal loss after transcervical chorionic villus sampling a review of 719 patients (Wade and Young). 1989;161:513-9
Aspirin differentially affects thromboxane and prostacyclin production by trophoblast and villous core compartments of human placental villi (Nelson and Walsh). 1989;161:1593-8
Chorionic villi sampling
Integration of the transabdominal technique into an on-

Chorionic villi sampling—cont'd
going chorionic villus sampling program (Copeland et al). 1989;161:1289-94
Pregnancy loss in a small chorionic villus sampling series (Clark et al). 1989;161:301-2
Chromosome abnormalities
Rapid chromosome analysis with spontaneously dividing cells derived from umbilical cord blood (fetal and neonatal) (Tipton et al). 1989;161:1546-8
Chromosomes, analysis
Rapid chromosome analysis with spontaneously dividing cells derived from umbilical cord blood (fetal and neonatal) (Tipton et al). 1989;161:1546-8
Chung Chong
In vitro uterine response to tetramethylpyrazine, the active constituent of *Chung Chong* (a traditional Chinese medicine) (Tuttle et al). 1989;161:1319-23
Chylomicrons
Absorption of oral progesterone is influenced by vehicle and particle size (Hargrove et al). 1989;161:948-51
Cisterna magna
Enlarged cisterna magna in trisomy 18: prenatal ultrasonographic diagnosis (Thurmond et al). 1989;161:83-5
Cleavage stage, ovum
Semen predictors of in vitro fertilization and embryo cleavage (Daya et al). 1989;161:1284-9
Clindamycin
Short course of antibiotic therapy in treatment of postpartum endomyometritis (Morales et al). 1989;161:568-72
Clinical opinion
Clinical opinion. 1989;161:1-24, 261-77, 843-60, 1095-1105
Clinical protocols
Clinical management of the fetus with markedly diminished umbilical artery end-diastolic flow (Divon et al). 1989;161:1523-7
Clinical trials
Controlled trial of hydration and bed rest versus bed rest alone in the evaluation of preterm uterine contractions (Pircon et al). 1989;161:775-9
Gestational trophoblastic disease: a case-control study from the People's Republic of China (Brinton et al). 1989;161:121-7
Magnesium supplementation during pregnancy: a double-blind randomized controlled clinical trial (Sibai et al). 1989;161:115-9
Removing a retained placenta by oxytocin a controlled study (Wilken-Jensen et al). 1989;161:155-6
Clitoris
A new surgical technique to treat refractory labial fusion in the elderly (Johnson et al). 1989;161:289-90
Clomiphene
Effects of clomiphene citrate on episodic luteinizing hormone secretion throughout the menstrual cycle (Archer et al). 1989;161:581-92
Peri-implantation phase endometrial estrogen and progesterone receptors: effect of ovulation induction with clomiphene citrate (Hecht et al). 1989;161:1688-93
Clone cells
Isolation and sequencing of a complementary deoxyribonucleic acid clone encoding human placental 17β-estradiol dehydrogenase: identification of the putative cofactor binding site (Gast et al). 1989;161:1726-31
Clostridium
Maternal deaths associated with *Clostridium sordellii* infection (McGregor et al). 1989;161:987-95
Cocaine
Cocaine abuse during pregnancy: peripartum prevalence and perinatal outcome (Neerhof et al). 1989;161:633-8
Effect of magnesium on cocaine-induced, catecholamine-mediated platelet and vascular response in term pregnant ewes (Weaver et al). 1989;161:1331-7
In utero cocaine exposure: observations of fetal behavioral state may predict neonatal outcome (Hume et al). 1989;161:685-90
Colposcopy
Colposcopic findings and intraepithelial neoplasia in diethylstilbestrol-exposed offspring (Helmerhorst et al). 1989;161:1191-4
Combination drug therapy; *see* **Drug therapy, combination**
Compliance
Compliance considerations with estrogen replacement: withdrawal bleeding and other factors (Hahn). 1989;161:1854-8 (Symposium)
The patient's reaction to side effects of oral contraceptives (Hillard). 1989;161:1412-5 (Symposium)
Computer assisted diagnosis; *see* **Diagnosis, computer assisted**
Congenital abnormality; *see* **Abnormalities**
Congenital defects; *see* **Abnormalities**
Consultants
Consultants for the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY. 1989;161:1431-8
Contraceptive devices, female
The importance of ultrasonography in infertile women with "forgotten" intrauterine contraceptive devices (Ron-El et al). 1989;161:211-2
Contraceptives, oral
A case-control study of epithelial ovarian cancer (Hartge et al). 1989;161:10-6 (Clin. opinion)
Correcting misconceptions about oral contraceptives (Mishell). 1989;161:1385-9 (Symposium)
The effect of triphasic oral contraceptives on plasma lipids and lipoproteins (Patsch et al). 1989;161:1396-401 (Symposium)
Effects of smoking and oral contraception on plasma β-carotene levels in healthy women (Palan et al). 1989;161:881-5
Evaluating triphasic oral contraceptives: the rationale for a randomized trial (Grimes). 1989;161:1390-2 (Symposium)
Longitudinal ultrasonographic study of the ovarian suppressive activity of a low-dose triphasic oral contraceptive during correct and incorrect pill intake (Hamilton and Hoogland). 1989;161:1159-62
Study design for the Triphasic Randomized Comparative Trial (Dickerson). 1989;161:1392-6 (Symposium)
Triphasic Randomized Clinical Trial: comparative frequency of intermenstrual bleeding (Droegemueller et al). 1989;161:1407-11 (Symposium)
Triphasic Randomized Clinical Trial: comparison of effects on carbohydrate metabolism (Bowes et al). 1989;161:1402-7 (Symposium)
Contraceptives, oral, adverse effects
Correcting misconceptions about oral contraceptives (Mishell). 1989;161:1385-9 (Symposium)
Gestational trophoblastic disease: a case-control study from the People's Republic of China (Brinton et al). 1989;161:121-7
Gestational trophoblastic disease and use of oral contraceptives (Rosenberg et al). 1989;161:1087-8 (Letter)

The patient's reaction to side effects of oral contraceptives (Hillard). 1989;161:1412-5 (Symposium)
Triphasic Randomized Clinical Trial: comparative frequency of intermenstrual bleeding (Droegemueller et al). 1989;161:1407-11 (Symposium)
Cordocentesis; *see* **Fetal blood**
Coronary disease
Cardiac isoenzymes and electrocardiographic changes during ritodrine tocolysis (Hadi and Albazzaz). 1989;161:318-21
Decreased in vitro production of 6-keto-prostaglandin $F_{1\alpha}$ by uterine arteries from postmenopausal women (Steinleitner et al). 1989;161:1677-81
Estrogen replacement and cardiovascular disease: serum lipids and blood pressure effects (Hazzard). 1989;161:1847-53 (Symposium)
Coronary heart disease; *see* **Coronary disease**
Corpus luteum maintenance
Effects of systemic administration of indomethacin on ovulation, luteinization, and steroidogenesis in the rabbit ovary (Katz et al). 1989;161:1361-6
Corrections
Effects of regular and decaffeinated coffee on fetal breathing and heart rate (Salvador HS, Koos BJ) (1989;160:1043-7). 1989;161:669
Identification of a deoxyribonucleic acid allelic variant for β1-4 galactosyltransferase expression associated with male sperm binding/penetration infertility (Humphreys-Beher MG, Blackwell RE) (1989;160:1160-5). 1989;161:816
Metabolic changes during medical treatment of endometriosis: Nafarelin acetate versus danazol (Burry et al) (1989;160:1454-61). 1989;161:1755
Perinatal factors influencing atrial natriuretic peptide levels in umbilical arterial plasma at the time of delivery (Castro et al). (1989;161:623-7). 1989;161:1238
Correspondence
Letters. 1989;161:254-9, 497-503, 834-40, 1087-92, 1416-27, 1744-54
Cortisol; *see* **Adrenal cortex hormones**
Counseling
Multidisciplinary team counseling for fetal anomalies (Lorenz and Kuhn). 1989;161:263-6 (Clin. opinion)
Crisis intervention
Court-ordered cesarean section: an analysis of ethical concerns in compelling cases (Elkins et al). 1989;161:150-4
Critical care
Central hemodynamic assessment of normal term pregnancy (Clark et al). 1989;161:1439-42
Cromer antigen
Isoimmunization to Cromer antigen in pregnancy (Sacks and Garratty). 1989;161:928-9
Cultured human fetal cells; *see* **Cells, cultured**
Current development
Current development. 1989;161:1025-34, 1375-84
Current investigation
Current investigation. 1989;161:1297-304
Cystic hygroma; *see* **Lymphangioma**
Cysts
Pelvic and paraaortic lymphocysts after radical surgery because of cervical and ovarian cancer (Petru et al). 1989;161:937-41
Cytobrush; *see* **Cervix uteri, cytology**
Cytochrome P-450
Aromatase in human fetal tissues (Doody and Carr). 1989;161:1694-7
Cytogenetics
Rapid chromosome analysis with spontaneously dividing cells derived from umbilical cord blood (fetal and neonatal) (Tipton et al). 1989;161:1546-8

D

Danazol
Buserelin versus danazol in the treatment of endometriosis-associated infertility (Fedele et al). 1989;161:871-6
Elevated serum levels of endometrial secretory protein PP14 in patients with advanced endometriosis: suppression by treatment with danazol and high-dose medroxyprogesterone acetate (Telimaa et al). 1989;161:866-71
Dandy-Walker syndrome
Dandy-Walker syndrome: a review of fifteen cases evaluated by prenatal sonography (Russ et al). 1989;161:401-6
Death rate, maternal; *see* **Maternal mortality**
Decidua
Vasopressin receptors in human pregnant myometrium and decidua: interactions with oxytocin and vasopressin agonists and antagonists (Ivanisevic et al). 1989;161:1637-43
Deglutition
Stimuli for fetal swallowing: systemic factors (Ross et al). 1989;161:1559-65
Deglutition, animal
Fetal blood volume, urine flow, swallowing, and amniotic fluid volume responses to long-term intravascular infusions of saline (Brace). 1989;161:1049-54
Delivery
Comparison of predelivery versus postdelivery Kleihauer–Betke stains in cases of fetal death (Owen et al). 1989;161:663-6
Increased myometrial responsiveness to oxytocin during term and preterm labor (Garfield and Beier). 1989;161:454-61
Management of the nonvertex second twin: primary cesarean section, external version, or primary breech extraction (Gocke et al). 1989;161:111-4
Perinatal factors influencing atrial natriuretic peptide levels in umbilical arterial plasma at the time of delivery (Castro et al). 1989;161:623-7
Perinatal factors influencing atrial natriuretic peptide levels in umbilical arterial plasma at the time of delivery (Castro et al). (1989;161:623-7). 1989;161:1238 (Correction)
Relaxin in paired samples of serum and milk from women after term and preterm delivery (Eddie et al). 1989;161:970-3
Vaginal birth after cesarean section: a reappraisal of risk (Yetman and Nolan). 1989;161:1119-23
Densitometry
Monitoring skeletal response to estrogen (Mazess et al). 1989;161:843-8 (Clin opinion)
Deoxyribonucleic acid; *see* **DNA**
Deoxyribonucleic acid probes; *see* **DNA probes**
Depression
Depressive episodes in premenstrual syndrome (Mortola et al). 1989;161:1682-7
Effect of nutrient intake on premenstrual depression (Wurtman et al). 1989;161:1228-34
Dexamethasone
Effects of fetal sex and dexamethasone on preterm maternal

Dexamethasone—cont'd
serum concentrations of human chorionic gonadotropin, progesterone, estrone, estradiol, and estriol (Haning et al). 1989;161:1549-53
Impact of antenatal dexamethasone administration on respiratory distress syndrome in surfactant-treated infants (Farrell et al). 1989;161:628-33
Dextrocardia
Anesthetic management of labor in a patient with dextrocardia, congenitally corrected transposition, Wolff-Parkinson-White syndrome, and congestive heart failure (Sellers et al). 1989;161:1001-3
Diabetes mellitus, animal
Gestational diabetes in the BHE rat: influence of dietary fat (Bue et al). 1989;161:234-40
Diabetes mellitus in pregnancy; *see* **Pregnancy in diabetes**
Diagnosis, computer-assisted
Fetal heart rate patterns in the small-for-gestational-age human fetus (Gagnon et al). 1989;161:779-84
Heuristic determination of relevant diagnostic procedures in a medical expert system for gynecology (Small and Muechler). 1989;161:17-24 (Clin. opinion)
Diagnosis, differential
Intrauterine growth retardation: standards for diagnosis (Goldenberg et al). 1989;161:271-7 (Clin. opinion)
Serial human chorionic gonadotropin determinations by fluoroimmunoassay for differentiation between intrauterine and ectopic gestation (Lindblom et al). 1989;161:397-400
Diagnostic services
Heuristic determination of relevant diagnostic procedures in a medical expert system for gynecology (Small and Muechler). 1989;161:17-24 (Clin. opinion)
Diet therapy
Randomized trial of diet versus diet plus cardiovascular conditioning on glucose levels in gestational diabetes (Jovanovic-Peterson et al). 1989;161:415-9
Dietary fats
Gestational diabetes in the BHE rat: influence of dietary fat (Bue et al). 1989;161:234-40
Dietary habits; *see* **Food habits**
Diethylstilbestrol
Colposcopic findings and intraepithelial neoplasia in diethylstilbestrol-exposed offspring (Helmerhorst et al). 1989;161:1191-4
Dilapan
Cervical dilation: a comparison of Lamicel and Dilapan (Wells and Hulka). 1989;161:1124-6
Dilatation and curettage
Cervical dilation: a comparison of Lamicel and Dilapan (Wells and Hulka). 1989;161:1124-6
DNA
Flow cytometry of normal, hyperplastic, and malignant human endometrium (Thornton et al). 1989;161:487-92
Increased frequency of detection of human papillomavirus deoxyribonucleic acid in exfoliated cervical cells during pregnancy (Rando et al). 1989;161:50-5
Mechanism for human papillomavirus transmission at birth (Sedlacek et al). 1989;161:55-9
Rapid determination of fetal sex by deoxyribonucleic acid amplification of Y chromosome–specific sequences (Pinckert et al). 1989;161:693-8
DNA, neoplasm
Flow cytometry of normal, hyperplastic, and malignant human endometrium (Thornton et al). 1989;161:487-92
DNA probes
Isolation and sequencing of a complementary deoxyribonucleic acid clone encoding human placental 17β-estradiol dehydrogenase: identification of the putative cofactor binding site (Gast et al). 1989;161:1726-31
Molecular scanning of Yq11 (interval 6) in men with Sertoli-cell-only syndrome (Johnson et al). 1989;161:1732-7
DNA probes, HPV
Increased frequency of detection of human papillomavirus deoxyribonucleic acid in exfoliated cervical cells during pregnancy (Rando et al). 1989;161:50-5
Presence of human papillomavirus deoxyribonucleic acid (Beckmann et al) (Letter); (Kaufman et al) (Reply). 1989;161:1419-20
Doppler echocardiography; *see* **Echocardiography, Doppler**
Doppler ultrasound; *see* **Ultrasonic diagnosis**
Doppler velocimetry; *see* **Flowmeters**
Down's syndrome
The efficacy of fetal sonographic biometry in Down syndrome screening (Peters et al). 1989;161:297-300
Long bone growth in fetuses with Down syndrome (Fitz-Simmons et al). 1989;161:1174-7
Predictive values, relative risks, and overall benefits of high and low maternal serum α-fetoprotein screening in singleton pregnancies: new epidemiologic data (Milunsky et al). 1989;161:291-7
Prenatal screening for Down syndrome with maternal serum human chorionic gonadotropin levels (Petrocik et al). 1989;161:1168-73
Doxycycline
Randomized treatment of mucopurulent cervicitis with doxycycline or amoxicillin (Paavonen et al). 1989;161:128-35
Drug antagonism
Antagonism of antihypertensive drug therapy in pregnancy by indomethacin? (Schoenfeld et al). 1989;161:1204-5
Drug induced abnormalities; *see* **Abnormalities, drug induced**
Drug interactions
Neuromuscular blockade with magnesium sulfate and nifedipine (Snyder and Cardwell). 1989;161:35-6
Drug resistance, microbial
Vancomycin during pregnancy: Does it cause hearing loss or nephrotoxicity in the infant? (Reyes et al). 1989;161:977-81
Drug therapy, combination
Number clarification requested (Hatjis) (Letter); (Wilkins and Lynch) (Reply). 1989;161:1746
Ductus arteriosus
Doppler assessment of the pulsatility index of the middle cerebral artery during constriction of the fetal ductus arteriosus after indomethacin therapy (Mari et al). 1989;161:1528-31
Dysgerminoma
Seminoma in pubertal patient with androgen insensitivity syndrome (Hurt et al). 1989;161:530-1
Dyspareunia
A new surgical technique to treat refractory labial fusion in the elderly (Johnson et al). 1989;161:289-90
Dysplasia, cervix; *see* **Cervix dysplasia**
Dystocia
The effect of continuous epidural analgesia on cesarean

section for dystocia in nulliparous women (Thorp et al). 1989;161:670-5

E

Echocardiography
Cardiovascular changes in early pregnancy (Capeless and Clapp). 1989;161:1449-53
Hemodynamic changes during twin pregnancy: a Doppler and M-mode echocardiographic study (Robson et al). 1989;161:1273-8

Echocardiography, Doppler
Evaluation of pulsed Doppler echocardiography for measurement of aortic blood flow in the fetal lamb (Veille et al). 1989;161:1610-4
Hemodynamic changes during twin pregnancy: a Doppler and M-mode echocardiographic study (Robson et al). 1989;161:1273-8
Human fetal tricuspid and mitral deceleration time: changes with normal pregnancy and intrauterine growth retardation (Reed et al). 1989;161:1532-5
Maternal hemodynamic effects of uterine contractions by M-mode and pulsed-Doppler echocardiography (Lee et al). 1989;161:974-7
Right ventricular dysfunction in human fetal compromise (Räsänen et al). 1989;161:136-40

Eclampsia
Pulmonary artery catheterization in severe eclampsia (Gibbs). 1989;161:1089 (Letter)

Ectoderm
CA 125 in the epithelium closely related to the embryonic ectoderm: the periderm and the amnion (Nanbu et al). 1989;161:462-7

Ectopic pregnancy; *see* **Pregnancy, ectopic**

Edema
Is a decrease in plasma oncotic pressure enough to explain the edema of pregnancy? (Valenzuela). 1989;161:1624-7

Education, medical
Breast disease programs in obstetrics and gynecology: a plea for training in mammography (Gleicher). 1989;161:267-70 (Clin. opinion)

Elderly; *see* **Aged**

Electrocardiography
Cardiac isoenzymes and electrocardiographic changes during ritodrine tocolysis (Hadi and Albazzaz). 1989;161:318-21

Electroencephalography
The effect of elevated blood glucose on the electroencephalogram and cerebral metabolism during short-term brain ischemia in fetal sheep (Chao et al). 1989;161:221-8

Electromyography
Power spectrum analysis of myometrial electromyogram and intrauterine pressure changes in the pregnant rhesus monkey in late gestation (Hsu et al). 1989;161:467-73

ELISA; *see* **Enzyme-linked immunosorbent assay**

Embolism
Death caused by air embolism associated with neodymium:yttrium-aluminum-garnet laser surgery and artificial sapphire tips (Baggish and Daniell). 1989;161:877-8

Embolism, amniotic fluid, animal
Effect of placental embolization on the umbilical arterial velocity waveform in fetal sheep (Morrow et al). 1989;161:1055-60

Embryo
CA 125 in the epithelium closely related to the embryonic ectoderm: the periderm and the amnion (Nanbu et al). 1989;161:462-7
First-trimester midgut herniation: a high-frequency transvaginal sonographic study (Timor-Tritsch et al). 1989;161:831-3
Semen predictors of in vitro fertilization and embryo cleavage (Daya et al). 1989;161:1284-9

Embryo development; *see* **Fetal development**

Embryo transfer
Cervical pregnancy after in vitro fertilization and embryo transfer (Weyerman et al). 1989;161:1145-6
Successful twin pregnancy after embryo donation to a patient with XY gonadal dysgenesis (Sauer et al). 1989;161:380-1

Endometrial hyperplasia
Flow cytometry of normal, hyperplastic, and malignant human endometrium (Thornton et al). 1989;161:487-92

Endometriosis
Buserelin versus danazol in the treatment of endometriosis-associated infertility (Fedele et al). 1989;161:871-6
Elevated serum levels of endometrial secretory protein PP14 in patients with advanced endometriosis: suppression by treatment with danazol and high-dose medroxyprogesterone acetate (Telimaa et al). 1989;161:866-71
Lymphocyte activity in the presence of peritoneal fluid from fertile women and infertile women with and without endometriosis (Hill and Anderson). 1989;161:861-4
Peritoneal blood painting: an aid in the diagnosis of endometriosis (Redwine). 1989;161:865-6

Endometritis
Antibiotics for postcesarean endometritis (Duff). 1989;161:1087 (Letter)

Endometrium
The cancer question: an overview of recent epidemiologic and retrospective data (Henderson). 1989;161:1859-64 (Symposium)
Epidermal growth factor receptor expression in normal and malignant endometrium (Berchuck et al). 1989;161:1247-52
Flow cytometry of normal, hyperplastic, and malignant human endometrium (Thornton et al). 1989;161:487-92
Immunocytochemical study of *ras* and *myc* proto-oncogene polypeptide expression in the human menstrual cycle (Odom et al). 1989;161:1663-8
Immunohistochemical expression of TAG-72 in normal and malignant endometrium: correlation of antigen expression with estrogen receptor and progesterone receptor levels (Soisson et al). 1989;161:1258-63
Peri-implantation phase endometrial estrogen and progesterone receptors: effect of ovulation induction with clomiphene citrate (Hecht et al). 1989;161:1688-93
Prognostic significance of steroid receptors measured in primary metastatic and recurrent endometrial carcinoma (Borazjani et al). 1989;161:1253-7

Endometrium, cytology
Double endometrial aspirative biopsy (Quilez et al). 1989;161:1426-7 (Letter)

Endomyometritis; *see* **Myometrium, microbiology**

Endorphins
Fetal intermediate lobe is stimulated by parturition (Facchinetti et al). 1989;161:1267-70

Endothelium, vascular
Lipid peroxidation in pregnancy: new perspectives on preeclampsia (Hubel et al). 1989;161:1025-34 (Curr. dev.)

Endothelium, vascular—cont'd
Preeclampsia: an endothelial cell disorder (Roberts et al). 1989;161:1200-4
Endothelium, vascular, animal
Effect of pregnancy on uterine and carotid artery response to norepinephrine, epinephrine, and phenylephrine in vessels with documented functional endothelium (Weiner et al). 1989;161:1605-10
In vitro release of endothelium-derived relaxing factor by acetylcholine is increased during the guinea pig pregnancy (Weiner et al). 1989;161:1599-605
Endothelium-derived relaxing factor
In vitro release of endothelium-derived relaxing factor by acetylcholine is increased during the guinea pig pregnancy (Weiner et al). 1989;161:1599-605
Enzyme-linked immunosorbent assay
Sperm antibodies and infertility (Shulman) (Letter); (Witkin) (Reply). 1989;161:497-9
Epidemiology
Gestational trophoblastic disease: a case-control study from the People's Republic of China (Brinton et al). 1989;161:121-7
Epidermal growth factor receptors; *see* **Receptors, epidermal growth factor-urogastrone**
Epidermal growth factor-urogastrone
Assessment of epidermal growth factor in the healing process of clean full-thickness skin wounds (Jijon et al). 1989;161:1658-62
Human uterus-derived growth substances for rat bone cells and fibroblasts (Koutsilieris). 1989;161:1313-7
Epidermal growth factors; *see* **Growth substances**
Epidural analgesia; *see* **Analgesia, epidural**
Epinephrine
Effect of pregnancy on uterine and carotid artery response to norepinephrine, epinephrine, and phenylephrine in vessels with documented functional endothelium (Weiner et al). 1989;161:1605-10
Erythroblastosis, fetal; *see also* **Rh isoimmunization**
Frequency and timing of intravascular intrauterine transfusions reconsidered (Voto and Margulies) (Letter); (Socol et al) (Reply). 1989;161:255-6
Isoimmunization to Cromer antigen in pregnancy (Sacks and Garratty). 1989;161:928-9
Pulsed Doppler flow-velocity waveforms in the prediction of fetal hematocrit of the severely isoimmunized pregnancy (Copel et al). 1989;161:341-4
Erythropoiesis
Cordocentesis in the investigation of fetal erythropoiesis (Nicolaides et al). 1989;161:1197-200
Esterases
Esterase activity in second- and third-trimester amniotic fluid: an indicator of chorioamnionitis (Hoskins et al). 1989;161:1543-5
Estradiol
Effect of a 48-hour Δ^4-androstenedione infusion on the pregnant rhesus monkey in the last third of gestation: changes in maternal plasma estradiol concentrations and myometrial contractility (Figueroa et al). 1989;161:481-6
Effects of fetal sex and dexamethasone on preterm maternal serum concentrations of human chorionic gonadotropin, progesterone, estrone, estradiol, and estriol (Haning et al). 1989;161:1549-53
Mechanisms that subserve estradiol's induction of increased prolactin concentrations: evidence of amplitude modulation of spontaneous prolactin secretory bursts (Veldhuis et al). 1989;161:1149-58
Treatment of oophorectomized guinea pigs with intrauterine 17β-estradiol pellets may modulate myometrial β-adrenergic receptor binding properties (Hatjis et al). 1989;161:1628-32
Up-regulation of guinea pig myometrial adenylate cyclase activity by intrauterine estradiol and progesterone pellets (Hatjis and Koritnik). 1989;161:1073-8
Estradiol dehydrogenase
Isolation and sequencing of a complementary deoxyribonucleic acid clone encoding human placental 17β-estradiol dehydrogenase: identification of the putative cofactor binding site (Gast et al). 1989;161:1726-31
Estradiol valerate
Pattern of human chorionic gonadotropin binding in the polycystic ovary (Brawer et al). 1989;161:474-80
Estriol
Effects of fetal sex and dexamethasone on preterm maternal serum concentrations of human chorionic gonadotropin, progesterone, estrone, estradiol, and estriol (Haning et al). 1989;161:1549-53
Estrogens
Effect of a 48-hour Δ^4-androstenedione infusion on the pregnant rhesus monkey in the last third of gestation: changes in maternal plasma estradiol concentrations and myometrial contractility (Figueroa et al). 1989;161:481-6
Estrogens, biosynthesis
Biosynthesis and physiologic effects of estrogen and pathophysiologic effects of estrogen deficiency: a review (Utian). 1989;161:1828-31 (Symposium)
Estrogens, deficiency
Biosynthesis and physiologic effects of estrogen and pathophysiologic effects of estrogen deficiency: a review (Utian). 1989;161:1828-31 (Symposium)
Decreased in vitro production of 6-keto-prostaglandin $F_{1\alpha}$ by uterine arteries from postmenopausal women (Steinleitner et al). 1989;161:1677-81
Estrogen replacement therapy: an overview (Mishell). 1989;161:1825-7 (Symposium)
Estrogens, physiology
Biosynthesis and physiologic effects of estrogen and pathophysiologic effects of estrogen deficiency: a review (Utian). 1989;161:1828-31 (Symposium)
Estrogens, therapeutic use
Biosynthesis and physiologic effects of estrogen and pathophysiologic effects of estrogen deficiency: a review (Utian). 1989;161:1828-31 (Symposium)
The cancer question: an overview of recent epidemiologic and retrospective data (Henderson). 1989;161:1859-64 (Symposium)
Compliance considerations with estrogen replacement: withdrawal bleeding and other factors (Hahn). 1989;161:1854-8 (Symposium)
Estrogen replacement and cardiovascular disease: serum lipids and blood pressure effects (Hazzard). 1989;161:1847-53 (Symposium)
Estrogen replacement therapy: indications, contraindications, and agent selection (Notelovitz). 1989;161:1832-41 (Symposium)
Estrogen replacement therapy: an overview (Mishell). 1989;161:1825-7 (Symposium)
Estrogen replacement therapy: what the future holds (Hammond). 1989;161:1864-8 (Symposium)

Estrogens in the prevention of osteoporosis in postmenopausal women (Genant et al). 1989;161:1842-6 (Symposium)
Monitoring skeletal response to estrogen (Mazess et al). 1989;161:843-8 (Clin opinion)
Twin pregnancy in premature ovarian failure after estrogen treatment: a case report (Tang and Sawers). 1989;161:172-3

Estrone
Effects of fetal sex and dexamethasone on preterm maternal serum concentrations of human chorionic gonadotropin, progesterone, estrone, estradiol, and estriol (Haning et al). 1989;161:1549-53

Ethics, medical
Basic reference gambles recommended for utility assessment (Thornton and Lilford) (Letter); (Silver and Minogue) (Reply). 1989;161:256-7
The challenge of change revisited (Greiss). 1989;161:505-9 (Pres. address)
Court-ordered cesarean section: an analysis of ethical concerns in compelling cases (Elkins et al). 1989;161:150-4

Ethnic groups
Cervical cancer in Jewish women (Glezerman et al). 1989;161:1186-90

Exercise
The effects of maternal exercise on early pregnancy outcome (Clapp). 1989;161:1453-7
Exercise prescription in pregnancy: weight-bearing versus non-weight-bearing exercise (Artal et al). 1989;161:1464-9

Exercise test
Exercise prescription in pregnancy: weight-bearing versus non-weight-bearing exercise (Artal et al). 1989;161:1464-9
Oxygen consumption during treadmill exercise before, during, and after pregnancy (Clapp). 1989;161:1458-64
Predicting maximal oxygen uptake from treadmill testing in trained and untrained women (Martin et al). 1989;161:1127-32

Exercise therapy
Randomized trial of diet versus diet plus cardiovascular conditioning on glucose levels in gestational diabetes (Jovanovic-Peterson et al). 1989;161:415-9

Experimental design; *see* **Research design**

F

False positive reactions
Specifics of specificity and sensitivity (Kadar) (Letter); (Kolins) (Reply). 1989;161:1416-7

Fatty acids
Absorption of oral progesterone is influenced by vehicle and particle size (Hargrove et al). 1989;161:948-51
Prolonged hyperinsulinemia reduces plasma fatty acid levels in the major lipid groups in fetal sheep (Ogburn et al). 1989;161:728-32

Fatty liver
Elevated liver enzymes and thrombocytopenia in the third trimester of pregnancy: an unusual case report and a review of the literature (Hannah et al). 1989;161:322-3

Femur
The efficacy of fetal sonographic biometry in Down syndrome screening (Peters et al). 1989;161:297-300
Race and gender factors omitted from results (Ansbacher) (Letter); (Goldstein) (Reply). 1989;161:502-3

Fenestration
Recurrence of functional ovarian cysts after laparoscopic fenestration (De Wilde). 1989;161:839 (Letter)

Fentanyl
Fentanyl citrate analgesia during labor (Rayburn et al). 1989;161:202-6

Ferritin
Role of ferritin supported in diagnosis of anemias of pregnancy (Goodlin) (Letter); (Schifman) (Reply). 1989;161:258-9

Fertility
Fertility after ectopic pregnancy (Mitchell et al). 1989;161:576-80
Gestational diabetes in the BHE rat: influence of dietary fat (Bue et al). 1989;161:234-40
Lymphocyte activity in the presence of peritoneal fluid from fertile women and infertile women with and without endometriosis (Hill and Anderson). 1989;161:861-4
Subsequent fertility in women who undergo cardiac surgery (Nunley et al). 1989;161:573-6

Fertilization
Significance of D-mannose as a sperm receptor site on the zona pellucida in human fertilization (Mori et al). 1989;161:207-11

Fertilization in vitro
Cervical pregnancy after in vitro fertilization and embryo transfer (Weyerman et al). 1989;161:1145-6
Effects of platelet activating factor on mouse oocyte fertilization in vitro (Minhas et al). 1989;161:1714-7
Ovarian hyperstimulation presenting as acute hydrothorax after in vitro fertilization (Kingsland et al). 1989;161:381-2
Semen predictors of in vitro fertilization and embryo cleavage (Daya et al). 1989;161:1284-9

Fetal anemia; *see* **Fetal hemoglobin**

Fetal anoxia
Renal artery flow-velocity waveforms in normal and hypoxemic fetuses (Vyas et al). 1989;161:168-72

Fetal anoxia, animal
Effects of cephalic hypotension, hypertension, and barbiturates on fetal cerebral blood flow and metabolism (Hohimer and Bissonnette). 1989;161:1344-51

Fetal blood
Blood gases, pH, and lactate in appropriate- and small-for-gestational-age fetuses (Nicolaides et al). 1989;161:996-1001
Clinical management of the fetus with markedly diminished umbilical artery end-diastolic flow (Divon et al). 1989;161:1523-7
Cordocentesis in the diagnosis of intrauterine starvation (Economides et al). 1989;161:1004-8
Cordocentesis in the investigation of fetal erythropoiesis (Nicolaides et al). 1989;161:1197-200
Detection of fetal blood (Leiberman et al) (Letter); (Cardwell) (Reply). 1989;161:257-8
Doppler assessment of the fetal and uteroplacental circulation during nifedipine therapy for preterm labor (Mari et al). 1989;161:1514-8
Doppler assessment of the pulsatility index of the middle cerebral artery during constriction of the fetal ductus arteriosus after indomethacin therapy (Mari et al). 1989;161:1528-31
Duplex Doppler ultrasonographic evaluation of the fetal renal artery in normal and abnormal fetuses (Veille and Kanaan). 1989;161:1502-7

Fetal blood—cont'd
Effect of acute intravascular volume expansion on human fetal prostaglandin concentrations (Weiner and Robillard). 1989;161:1494-7
Effect of diagnostic and therapeutic cordocentesis on maternal serum α-fetoprotein concentration (Weiner et al). 1989;161:706-8
The effect of intravascular transfusion on umbilical venous pressure in anemic fetuses with and without hydrops (Weiner et al). 1989;161:1498-501
Fetal blood gas analysis from cordocentesis for abnormal fetal heart rate patterns (Shah and Boehm). 1989;161:374-6
Fetal hyperinsulinism in anencephaly (Hubinont et al). 1989;161:306-7
Fetal intermediate lobe is stimulated by parturition (Facchinetti et al). 1989;161:1267-70
Human fetal tricuspid and mitral deceleration time: changes with normal pregnancy and intrauterine growth retardation (Reed et al). 1989;161:1532-5
Maternal and fetal blood flow velocity waveforms in patients with preterm labor: relationship to outcome (Brar et al). 1989;161:1519-22
Normal values for human umbilical venous and amniotic fluid pressures and their alteration by fetal disease (Weiner et al). 1989;161:714-7
Plasma atrial natriuretic peptide in human fetus: response to intravascular blood transfusion (Panos et al). 1989;161:357-61
Prediction of hematocrit decline after intravascular fetal transfusion (MacGregor et al). 1989;161:1491-3
Prolactin levels in umbilical cord blood of human infants: relation to gestational age, maternal complications, and neonatal lung function (Parker et al). 1989;161:795-802
Rapid chromosome analysis with spontaneously dividing cells derived from umbilical cord blood (fetal and neonatal) (Tipton et al). 1989;161:1546-8
Routine umbilical cord blood gas determinations? (Thorp et al). 1989;161:600-5
Techniques differ in use of Doppler ultrasonography (Easterling and Benedetti) (Letter); (Lee and Rokey) (Reply). 1989;161:1748-50
Thrombocytopenia and cordocentesis clarifications requested (Weiner). 1989;161:1091-2 (Letter)
Transfer of prostaglandins to the fetus after prostaglandin E_2 vaginal pessary administration (MacKenzie et al). 1989;161:920-3

Fetal blood, animal
Evaluation of pulsed Doppler echocardiography for measurement of aortic blood flow in the fetal lamb (Veille et al). 1989;161:1610-4
Fetal blood volume, urine flow, swallowing, and amniotic fluid volume responses to long-term intravascular infusions of saline (Brace). 1989;161:1049-54
Intrauterine growth retardation and the circulatory responses to acute hypoxemia in fetal sheep (Block et al). 1989;161:1576-9
Ovine fetal cardiovascular responses to packed red blood cell transfusions (Brace). 1989;161:1367-74

Fetal blood, immunology
Fetal cardiac output in the isoimmunized pregnancy: a pulsed Doppler-echocardiographic study of patients undergoing intravascular intrauterine transfusion (Copel et al). 1989;161:361-5
Fetal heart rate patterns in red blood cell isoimmunized pregnancies (Nicolaides et al). 1989;161:351-6
Maternal-fetal glucose gradient in normal pregnancies and in pregnancies complicated by alloimmunization and fetal growth retardation (Nicolini et al). 1989;161:924-7
Pulsed Doppler flow-velocity waveforms in the prediction of fetal hematocrit of the severely isoimmunized pregnancy (Copel et al). 1989;161:341-4

Fetal death
Analysis of fetal loss after transcervical chorionic villus sampling a review of 719 patients (Wade and Young). 1989;161:513-9
Comparison of predelivery versus postdelivery Kleihauer–Betke stains in cases of fetal death (Owen et al). 1989;161:663-6
Does midtrimester ΔOD_{450} of amniotic fluid reflect severity of Rh disease? (Ananth and Queenan). 1989;161:47-9
Fetal death: diagnosis and management (Merlob). 1989;161:254 (Letter)
Fetal death from chlamydial infection across intact amniotic membranes (Thorp et al). 1989;161:1245-6
Fetomaternal hemorrhage possible cause of fetal distress and death (Cardwell and Snyder) (Letter); (Biddinger et al) (Reply). 1989;161:1744
Missed abortion: Still appropriate terminology? (Pridjian and Moawad). 1989;161:261-2 (Clin. opinion)
Perinatal death as a result of social factors (Fribourg) (Letter); (Delke and Minkoff) (Reply). 1989;161:1748
Predictive value of increased plasma levels of fibronectin in gestational hypertension (Ballegeer et al). 1989;161:432-6
Predictive values, relative risks, and overall benefits of high and low maternal serum α-fetoprotein screening in singleton pregnancies: new epidemiologic data (Milunsky et al). 1989;161:291-7
Pregnancy loss in a small chorionic villus sampling series (Clark et al). 1989;161:301-2
The prevalence and biologic significance of lupus anticoagulant and anticardiolipin antibodies in a general obstetric population (Lockwood et al). 1989;161:369-73
Twin-to-twin transfusion syndrome: What are appropriate diagnostic criteria? (Danskin and Neilson). 1989;161:365-9

Fetal death, animal
Effect of inhibiting prostaglandin synthesis in pregnant sheep with 4-aminoantipyrine under normothermic and hyperthermic conditions (Andrianakis et al). 1989;161:241-7

Fetal development
Concurrence of mouthing movement and rapid eye movement/non–rapid eye movement phases with advance in gestation of the human fetus (Horimoto et al). 1989;161:344-51
Dating the early pregnancy by sequential appearance of embryonic structures (Warren et al). 1989;161:747-53
Estimating gestational age in the term pregnancy with a model based on multiple indices of fetal maturity (Goldstein et al). 1989;161:1235-8
Fetal and neonatal cardiovascular complications from β-sympathomimetic therapy for tocolysis (Katz and Seeds). 1989;161:1-9 (Clin. opinion)
Fetal radius length: a critical evaluation of race as a factor in gestational age assessment (Hill et al). 1989;161:193-9
Individual growth curve standards in twins: prediction of

third-trimester growth and birth characteristics (Stefos et al). 1989;161:179-83
Long bone growth in fetuses with Down syndrome (Fitz-Simmons et al). 1989;161:1174-7
A longitudinal study of the relationship between placental and fetal growth as measured by ultrasonography (Wolf et al). 1989;161:1140-5
Low amniotic pressure in oligohydramnios Is this the cause of pulmonary hypoplasia? (Nicolini et al). 1989;161:1098-101 (Clin. opinion)
Normal amniotic fluid volume changes throughout pregnancy (Brace and Wolf). 1989;161:382-8
Plasma amino acids in appropriate- and small-for-gestational-age fetuses (Economides et al). 1989;161:1219-27
The predictive value of fetal breathing movement and Bishop score in the diagnosis of "true" preterm labor (Schreyer et al). 1989;161:886-9
Stimuli for fetal swallowing: systemic factors (Ross et al). 1989;161:1559-65
Ultrasonographic measurement of fetal femur length in growth disturbances (Abramowicz et al). 1989;161:1137-40

Fetal development, animal

In vivo administration of allopurinol affects ovulation and early embryonic development in rabbits (Miyazaki et al). 1989;161:1709-14

Fetal development, epidemiology

Fetal radius length: a critical evaluation of race as a factor in gestational age assessment (Hill et al). 1989;161:193-9

Fetal diseases; *see also* **Abnormalities**

Abscess of allantoic duct remnant (Baill et al). 1989;161:334-6
Amniotic fluid acetylcholinesterase ratios in prenatal diagnosis of fetal abnormalities (Kelly et al). 1989;161:703-5
Antenatal ultrasonographic diagnosis of fetal seizure activity (Landy et al). 1989;161:308
Candida warrants concern (Elliott) (Letter); (Smith) (Reply). 1989;161:503
First-trimester and early second-trimester diagnosis of nuchal cystic hygroma by transvaginal sonography: diverse prognosis of the septated from the nonseptated lesion (Bronshtein et al). 1989;161:78-82
First-trimester midgut herniation: a high-frequency transvaginal sonographic study (Timor-Tritsch et al). 1989;161:831-3
In utero cocaine exposure: observations of fetal behavioral state may predict neonatal outcome (Hume et al). 1989;161:685-90
Isolation of human immunodeficiency virus from the placenta or from maternal blood contaminating the placenta? (Greenspoon and Settlage) (Letter); (Hill et al) (Reply). 1989;161:501-2
Noninvasive management of isolated bilateral fetal hydrothorax (Pijpers et al). 1989;161:330-2
Normal values for human umbilical venous and amniotic fluid pressures and their alteration by fetal disease (Weiner et al). 1989;161:714-7

Fetal distress

Acid-base status at birth and neurodevelopmental outcome at four and one-half years (Dennis et al). 1989;161:213-20
Effects of maternal hyperoxia on the biophysical assessment of fetuses with suspected intrauterine growth retardation (Ruedrich et al). 1989;161:188-92
Fetal blood gas analysis from cordocentesis for abnormal fetal heart rate patterns (Shah and Boehm). 1989;161:374-6
Fetomaternal hemorrhage possible cause of fetal distress and death (Cardwell and Snyder) (Letter); (Biddinger et al) (Reply). 1989;161:1744
Intrapartum Doppler velocimetry, amniotic fluid volume, and fetal heart rate as predictors of subsequent fetal distress. I. An initial report (Sarno et al). 1989;161:1508-14
Perinatal factors influencing atrial natriuretic peptide levels in umbilical arterial plasma at the time of delivery (Castro et al). 1989;161:623-7
Perinatal factors influencing atrial natriuretic peptide levels in umbilical arterial plasma at the time of delivery (Castro et al). (1989;161:623-7). 1989;161:1238 (Correction)
Right ventricular dysfunction in human fetal compromise (Räsänen et al). 1989;161:136-40
Usefulness of continuous fetal heart rate monitoring during cesarean section (Petrikovsky et al). 1989;161:36-7

Fetal distress, animal

Effects of cephalic hypotension, hypertension, and barbiturates on fetal cerebral blood flow and metabolism (Hohimer and Bissonnette). 1989;161:1344-51
The use of an indwelling Doppler probe to study acute changes in umbilical artery waveforms in the fetal sheep (Abitbol et al). 1989;161:1324-31

Fetal growth retardation

Blood gases, pH, and lactate in appropriate- and small-for-gestational-age fetuses (Nicolaides et al). 1989;161:996-1001
Doppler velocimetry and placental disease (Bracero et al). 1989;161:388-93
Duplex Doppler ultrasonographic evaluation of the fetal renal artery in normal and abnormal fetuses (Veille and Kanaan). 1989;161:1502-7
Effect of placental laterality on uterine artery resistance and development of preeclampsia and intrauterine growth retardation (Kofinas et al). 1989;161:1536-9
Effects of maternal hyperoxia on the biophysical assessment of fetuses with suspected intrauterine growth retardation (Ruedrich et al). 1989;161:188-92
Fetal heart rate patterns in the small-for-gestational-age human fetus (Gagnon et al). 1989;161:779-84
Glycemic control in gestational diabetes mellitus How tight is tight enough: Small for gestational age versus large for gestational age? (Langer et al). 1989;161:646-53
Human fetal tricuspid and mitral deceleration time: changes with normal pregnancy and intrauterine growth retardation (Reed et al). 1989;161:1532-5
Intrauterine growth retardation: standards for diagnosis (Goldenberg et al). 1989;161:271-7 (Clin. opinion)
Maternal-fetal glucose gradient in normal pregnancies and in pregnancies complicated by alloimmunization and fetal growth retardation (Nicolini et al). 1989;161:924-7
Plasma amino acids in appropriate- and small-for-gestational-age fetuses (Economides et al). 1989;161:1219-27
Ultrasonographic measurement of fetal femur length in growth disturbances (Abramowicz et al). 1989;161:1137-40
Which is better, being small or thin? (Nikliński) (Letter); (Patterson) (Reply). 1989;161:1089-90

Fetal growth retardation, animal

Effect of placental embolization on the umbilical arterial velocity waveform in fetal sheep (Morrow et al). 1989;161:1055-60

Fetal growth retardation, animal—cont'd
Intrauterine growth retardation and the circulatory responses to acute hypoxemia in fetal sheep (Block et al). 1989;161:1576-9
Pulmonary β-adrenergic receptors and response do not mature precociously in growth-retarded rabbit fetuses (Lee et al). 1989;161:1044-9
Fetal heart
Fetal and neonatal cardiovascular complications from β-sympathomimetic therapy for tocolysis (Katz and Seeds). 1989;161:1-9 (Clin. opinion)
Fetal cardiac output in the isoimmunized pregnancy: a pulsed Doppler-echocardiographic study of patients undergoing intravascular intrauterine transfusion (Copel et al). 1989;161:361-5
Fetal heart rate patterns in red blood cell isoimmunized pregnancies (Nicolaides et al). 1989;161:351-6
Right ventricular dysfunction in human fetal compromise (Räsänen et al). 1989;161:136-40
Fetal heart rate; *see* **Heart rate, fetal**
Fetal hemoglobin
Detection of fetal blood (Leiberman et al) (Letter); (Cardwell) (Reply). 1989;161:257-8
Does midtrimester ΔOD_{450} of amniotic fluid reflect severity of Rh disease? (Ananth and Queenan). 1989;161:47-9
Fetal liver length in normal and isoimmunized pregnancies (Roberts et al). 1989;161:42-6
Fetal hydrops; *see* **Hydrops fetalis**
Fetal hydrothorax; *see* **Hydrothorax**
Fetal macrosomia
Glycemic control in gestational diabetes mellitus How tight is tight enough: Small for gestational age versus large for gestational age? (Langer et al). 1989;161:646-53
Macrosomia in the postdate pregnancy: Is routine ultrasonographic screening indicated? (Chervenak et al). 1989;161:753-6
Fetal maturity, chronologic; *see* **Gestational age**
Fetal membranes
Abscess of allantoic duct remnant (Baill et al). 1989;161:334-6
Fetal death from chlamydial infection across intact amniotic membranes (Thorp et al). 1989;161:1245-6
Prostaglandin E_2 metabolism in the human fetal membranes (Cheung and Challis). 1989;161:1580-5
Fetal membranes, premature rupture
Impact of residual amniotic fluid volume in patients receiving parenteral tocolysis after premature rupture of the membranes (Silver et al). 1989;161:784-7
Prolongation of pregnancy and survival of twin B after loss of twin A at 21 weeks' gestation (Feichtinger et al). 1989;161:891-3
Risk factors for intraamniotic infection: a prospective epidemiologic study (Soper et al). 1989;161:562-8
Saline solution amnioinfusion for oligohydramnios after premature rupture of the membranes (Imanaka et al). 1989;161:102-6
Fetal monitoring
Continuous fetal monitoring with a pulse oximeter: a case of cord compression (Johnson and Johnson). 1989;161:1295-6
Continuous tissue pH monitoring in the term fetus (Small et al). 1989;161:323-9
Effects of maternal hyperoxia on the biophysical assessment of fetuses with suspected intrauterine growth retardation (Ruedrich et al). 1989;161:188-92
Fetal blood gas analysis from cordocentesis for abnormal fetal heart rate patterns (Shah and Boehm). 1989;161:374-6
Fetal heart rate patterns in red blood cell isoimmunized pregnancies (Nicolaides et al). 1989;161:351-6
Postdate fetal surveillance: Is 41 weeks too early? (Guidetti et al). 1989;161:91-3
Preterm and term fetal cardiac and movement responses to vibratory acoustic stimulation (Thomas et al). 1989;161:141-5
Renal artery flow-velocity waveforms in normal and hypoxemic fetuses (Vyas et al). 1989;161:168-72
Risk factors for intraamniotic infection: a prospective epidemiologic study (Soper et al). 1989;161:562-8
Usefulness of continuous fetal heart rate monitoring during cesarean section (Petrikovsky et al). 1989;161:36-7
Fetal movement
Concurrence of mouthing movement and rapid eye movement/non–rapid eye movement phases with advance in gestation of the human fetus (Horimoto et al). 1989;161:344-51
The effect of vibroacoustic stimulation on the nonstress test at gestational ages of thirty-two weeks or less (Druzin et al). 1989;161:1476-8
Effects of low-frequency vibration on human term fetuses (Gagnon et al). 1989;161:1479-85
The effects of vibroacoustic stimulation on baseline heart rate, breathing activity, and body movements of normal term fetuses (Devoe et al). 1989;161:524-9
Human fetal behavioral states after vibratory stimulation (Gagnon et al). 1989;161:1470-6
Preterm and term fetal cardiac and movement responses to vibratory acoustic stimulation (Thomas et al). 1989;161:141-5
Fetal organ maturity
Measurement of fetal urine production in normal pregnancy by real-time ultrasonography (Rabinowitz et al). 1989;161:1264-6
Ovine fetal lung fluid response to atrial natriuretic factor (Castro et al). 1989;161:1337-43
Pulmonary β-adrenergic receptors and response do not mature precociously in growth-retarded rabbit fetuses (Lee et al). 1989;161:1044-9
Surfactant proteins in the diagnosis of fetal lung maturity. II. The 35 kd protein and phospholipids in complicated pregnancy (Hallman et al). 1989;161:965-9
Uselessness of the phosphatidylglycerol assay for prediction of lung maturity (Egberts and Wijnands) (Letter); (Fadel) (Reply). 1989;161:1417-8
Fetal presentation, breech; *see* **Breech presentation**
Fetal proteins
Immunoregulatory activity in supernatants from cultures of normal human trophoblast cells of the first trimester (Sanyal et al). 1989;161:446-53
Fetal scalp monitoring; *see* **Fetal monitoring**
Fetal transfusion; *see* **Blood transfusion, intrauterine**
Fetal viability
Renal artery flow-velocity waveforms in normal and hypoxemic fetuses (Vyas et al). 1989;161:168-72
Fetofetal transfusion
Perinatal outcome in very preterm births with twin-twin transfusion syndrome (Shan and Chaffin). 1989;161:1111-3
Twin-to-twin transfusion syndrome: What are appropriate

diagnostic criteria? (Danskin and Neilson). 1989;161:365-9

Fetomaternal transfusion
Comparison of predelivery versus postdelivery Kleihauer–Betke stains in cases of fetal death (Owen et al). 1989;161:663-6
Fetomaternal hemorrhage possible cause of fetal distress and death (Cardwell and Snyder) (Letter); (Biddinger et al) (Reply). 1989;161:1744

α-Fetoproteins; *see* **Alpha fetoproteins**

Fetus
Effects of fetal sex and dexamethasone on preterm maternal serum concentrations of human chorionic gonadotropin, progesterone, estrone, estradiol, and estriol (Haning et al). 1989;161:1549-53
Effects of maternal hyperoxia on the biophysical assessment of fetuses with suspected intrauterine growth retardation (Ruedrich et al). 1989;161:188-92
The efficacy of fetal sonographic biometry in Down syndrome screening (Peters et al). 1989;161:297-300
Fetal liver length in normal and isoimmunized pregnancies (Roberts et al). 1989;161:42-5

Fetus, abnormalities
Comparison of six different ultrasonographic methods for predicting lethal fetal pulmonary hypoplasia (Vintzileos et al). 1989;161:606-12
Early pregnancy glycosylated hemoglobin, severity of diabetes, and fetal malformations (Lucas et al). 1989;161:426-31
Fetal hyperinsulinism in anencephaly (Hubinont et al). 1989;161:306-7
Multidisciplinary team counseling for fetal anomalies (Lorenz and Kuhn). 1989;161:263-6 (Clin. opinion)

Fetus, animal
Does furosemide alter the hemodynamic response to rapid intravascular transfusion of the anemic fetal lamb (Chestnut et al). 1989;161:1571-5
The effect of elevated blood glucose on the electroencephalogram and cerebral metabolism during short-term brain ischemia in fetal sheep (Chao et al). 1989;161:221-8
Effect of prolonged hypoxemia on fetal heart rate accelerations and decelerations in sheep (Bocking et al). 1989;161:722-7
Effects of cephalic hypotension, hypertension, and barbiturates on fetal cerebral blood flow and metabolism (Hohimer and Bissonnette). 1989;161:1344-51
Fetal vascular responses to maternal nicardipine administration in the hypertensive ewe (Parisi et al). 1989;161:1035-9
Ovine fetal cardiorespiratory response to nicardipine (Holbrook et al). 1989;161:718-21
Prolonged hyperinsulinemia reduces plasma fatty acid levels in the major lipid groups in fetal sheep (Ogburn et al). 1989;161:728-32

Fetus, cytology
Aromatase in human fetal tissues (Doody and Carr). 1989;161:1694-7
High glucose level decreases proliferation of cultured human fetal cells from placenta (Nelson and Curran). 1989;161:1553-8

Fever
Effect of inhibiting prostaglandin synthesis in pregnant sheep with 4-aminoantipyrine under normothermic and hyperthermic conditions (Andrianakis et al). 1989;161:241-7

Fibroblasts
Human uterus-derived growth substances for rat bone cells and fibroblasts (Koutsilieris). 1989;161:1313-7

Fibronectins
Predictive value of increased plasma levels of fibronectin in gestational hypertension (Ballegeer et al). 1989;161:432-6

First trimester pregnancy; *see* **Pregnancy trimester, first**

Flow cytometry
Flow cytometry of normal, hyperplastic, and malignant human endometrium (Thornton et al). 1989;161:487-92
A monoclonal antibody (MSN-1) against a newly established uterine endometrial cancer cell line (SNG-II) and its application to immunohistochemistry and flow cytometry (Nozawa et al). 1989;161:1079-86

Flowmeters
Clinical management of the fetus with markedly diminished umbilical artery end-diastolic flow (Divon et al). 1989;161:1523-7
Discordant twins a prospective study of the diagnostic value of real-time ultrasonography combined with umbilical artery velocimetry (Divon et al). 1989;161:757-60
Doppler assessment of the fetal and uteroplacental circulation during nifedipine therapy for preterm labor (Mari et al). 1989;161:1514-8
Doppler assessment of the pulsatility index of the middle cerebral artery during constriction of the fetal ductus arteriosus after indomethacin therapy (Mari et al). 1989;161:1528-31
Doppler umbilical artery waveform indices normal values from fourteen to forty-two weeks (Hendricks et al). 1989;161:761-5
Duplex Doppler ultrasonographic evaluation of the fetal renal artery in normal and abnormal fetuses (Veille and Kanaan). 1989;161:1502-7
Evaluation of pulsed Doppler echocardiography for measurement of aortic blood flow in the fetal lamb (Veille et al). 1989;161:1610-4
Intrapartum Doppler velocimetry, amniotic fluid volume, and fetal heart rate as predictors of subsequent fetal distress. I. An initial report (Sarno et al). 1989;161:1508-14
Maternal and fetal blood flow velocity waveforms in patients with preterm labor: relationship to outcome (Brar et al). 1989;161:1519-22
Renal artery flow-velocity waveforms in normal and hypoxemic fetuses (Vyas et al). 1989;161:168-72
Screening with Doppler velocimetry in labor (Feinkind et al). 1989;161:765-70
Systolic/diastolic ratios and waveform shapes (Bewley) (Letter); (Schulman) (Reply). 1989;161:1420-1
Techniques differ in use of Doppler ultrasonography (Easterling and Benedetti) (Letter); (Lee and Rokey) (Reply). 1989;161:1748-50
The use of an indwelling Doppler probe to study acute changes in umbilical artery waveforms in the fetal sheep (Abitbol et al). 1989;161:1324-31
The use of color Doppler imaging for prenatal diagnosis of umbilical cord anomalies: report of three cases (Jauniaux et al). 1989;161:1195-7

Fluid balance; *see* **Water-electrolyte balance**

Fluid therapy
Controlled trial of hydration and bed rest versus bed rest alone in the evaluation of preterm uterine contractions (Pircon et al). 1989;161:775-9

Fluoroimmunoassay
Serial human chorionic gonadotropin determinations by fluoroimmunoassay for differentiation between intrauterine and ectopic gestation (Lindblom et al). 1989;161:397-400
Follicular cyst
Pattern of human chorionic gonadotropin binding in the polycystic ovary (Brawer et al). 1989;161:474-80
Food habits
Risk factors for vulvar lichen sclerosus (Sideri et al). 1989;161:38-42
Forceps; *see* Obstetrical forceps
Forskolin
Uncoupling of excitation from contraction in uterine smooth muscle in near-term ewes (Reid et al). 1989;161:229-33
Free radicals
In vivo administration of allopurinol affects ovulation and early embryonic development in rabbits (Miyazaki et al). 1989;161:1709-14
Frontal bone, growth
In utero diagnosis of benign fetal macrocephaly (DeRosa et al). 1989;161:690-2
Funicitis; *see* Umbilical cord
Furosemide
Does furosemide alter the hemodynamic response to rapid intravascular transfusion of the anemic fetal lamb (Chestnut et al). 1989;161:1571-5

G

Gene amplification
Determination of cellular oncogene rearrangement or amplification in ovarian adenocarcinomas (Smith et al). 1989;161:911-5
Gene rearrangement
Determination of cellular oncogene rearrangement or amplification in ovarian adenocarcinomas (Smith et al). 1989;161:911-5
Genetic counseling
Multidisciplinary team counseling for fetal anomalies (Lorenz and Kuhn). 1989;161:263-6 (Clin. opinion)
Genetic screening
Prenatal screening for Down syndrome with maternal serum human chorionic gonadotropin levels (Petrocik et al). 1989;161:1168-73
Screening for gestational diabetes (Hunter) (Letter); (Weiner) (Reply). 1989;161:1423-4
Genital diseases, female
Phosphorus 31 nuclear magnetic resonance examination of female reproductive tissues (Noyszewski et al). 1989;161:282-8
Gestational age
Concurrence of mouthing movement and rapid eye movement/non–rapid eye movement phases with advance in gestation of the human fetus (Horimoto et al). 1989;161:344-51
Dating the early pregnancy by sequential appearance of embryonic structures (Warren et al). 1989;161:747-53
Doppler umbilical artery waveform indices normal values from fourteen to forty-two weeks (Hendricks et al). 1989;161:761-5
Estimating gestational age in the term pregnancy with a model based on multiple indices of fetal maturity (Goldstein et al). 1989;161:1235-8
Fetal radius length: a critical evaluation of race as a factor in gestational age assessment (Hill et al). 1989;161:193-9
Intrauterine growth retardation: standards for diagnosis (Goldenberg et al). 1989;161:271-7 (Clin. opinion)
Normal amniotic fluid volume changes throughout pregnancy (Brace and Wolf). 1989;161:382-8
Prolactin levels in umbilical cord blood of human infants: relation to gestational age, maternal complications, and neonatal lung function (Parker et al). 1989;161:795-802
Race and gender factors omitted from results (Ansbacher) (Letter); (Goldstein) (Reply). 1989;161:502-3
Gestational trophoblastic tumors; *see* Trophoblastic tumor
Glucose, blood; *see* Blood glucose
Glucose tolerance test
Carbohydrate metabolism in the course of intrahepatic cholestasis in pregnancy (Wójcicka-Jagodzińska et al). 1989;161:959-64
Do the current standards for glucose tolerance testing in pregnancy represent a valid conversion of O'Sullivan's original criteria? (Sacks et al). 1989;161:638-41
Gestational diabetes in the BHE rat: influence of dietary fat (Bue et al). 1989;161:234-40
How reliable is the fifty-gram, one-hour glucose screening test? (Sacks et al). 1989;161:642-5
Management of women with one abnormal oral glucose tolerance test value reduces adverse outcome in pregnancy (Langer et al). 1989;161:593-9
Screening for gestational diabetes (Hunter) (Letter); (Weiner) (Reply). 1989;161:1423-4
Glucuronates
Androgen sulfate and glucuronide conjugates in nonhirsute and hirsute women with polycystic ovarian syndrome (Matteri et al). 1989;161:1704-9
Glycolipids
Ceramide lactoside in amniotic fluid: high concentration in chorioamnionitis and in preterm labor (Hallman et al). 1989;161:313-8
Glycoproteins
Comparison of in vivo bioavailability of progestational agents into the rat uterus and liver and the effect of plasma protein binding (Van Voorhis et al). 1989;161:1698-703
Glycosylated hemoglobin; *see* Hemoglobin A, glycosylated
Gonadal dysgenesis, 46,XY
Successful twin pregnancy after embryo donation to a patient with XY gonadal dysgenesis (Sauer et al). 1989;161:380-1
Gonadotropin-releasing hormone, analogs; *see* Pituitary hormone releasing hormone, analogs
Gonadotropins
Localization of gonadotropin binding sites in human ovarian neoplasms (Nakano et al). 1989;161:905-10
Gonadotropins, chorionic
Effects of fetal sex and dexamethasone on preterm maternal serum concentrations of human chorionic gonadotropin, progesterone, estrone, estradiol, and estriol (Haning et al). 1989;161:1549-53
Effects of systemic administration of indomethacin on ovulation, luteinization, and steroidogenesis in the rabbit ovary (Katz et al). 1989;161:1361-6
Localization of gonadotropin binding sites in human ovarian neoplasms (Nakano et al). 1989;161:905-10
Pattern of human chorionic gonadotropin binding in the polycystic ovary (Brawer et al). 1989;161:474-80
Prenatal screening for Down syndrome with maternal se-

rum human chorionic gonadotropin levels (Petrocik et al). 1989;161:1168-73
Serial human chorionic gonadotropin determinations by fluoroimmunoassay for differentiation between intrauterine and ectopic gestation (Lindblom et al). 1989;161:397-400
Spontaneously resorbing ectopic pregnancy: preservation of human chorionic gonadotropin bioactivity despite declining steroid hormone levels (Sauer et al). 1989;161:1673-6

Graft rejection
A new surgical technique to treat refractory labial fusion in the elderly (Johnson et al). 1989;161:289-90

Grief
Multidisciplinary team counseling for fetal anomalies (Lorenz and Kuhn). 1989;161:263-6 (Clin. opinion)

Growth retardation, intrauterine; *see* **Fetal growth retardation**

Growth substances
Human uterus-derived growth substances for rat bone cells and fibroblasts (Koutsilieris). 1989;161:1313-7

Gynecologic diseases; *see* **Genital diseases, female**

Gynecology
Breast disease programs in obstetrics and gynecology: a plea for training in mammography (Gleicher). 1989;161:267-70 (Clin. opinion)

H

Haemophilus influenzae
Haemophilus influenzae culture (Winn and Egley). 1989;161:1419 (Letter)

HCG; *see* **Gonadotropins, chorionic**

Hearing loss, sensorineural
Vancomycin during pregnancy: Does it cause hearing loss or nephrotoxicity in the infant? (Reyes et al). 1989;161:977-81

Heart defects, congenital
Fetal and neonatal cardiovascular complications from β-sympathomimetic therapy for tocolysis (Katz and Seeds). 1989;161:1-9 (Clin. opinion)

Heart failure, congestive
Anesthetic management of labor in a patient with dextrocardia, congenitally corrected transposition, Wolff-Parkinson-White syndrome, and congestive heart failure (Sellers et al). 1989;161:1001-3

Heart rate, fetal
The effects of vibroacoustic stimulation on baseline heart rate, breathing activity, and body movements of normal term fetuses (Devoe et al). 1989;161:524-9
Fetal and neonatal cardiovascular complications from β-sympathomimetic therapy for tocolysis (Katz and Seeds). 1989;161:1-9 (Clin. opinion)
Fetal blood gas analysis from cordocentesis for abnormal fetal heart rate patterns (Shah and Boehm). 1989;161:374-6
Fetal heart rate patterns in red blood cell isoimmunized pregnancies (Nicolaides et al). 1989;161:351-6
Fetal heart rate patterns in the small-for-gestational-age human fetus (Gagnon et al). 1989;161:779-84
Human fetal behavioral states after vibratory stimulation (Gagnon et al). 1989;161:1470-6
Human fetal tricuspid and mitral deceleration time: changes with normal pregnancy and intrauterine growth retardation (Reed et al). 1989;161:1532-5
Intrapartum Doppler velocimetry, amniotic fluid volume, and fetal heart rate as predictors of subsequent fetal distress. I. An initial report (Sarno et al). 1989;161:1508-14
Preterm and term fetal cardiac and movement responses to vibratory acoustic stimulation (Thomas et al). 1989;161:141-5
Umbilical artery flow velocity waveforms during acute hypoxemia and the relationship with hemodynamic changes in the fetal lamb (van Huisseling et al). 1989;161:1061-4
Usefulness of continuous fetal heart rate monitoring during cesarean section (Petrikovsky et al). 1989;161:36-7

Heart rate, fetal, animal
Effect of prolonged hypoxemia on fetal heart rate accelerations and decelerations in sheep (Bocking et al). 1989;161:722-7
Effects of terbutaline sulfate on fetal cardiac function (Petersen et al). 1989;161:509-12
Ovine fetal cardiorespiratory response to nicardipine (Holbrook et al). 1989;161:718-21
Ovine fetal cardiovascular responses to packed red blood cell transfusions (Brace). 1989;161:1367-74

Heart surgery
Subsequent fertility in women who undergo cardiac surgery (Nunley et al). 1989;161:573-6

Hematocrit
Maternal hematocrit and premature labor (Goodlin) (Letter); (Lieberman et al) (Reply). 1989;161:1750-1
Ovine fetal cardiovascular responses to packed red blood cell transfusions (Brace). 1989;161:1367-74
Prediction of hematocrit decline after intravascular fetal transfusion (MacGregor et al). 1989;161:1491-3
Pulsed Doppler flow-velocity waveforms in the prediction of fetal hematocrit of the severely isoimmunized pregnancy (Copel et al). 1989;161:341-4

Hemodynamics
Cardiovascular changes in early pregnancy (Capeless and Clapp). 1989;161:1449-53
Central hemodynamic assessment of normal term pregnancy (Clark et al). 1989;161:1439-42
The central hemodynamics of severe preeclampsia (Mabie et al). 1989;161:1443-8
Does furosemide alter the hemodynamic response to rapid intravascular transfusion of the anemic fetal lamb (Chestnut et al). 1989;161:1571-5
Hemodynamic changes during twin pregnancy: a Doppler and M-mode echocardiographic study (Robson et al). 1989;161:1273-8
Maternal hemodynamic effects of uterine contractions by M-mode and pulsed-Doppler echocardiography (Lee et al). 1989;161:974-7

Hemoglobin A, glycosylated
Early pregnancy glycosylated hemoglobin, severity of diabetes, and fetal malformations (Lucas et al). 1989;161:426-31

Hemoglobin F; *see* **Fetal hemoglobin**
Maternal hemoglobin F levels may have an adverse effect on neonatal birth weight in pregnancies with sickle cell disease (Anyaegbunam et al). 1989;161:654-6

Hemolytic disease of newborn; *see* **Erythroblastosis, fetal**

Hemorrhage, fetomaternal; *see* **Fetomaternal transfusion**

Heparin
Thrombosis prophylaxis in pregnancy with use of subcutaneous heparin adjusted by monitoring heparin concentration in plasma (Dahlman et al). 1989;161:420-5

Hepatitis B
High hepatitis B carrier rate among non-Oriental patients with acute salpingitis (Smith et al). 1989;161:879-80
Hernia, ventral
First-trimester midgut herniation: a high-frequency transvaginal sonographic study (Timor-Tritsch et al). 1989;161:831-3
Hexoprenaline
Introduction of hexoprenaline into the treatment of preterm labor (Lechner). 1989;161:1090-1 (Letter)
Hirsutism
Androgen sulfate and glucuronide conjugates in nonhirsute and hirsute women with polycystic ovarian syndrome (Matteri et al). 1989;161:1704-9
Histocompatibility antigens
Differences between white and Chinese populations in human leukocyte antigen sharing and gestational trophoblastic tumors (Ho et al). 1989;161:942-8
HIV
Immunosuppression in pregnant women infected with human immunodeficiency virus (Biggar et al). 1989;161:1239-44
Pharmacokinetics of azidothymidine during late pregnancy in Long-Evans rats (Little et al). 1989;161:732-4
HIV seropositivity
Isolation of human immunodeficiency virus from the placenta or from maternal blood contaminating the placenta? (Greenspoon and Settlage) (Letter); (Hill et al) (Reply). 1989;161:501-2
Homeostasis
Effects of thromboxane synthetase inhibition on maternal-fetal homeostasis in gravid ewes with ovine pregnancy-induced hypertension (Keith et al). 1989;161:1305-13
Hospitals, community
Neonatal resuscitation in community hospitals: a regional-based, team-oriented training program coordinated by the tertiary center (Moore et al). 1989;161:849-55 (Clin. opinion)
Human chorionic gonadotropin; *see* **Gonadotropins, chorionic**
Human immunodeficiency viruses; *see* **HIV**
Human leukocyte antigens; *see* **Histocompatibility antigens**
Human papillomavirus DNA probes; *see* **DNA probes, HPV**
Hyaline membrane disease; *see also* **Respiratory distress syndrome**
Prolactin levels in umbilical cord blood of human infants: relation to gestational age, maternal complications, and neonatal lung function (Parker et al). 1989;161:795-802
Hybridization
Increased frequency of detection of human papillomavirus deoxyribonucleic acid in exfoliated cervical cells during pregnancy (Rando et al). 1989;161:50-5
Mechanism for human papillomavirus transmission at birth (Sedlacek et al). 1989;161:55-9
Hydatidiform mole
Gestational trophoblastic disease: a case-control study from the People's Republic of China (Brinton et al). 1989;161:121-7
Gestational trophoblastic disease and use of oral contraceptives (Rosenberg et al). 1989;161:1087-8 (Letter)
Hydramnios
Noninvasive management of isolated bilateral fetal hydrothorax (Pijpers et al). 1989;161:330-2
Saline solution amnioinfusion for oligohydramnios after premature rupture of the membranes (Imanaka et al). 1989;161:102-6
Hydration; *see* **Fluid therapy**
Hydrogen-ion concentration
Blood gases, pH, and lactate in appropriate- and small-for-gestational-age fetuses (Nicolaides et al). 1989;161:996-1001
Continuous tissue pH monitoring in the term fetus (Small et al). 1989;161:323-9
Diagnosis of birth asphyxia on the basis of fetal pH, Apgar score, and newborn cerebral dysfunction (Gilstrap et al). 1989;161:825-30
Effect of whole-body pH changes on thoracic duct lymph flow in anesthetized sheep (Valenzuela et al). 1989;161:1566-70
Routine umbilical cord blood gas determinations? (Thorp et al). 1989;161:600-5
Hydrops fetalis
The effect of intravascular transfusion on umbilical venous pressure in anemic fetuses with and without hydrops (Weiner et al). 1989;161:1498-501
Fetal cardiac output in the isoimmunized pregnancy: a pulsed Doppler-echocardiographic study of patients undergoing intravascular intrauterine transfusion (Copel et al). 1989;161:361-5
Frequency and timing of intravascular intrauterine transfusions reconsidered (Voto and Margulies) (Letter); (Socol et al) (Reply). 1989;161:255-6
Normal values for human umbilical venous and amniotic fluid pressures and their alteration by fetal disease (Weiner et al). 1989;161:714-7
Two hundred intrauterine exchange transfusions in severe blood incompatibilities (Poissonnier et al). 1989;161:709-13
Hydrostatic pressure
Is a decrease in plasma oncotic pressure enough to explain the edema of pregnancy? (Valenzuela). 1989;161:1624-7
Hydrothorax
Leiomyoma of the ovary with ascites and hydrothorax (Nicoll and Cox). 1989;161:177-8
Noninvasive management of isolated bilateral fetal hydrothorax (Pijpers et al). 1989;161:330-2
Ovarian hyperstimulation presenting as acute hydrothorax after in vitro fertilization (Kingsland et al). 1989;161:381-2
Hydroxyeicosatetraenoic acids
5-Hydroxyeicosatetraenoic acid, leukotriene C_4, and prostaglandin $F_{2\alpha}$ in amniotic fluid before and during term and preterm labor (Walsh). 1989;161:1352-60
Hydroxyurea
Standard administration of hydroxyurea recommended (Piver) (Letter); (Stehman and Bundy) (Reply). 1989;161:1425-6
Hyperglycemia
Glycemic control in gestational diabetes mellitus How tight is tight enough: Small for gestational age versus large for gestational age? (Langer et al). 1989;161:646-53
Hyperinsulinism
The central role of obesity (hyperinsulinemia) in the pathogenesis of the polycystic ovary syndrome (Nestler et al). 1989;161:1095-7 (Clin. opinion)
Effects of chronic fetal hyperinsulinemia on plasma arachidonic acid and prostaglandin concentrations (Stonestreet et al). 1989;161:894-9

Hyperinsulinism, animal
Prolonged hyperinsulinemia reduces plasma fatty acid levels in the major lipid groups in fetal sheep (Ogburn et al). 1989;161:728-32
Hyperphenylalaninemia; *see* **Phenylalanine**
Hypersensitivity
Allergic vaginitis (Dworetzky) (Letter); (Galland) (Reply). 1989;161:1752-3
Hypersensitivity to magnesium sulfate (Thorp et al). 1989;161:889-90
Hypertension
Calcium intake and pregnancy-induced hypertension (Semchyshyn) (Letter); (Belizán and Villar) (Reply). 1989;161:834-5
Can platelet volume predict progressive hypertensive disease in pregnancy? (Walker et al). 1989;161:676-9
Estrogen replacement and cardiovascular disease: serum lipids and blood pressure effects (Hazzard). 1989;161:1847-53 (Symposium)
Predictive value of increased plasma levels of fibronectin in gestational hypertension (Ballegeer et al). 1989;161:432-6
Zinc, cadmium, and hypertension in parturient women (Lazebnik et al). 1989;161:437-40
Hypertension, animal
Effect of magnesium on cocaine-induced, catecholamine-mediated platelet and vascular response in term pregnant ewes (Weaver et al). 1989;161:1331-7
Effects of cephalic hypotension, hypertension, and barbiturates on fetal cerebral blood flow and metabolism (Hohimer and Bissonnette). 1989;161:1344-51
Effects of thromboxane synthetase inhibition on maternal-fetal homeostasis in gravid ewes with ovine pregnancy-induced hypertension (Keith et al). 1989;161:1305-13
Fetal vascular responses to maternal nicardipine administration in the hypertensive ewe (Parisi et al). 1989;161:1035-9
Lack of evidence of a vasodepressor role for relaxin in spontaneously hypertensive and normotensive pregnant rats (Ahokas et al). 1989;161:618-22
Placental vascular responses to nicardipine in the hypertensive ewe (Parisi et al). 1989;161:1039-43
Hypertension, classification
Semantic changes suggested in hypertensive disorders (Reamy) (Letter); (Davey and MacGillivray) (Reply). 1989;161:1422-3
Hyperthermia; *see* **Fever**
Hypotension, animal
Effects of cephalic hypotension, hypertension, and barbiturates on fetal cerebral blood flow and metabolism (Hohimer and Bissonnette). 1989;161:1344-51
Hypoxemia; *see* **Anoxemia**
Hysterectomy
Basic reference gambles recommended for utility assessment (Thornton and Lilford) (Letter); (Silver and Minogue) (Reply). 1989;161:255-7
Hysterosalpingography
Fertility after ectopic pregnancy (Mitchell et al). 1989;161:576-80

I

Ifosfamide
Phase II trial of ifosfamide and mesna in mixed mesodermal tumors of the uterus (A Gynecologic Oncology Group Study) (Sutton et al). 1989;161:309-12
IgE
Allergic vaginitis (Dworetzky) (Letter); (Galland) (Reply). 1989;161:1752-3
IgG
Presence of autoantibodies in women with unexplained infertility (Taylor et al). 1989;161:377-9
The prevalence and biologic significance of lupus anticoagulant and anticardiolipin antibodies in a general obstetric population (Lockwood et al). 1989;161:369-73
IgM
Presence of autoantibodies in women with unexplained infertility (Taylor et al). 1989;161:377-9
The prevalence and biologic significance of lupus anticoagulant and anticardiolipin antibodies in a general obstetric population (Lockwood et al). 1989;161:369-73
Immunity, cellular
Candida albicans: cellular immune system interactions during different stages of menstrual cycle (Kalo-Klein and Witkin). 1989;161:1132-6
Immunization, passive
Successful treatment of two women with antiphospholipid antibodies and refractory pregnancy losses with intravenous immunoglobulin infusions (Wapner et al). 1989;161:1271-2
Immunocytochemistry; *see* **Immunohistochemistry**
Immunoglobulins
Successful treatment of two women with antiphospholipid antibodies and refractory pregnancy losses with intravenous immunoglobulin infusions (Wapner et al). 1989;161:1271-2
Immunohistochemistry
CA 125 in the epithelium closely related to the embryonic ectoderm: the periderm and the amnion (Nanbu et al). 1989;161:462-7
Immunocytochemical study of *ras* and *myc* proto-oncogene polypeptide expression in the human menstrual cycle (Odom et al). 1989;161:1663-8
Immunohistochemical expression of TAG-72 in normal and malignant endometrium: correlation of antigen expression with estrogen receptor and progesterone receptor levels (Soisson et al). 1989;161:1258-63
A monoclonal antibody (MSN-1) against a newly established uterine endometrial cancer cell line (SNG-II) and its application to immunohistochemistry and flow cytometry (Nozawa et al). 1989;161:1079-86
Immunologic suppression; *see* **Immunosuppression**
Immunosuppression
Histologic and immunologic study of uterine biopsy tissue of women with incipient abortion (Michel et al). 1989;161:409-14
Immunoregulatory activity in supernatants from cultures of normal human trophoblast cells of the first trimester (Sanyal et al). 1989;161:446-53
Immunosuppression in pregnant women infected with human immunodeficiency virus (Biggar et al). 1989;161:1239-44
Indomethacin, adverse effects
Antagonism of antihypertensive drug therapy in pregnancy by indomethacin? (Schoenfeld et al). 1989;161:1204-5
Doppler assessment of the pulsatility index of the middle cerebral artery during constriction of the fetal ductus arteriosus after indomethacin therapy (Mari et al). 1989;161:1528-31

Indomethacin, adverse effects—cont'd
Effects of systemic administration of indomethacin on ovulation, luteinization, and steroidogenesis in the rabbit ovary (Katz et al). 1989;161:1361-6
Infant, low birth weight
Data sought to link adverse pregnancy outcome with maternal serum α-fetoprotein levels in black gravid women (Lenke) (Letter); (Macri et al) (Reply). 1989;161:254-5
Effect of birth weight, race, and sex on survival of low-birth-weight infants in neonatal intensive care (Resnick et al). 1989;161:184-7
Intrauterine growth retardation: standards for diagnosis (Goldenberg et al). 1989;161:271-7 (Clin. opinion)
Maternity shelter care for adolescents: its effect on incidence of low birth weight (LaGuardia et al). 1989;161:303-6
Pregnancy in obstetrics residents (Milet). 1989;161:1422 (Letter)
Infant, newborn
Acid-base status at birth and neurodevelopmental outcome at four and one-half years (Dennis et al). 1989;161:213-20
Fetal and neonatal cardiovascular complications from β-sympathomimetic therapy for tocolysis (Katz and Seeds). 1989;161:1-9 (Clin. opinion)
In utero cocaine exposure: observations of fetal behavioral state may predict neonatal outcome (Hume et al). 1989;161:685-90
Neonatal resuscitation in community hospitals: a regional-based, team-oriented training program coordinated by the tertiary center (Moore et al). 1989;161:849-55 (Clin. opinion)
Routine umbilical cord blood gas determinations? (Thorp et al). 1989;161:600-5
Significant decrease in congenital malformations in newborn infants of an unselected population of diabetic women (Damm and Mølsted-Pedersen). 1989;161:1163-7
Infant, newborn, diseases
Antepartum infection as a result of *Streptococcus pneumoniae* and sepsis in neonate (Andreu et al). 1989;161:1424-5 (Letter)
Diagnosis of birth asphyxia on the basis of fetal pH, Apgar score, and newborn cerebral dysfunction (Gilstrap et al). 1989;161:825-30
Meconium aspiration syndrome: intrapartum and neonatal attributes (Rossi et al). 1989;161:1106-10
Neonatal seizures and cesarean section (Boylan) (Letter); (Spellacy) (Reply). 1989;161:836-7
Vancomycin during pregnancy: Does it cause hearing loss or nephrotoxicity in the infant? (Reyes et al). 1989;161:977-81
Infant, postmature; *see also* **Pregnancy, prolonged**
Postdate fetal surveillance: Is 41 weeks too early? (Guidetti et al). 1989;161:91-3
Infant, premature
Changing outcome of extremely premature infants (≤26 weeks' gestation and ≤750 gm): survival and follow-up at a tertiary center (Ferrara et al). 1989;161:1114-8
Effect of birth weight, race, and sex on survival of low-birth-weight infants in neonatal intensive care (Resnick et al). 1989;161:184-7
The effect of vibroacoustic stimulation on the nonstress test at gestational ages of thirty-two weeks or less (Druzin et al). 1989;161:1476-8
Perinatal outcome in very preterm births with twin-twin transfusion syndrome (Shan and Chaffin). 1989;161:1111-3
Prematurity among insulin-requiring diabetic gravid women (Greene et al). 1989;161:106-11
Infant, small for gestational age
Glycemic control in gestational diabetes mellitus How tight is tight enough: Small for gestational age versus large for gestational age? (Langer et al). 1989;161:646-53
Intrauterine growth retardation: standards for diagnosis (Goldenberg et al). 1989;161:271-7 (Clin. opinion)
Infant mortality
Effect of birth weight, race, and sex on survival of low-birth-weight infants in neonatal intensive care (Resnick et al). 1989;161:184-7
Neonatal mortality in normal birth weight babies: Does the level of hospital care make a difference? (Berg et al). 1989;161:86-91
Infection, intraamniotic; *see* **Amniotic fluid**
Infertility, female
Buserelin versus danazol in the treatment of endometriosis-associated infertility (Fedele et al). 1989;161:871-6
A case-control study of epithelial ovarian cancer (Hartge et al). 1989;161:10-6 (Clin. opinion)
Gestational trophoblastic disease: a case-control study from the People's Republic of China (Brinton et al). 1989;161:121-7
The importance of ultrasonography in infertile women with "forgotten" intrauterine contraceptive devices (Ron-El et al). 1989;161:211-2
Lymphocyte activity in the presence of peritoneal fluid from fertile women and infertile women with and without endometriosis (Hill and Anderson). 1989;161:861-4
Presence of autoantibodies in women with unexplained infertility (Taylor et al). 1989;161:377-9
Relationship between circulating antisperm antibodies in women and autoantibodies on the ejaculated sperm of their partners (Witkin and Chaudhry). 1989;161:900-3
Subsequent fertility in women who undergo cardiac surgery (Nunley et al). 1989;161:573-6
Infertility, male
Relationship between circulating antisperm antibodies in women and autoantibodies on the ejaculated sperm of their partners (Witkin and Chaudhry). 1989;161:900-3
Sperm antibodies and infertility (Shulman) (Letter); (Witkin) (Reply). 1989;161:497-9
Informed consent
Pelvic examinations by medical students (Cohen et al). 1989;161:1013-4
Prenatal informed consent for sonogram: an indication for obstetric ultrasonography (Chervenak et al). 1989;161:857-60 (Clin. opinion)
Infusions, intravenous
Fetal blood volume, urine flow, swallowing, and amniotic fluid volume responses to long-term intravascular infusions of saline (Brace). 1989;161:1049-54
Insemination, artificial
Multiple gestation with intrauterine insemination and menotropin therapy (Corsan et al) (Letter); (Dodson et al) (Reply). 1989;161:1751-2
Insulin
Does insulin affect placental glucose metabolism and transfer? (Urbach et al). 1989;161:953-9

Fetal hyperinsulinism in anencephaly (Hubinont et al). 1989;161:306-7
Prolonged hyperinsulinemia reduces plasma fatty acid levels in the major lipid groups in fetal sheep (Ogburn et al). 1989;161:728-32
Triphasic Randomized Clinical Trial: comparison of effects on carbohydrate metabolism (Bowes et al). 1989;161: 1402-7 (Symposium)
Insulin receptors; *see* **Receptors, insulin**
Intensive care units, neonatal
Effect of birth weight, race, and sex on survival of low-birth-weight infants in neonatal intensive care (Resnick et al). 1989;161:184-7
Interleukin 1
Infection and labor. IV. Cachectin–tumor necrosis factor in the amniotic fluid of women with intraamniotic infection and preterm labor (Romero et al). 1989;161: 336-41
Intermenstrual bleeding; *see* **Menstruation, drug effects**
Internship and residency
Pregnancy in obstetrics residents (Milet). 1989;161:1422 (Letter)
Intraamniotic infection; *see* **Amniotic fluid**
Intraepithelial neoplasia; *see* **Cervix dysplasia**
Intraoperative complications
Does terbutaline cause uterine atony and increase intraoperative blood loss? (Brown) (Letter); (Patriarco) (Reply). 1989;161:259
Intrauterine blood transfusion; *see* **Blood transfusion, intrauterine**
Intrauterine devices
Candida warrants concern (Elliott) (Letter); (Smith) (Reply). 1989;161:503
The importance of ultrasonography in infertile women with "forgotten" intrauterine contraceptive devices (Ron-El et al). 1989;161:211-2
Uterine rupture: Are intrauterine pressure catheters useful in the diagnosis? (Rodriguez et al). 1989;161: 666-9
Intrauterine growth retardation; *see* **Fetal growth retardation**
Iron deficiency anemia; *see* **Anemia, hypochromic**
Ischemic heart disease; *see* **Coronary disease**
Isoenzymes
Cardiac isoenzymes and electrocardiographic changes during ritodrine tocolysis (Hadi and Albazzaz). 1989;161: 318-21
Isoimmunization; *see* **Rh isoimmunization**
Isoproterenol
Pregnancy-induced changes in the interaction of guinea pig myometrial β-adrenergic receptors with *l*-isoproterenol (Hatjis and Grogan). 1989;161:1633-6
Items
Items. 1989;161:260, 504, 842, 1094, 1430, 1756

K

Karyotyping
Rapid chromosome analysis with spontaneously dividing cells derived from umbilical cord blood (fetal and neonatal) (Tipton et al). 1989;161:1546-8
Kidney diseases
Vancomycin during pregnancy: Does it cause hearing loss or nephrotoxicity in the infant? (Reyes et al). 1989;161:977-81
Kleihauer-Betke stains; *see* **Stains and staining**
Kwashiorkor
Cordocentesis in the diagnosis of intrauterine starvation (Economides et al). 1989;161:1004-8

L

Labor
Fentanyl citrate analgesia during labor (Rayburn et al). 1989;161:202-6
Fetal intermediate lobe is stimulated by parturition (Facchinetti et al). 1989;161:1267-70
Oxytocin may explain neonatal seizures (Goodlin). 1989;161:259 (Letter)
Prostaglandin E_2 metabolism in the human fetal membranes (Cheung and Challis). 1989;161:1580-5
Screening with Doppler velocimetry in labor (Feinkind et al). 1989;161:765-70
Labor, animal
The antagonistic effect of oxytocin and relaxin on rat uterine segment contractility (Goldsmith et al). 1989;161:1644-9
5-Hydroxyeicosatetraenoic acid, leukotriene C_4, and prostaglandin $F_{2\alpha}$ in amniotic fluid before and during term and preterm labor (Walsh). 1989;161:1352-60
Increased myometrial responsiveness to oxytocin during term and preterm labor (Garfield and Beier). 1989;161:454-61
Labor, premature
Cardiac isoenzymes and electrocardiographic changes during ritodrine tocolysis (Hadi and Albazzaz). 1989;161:318-21
Cardiovascular and metabolic effects associated with nifedipine and ritodrine tocolysis (Ferguson et al). 1989;161:788-95
Ceramide lactoside in amniotic fluid: high concentration in chorioamnionitis and in preterm labor (Hallman et al). 1989;161:313-8
Controlled trial of hydration and bed rest versus bed rest alone in the evaluation of preterm uterine contractions (Pircon et al). 1989;161:775-9
Doppler assessment of the fetal and uteroplacental circulation during nifedipine therapy for preterm labor (Mari et al). 1989;161:1514-8
The effect of magnesium sulfate tocolysis on the fetal biophysical profile (Peaceman et al). 1989;161:771-4
The incidence of positive amniotic fluid cultures in patients in preterm labor with intact membranes (Skoll et al). 1989;161:813-6
Infection and labor. IV. Cachectin–tumor necrosis factor in the amniotic fluid of women with intraamniotic infection and preterm labor (Romero et al). 1989;161: 336-41
Infection and labor. V. Prevalence, microbiology, and clinical significance of intraamniotic infection in women with preterm labor and intact membranes (Romero et al). 1989;161:817-24
Introduction of hexoprenaline into the treatment of preterm labor (Lechner). 1989;161:1090-1 (Letter)
Maternal and fetal blood flow velocity waveforms in patients with preterm labor: relationship to outcome (Brar et al). 1989;161:1519-22
Maternal hematocrit and premature labor (Goodlin) (Letter); (Lieberman et al) (Reply). 1989;161:1750-1
Nifedipine pharmacokinetics during preterm labor tocolysis (Ferguson et al). 1989;161:1485-90
Pneumonia during pregnancy: Has modern technology im-

Labor, premature—cont'd
proved maternal and fetal outcome? (Madinger et al). 1989;161:657-62
The predictive value of fetal breathing movement and Bishop score in the diagnosis of "true" preterm labor (Schreyer et al). 1989;161:886-9
Relaxin in paired samples of serum and milk from women after term and preterm delivery (Eddie et al). 1989;161:970-3
Unexplained elevated maternal serum α-fetoprotein level associated with third-trimester problems (Stephens). 1989;161:500 (Letter)

Labor, premature, animal
5-Hydroxyeicosatetraenoic acid, leukotriene C_4, and prostaglandin $F_{2\alpha}$ in amniotic fluid before and during term and preterm labor (Walsh). 1989;161:1352-60
Increased myometrial responsiveness to oxytocin during term and preterm labor (Garfield and Beier). 1989;161:454-61
Ovine fetal cardiorespiratory response to nicardipine (Holbrook et al). 1989;161:718-21

Labor complications; *see also* **Amniotic fluid**
Defining "primiparas" (Gooden) (Letter); (Thomson) (Reply). 1989;161:1417
The effect of continuous epidural analgesia on cesarean section for dystocia in nulliparous women (Thorp et al). 1989;161:670-5
Neonatal mortality in normal birth weight babies: Does the level of hospital care make a difference? (Berg et al). 1989;161:86-91
Prophylactic amnioinfusion during labor complicated by meconium: a preliminary report (Sadovsky et al). 1989;161:613-7
Risk factors for intraamniotic infection: a prospective epidemiologic study (Soper et al). 1989;161:562-8

Lactates
Blood gases, pH, and lactate in appropriate- and small-for-gestational-age fetuses (Nicolaides et al). 1989;161:996-1001

Lamicel
Cervical dilation: a comparison of Lamicel and Dilapan (Wells and Hulka). 1989;161:1124-6

Laparoscopy; *see* **Peritoneoscopy**

Laparotomy
Nerve entrapment after Pfannenstiel incision (Piura) (Letter); (Sippo) (Reply). 1989;161:499-500

Laser surgery
Death caused by air embolism associated with neodymium:yttrium-aluminum-garnet laser surgery and artificial sapphire tips (Baggish and Daniell). 1989;161:877-8
A ten-year experience treating cervical intraepithelial neoplasia with the CO_2 laser (Baggish et al). 1989;161:60-8

Leiomyoma
Decreased β-carotene tissue levels in uterine leiomyomas and cancers of reproductive and nonreproductive organs (Palan et al). 1989;161:1649-52
Leiomyoma of the ovary with ascites and hydrothorax (Nicoll and Cox). 1989;161:177-8

Leiomyosarcoma; *see* **Sarcoma**

Letters
Letters. 1989;161:254-9, 497-503, 834-40, 1087-92, 1416-27, 1744-54

Leukocytes
Esterase activity in second- and third-trimester amniotic fluid: an indicator of chorioamnionitis (Hoskins et al). 1989;161:1543-5

Leukotaxis; *see* **Chemotaxis, leukocyte**

Leukotrienes
5-Hydroxyeicosatetraenoic acid, leukotriene C_4, and prostaglandin $F_{2\alpha}$ in amniotic fluid before and during term and preterm labor (Walsh). 1989;161:1352-60

Leydig cell tumor
Steroid biosynthesis in the Sertoli-Leydig cell tumor: effects of insulin and luteinizing hormone (Nagamani et al). 1989;161:1738-43

LH
Effects of clomiphene citrate on episodic luteinizing hormone secretion throughout the menstrual cycle (Archer et al). 1989;161:581-92
Pattern of human chorionic gonadotropin binding in the polycystic ovary (Brawer et al). 1989;161:474-80

Light
Vitamin D (soltriol), light, and reproduction (Stumpf and Denny). 1989;161:1375-84 (Curr. dev.)

Lilopristone; *see* **Abortifacient agents**

***Limulus* test**
Infection and labor. V. Prevalence, microbiology, and clinical significance of intraamniotic infection in women with preterm labor and intact membranes (Romero et al). 1989;161:817-24

Lipid peroxidation
Lipid peroxidation in pregnancy: new perspectives on preeclampsia (Hubel et al). 1989;161:1025-34 (Curr. dev.)

Lipids
Estrogen replacement and cardiovascular disease: serum lipids and blood pressure effects (Hazzard). 1989;161:1847-53 (Symposium)
Prolonged hyperinsulinemia reduces plasma fatty acid levels in the major lipid groups in fetal sheep (Ogburn et al). 1989;161:728-32

Lipopolysaccharides
Infection and labor. V. Prevalence, microbiology, and clinical significance of intraamniotic infection in women with preterm labor and intact membranes (Romero et al). 1989;161:817-24

Lipoproteins
The effect of triphasic oral contraceptives on plasma lipids and lipoproteins (Patsch et al). 1989;161:1396-401 (Symposium)

Liver
Elevated liver enzymes and thrombocytopenia in the third trimester of pregnancy: an unusual case report and a review of the literature (Hannah et al). 1989;161:322-3
Fetal liver length in normal and isoimmunized pregnancies (Roberts et al). 1989;161:42-6

Liver, animal
Comparison of in vivo bioavailability of progestational agents into the rat uterus and liver and the effect of plasma protein binding (Van Voorhis et al). 1989;161:1698-703
Compensatory ovarian hypertrophy occurs by a mechanism distinct from compensatory growth in the regenerating liver (Alvarez et al). 1989;161:1653-7

Liver, transplantation
Successful pregnancy after a liver transplant (Sims et al). 1989;161:532-3

Liver regeneration
Compensatory ovarian hypertrophy occurs by a mechanism distinct from compensatory growth in the regenerating liver (Alvarez et al). 1989;161:1653-7
Longitudinal studies
A longitudinal study of the relationship between placental and fetal growth as measured by ultrasonography (Wolf et al). 1989;161:1140-5
Longitudinal ultrasonographic study of the ovarian suppressive activity of a low-dose triphasic oral contraceptive during correct and incorrect pill intake (Hamilton and Hoogland). 1989;161:1159-62
Low birth weight; *see* **Infant, low birth weight**
Lung
Surfactant proteins in the diagnosis of fetal lung maturity. II. The 35 kd protein and phospholipids in complicated pregnancy (Hallman et al). 1989;161:965-9
Uselessness of the phosphatidylglycerol assay for prediction of lung maturity (Egberts and Wijnands) (Letter); (Fadel) (Reply). 1989;161:1417-8
Lung, abnormalities
Comparison of six different ultrasonographic methods for predicting lethal fetal pulmonary hypoplasia (Vintzileos et al). 1989;161:606-12
Low amniotic pressure in oligohydramnios Is this the cause of pulmonary hypoplasia? (Nicolini et al). 1989;161:1098-101 (Clin. opinion)
Lung, embryology
Low amniotic pressure in oligohydramnios Is this the cause of pulmonary hypoplasia? (Nicolini et al). 1989;161:1098-101 (Clin. opinion)
Lung, secretions, animal
Ovine fetal lung fluid response to atrial natriuretic factor (Castro et al). 1989;161:1337-43
Luteal phase
Optimal time in the menstrual cycle for serum progesterone measurement to diagnose luteal phase defects (Daya). 1989;161:1009-11
Luteinizing hormone; *see* **LH**
Lymph
Effect of whole-body pH changes on thoracic duct lymph flow in anesthetized sheep (Valenzuela et al). 1989;161:1566-70
Lymph flow rate response to angiotensin II is decreased in pregnant sheep (Valenzuela and Longo). 1989;161:1615-9
Lymphangioma
First-trimester and early second-trimester diagnosis of nuchal cystic hygroma by transvaginal sonography: diverse prognosis of the septated from the nonseptated lesion (Bronshtein et al). 1989;161:78-82
Lymphatic diseases
Pelvic and paraaortic lymphocysts after radical surgery because of cervical and ovarian cancer (Petru et al). 1989;161:937-41
Lymphatic system, animal
Effect of whole-body pH changes on thoracic duct lymph flow in anesthetized sheep (Valenzuela et al). 1989;161:1566-70
Lymph flow rate response to angiotensin II is decreased in pregnant sheep (Valenzuela and Longo). 1989;161:1615-9
Lymphocysts; *see* **Lymphatic diseases**
Lymphocytes
Lymphocyte activity in the presence of peritoneal fluid from fertile women and infertile women with and without endometriosis (Hill and Anderson). 1989;161:861-4

M

M-mode echocardiography; *see* **Echocardiography**
Macrocephaly; *see* **Skull, growth**
Macrophages
Release of tumor necrosis factor-α by human peritoneal macrophages in vivo and in vitro (Halme). 1989;161:1718-25
Macrosomia; *see* **Fetal macrosomia**
Magnesium
Effect of magnesium on cocaine-induced, catecholamine-mediated platelet and vascular response in term pregnant ewes (Weaver et al). 1989;161:1331-7
Effect of magnesium and preeclampsia on plasma cholinesterase activity (Marx) (Letter); (Kambam et al) (Reply). 1989;161:1754-5
Magnesium supplementation during pregnancy: a double-blind randomized controlled clinical trial (Sibai et al). 1989;161:115-9
Magnesium sulfate
The effect of magnesium sulfate tocolysis on the fetal biophysical profile (Peaceman et al). 1989;161:771-4
Hypersensitivity to magnesium sulfate (Thorp et al). 1989;161:889-90
Neuromuscular blockade with magnesium sulfate and nifedipine (Snyder and Cardwell). 1989;161:35-6
Number clarification requested (Hatjis) (Letter); (Wilkins and Lynch) (Reply). 1989;161:1746
Mammography
Breast disease programs in obstetrics and gynecology: a plea for training in mammography (Gleicher). 1989;161:267-70 (Clin. opinion)
Mannose
Significance of D-mannose as a sperm receptor site on the zona pellucida in human fertilization (Mori et al). 1989;161:207-11
Marmosets; *see* **Callithricidae**
Mastitis
Methicillin-resistant staphylococcal mastitis (Kalstone). 1989;161:120
Maternal-fetal exchange; *see also* **Tocolytic agents**
Candida warrants concern (Elliott) (Letter); (Smith) (Reply). 1989;161:503
Effects of thromboxane synthetase inhibition on maternal-fetal homeostasis in gravid ewes with ovine pregnancy-induced hypertension (Keith et al). 1989;161:1305-13
Isolation of human immunodeficiency virus from the placenta or from maternal blood contaminating the placenta? (Greenspoon and Settlage) (Letter); (Hill et al) (Reply). 1989;161:501-2
Maternal-fetal glucose gradient in normal pregnancies and in pregnancies complicated by alloimmunization and fetal growth retardation (Nicolini et al). 1989;161:924-7
Maternal health services
Maternity shelter care for adolescents: its effect on incidence of low birth weight (LaGuardia et al). 1989;161:303-6
Maternal mortality
Basic reference gambles recommended for utility assessment (Thornton and Lilford) (Letter); (Silver and Minogue) (Reply). 1989;161:256-7

Maternal mortality—cont'd
Maternal deaths associated with *Clostridium sordellii* infection (McGregor et al). 1989;161:987-95
Maternal mortality in North Carolina: a forty-year experience (May and Greiss). 1989;161:555-61
Pneumonia during pregnancy: Has modern technology improved maternal and fetal outcome? (Madinger et al). 1989;161:657-62
Maternal welfare
Maternity shelter care for adolescents: its effect on incidence of low birth weight (LaGuardia et al). 1989;161:303-6
Maximal oxygen uptake; *see* **Oxygen consumption**
Mayer-Rokitansky syndrome; *see* **Vagina, abnormalities**
Meconium
Prophylactic amnioinfusion during labor complicated by meconium: a preliminary report (Sadovsky et al). 1989;161:613-7
Meconium aspiration
Meconium aspiration syndrome: intrapartum and neonatal attributes (Rossi et al). 1989;161:1106-10
Prophylactic amnioinfusion during labor complicated by meconium: a preliminary report (Sadovsky et al). 1989;161:613-7
Medroxyprogesterone
Elevated serum levels of endometrial secretory protein PP14 in patients with advanced endometriosis: suppression by treatment with danazol and high-dose medroxyprogesterone acetate (Telimaa et al). 1989;161:866-71
Melanocyte stimulating hormone; *see* **MSH**
Menarche
Uterine development and endocrine relationships after menarche (Porcu et al). 1989;161:174-7
Menopause; *see also* **Estrogens, therapeutic use**
Biosynthesis and physiologic effects of estrogen and pathophysiologic effects of estrogen deficiency: a review (Utian). 1989;161:1828-31 (Symposium)
Decreased in vitro production of 6-keto-prostaglandin $F_{1\alpha}$ by uterine arteries from postmenopausal women (Steinleitner et al). 1989;161:1677-81
Estrogen replacement therapy: indications, contraindications, and agent selection (Notelovitz). 1989;161:1832-41 (Symposium)
Estrogen replacement therapy: an overview (Mishell). 1989;161:1825-7 (Symposium)
Estrogen replacement therapy: what the future holds (Hammond). 1989;161:1842-6 (Symposium)
Estrogens in the prevention of osteoporosis in postmenopausal women (Genant et al). 1989;161:1842-6 (Symposium)
A new surgical technique to treat refractory labial fusion in the elderly (Johnson et al). 1989;161:289-90
Menotropins
Multiple gestation with intrauterine insemination and menotropin therapy (Corsan et al) (Letter); (Dodson et al) (Reply). 1989;161:1751-2
Menstrual cycle
Atrial natriuretic peptide during the menstrual cycle (Jensen et al). 1989;161:951-2
Candida albicans: cellular immune system interactions during different stages of menstrual cycle (Kalo-Klein and Witkin). 1989;161:1132-6
Effects of clomiphene citrate on episodic luteinizing hormone secretion throughout the menstrual cycle (Archer et al). 1989;161:581-92
Immunocytochemical study of *ras* and *myc* proto-oncogene polypeptide expression in the human menstrual cycle (Odom et al). 1989;161:1663-8
Optimal time in the menstrual cycle for serum progesterone measurement to diagnose luteal phase defects (Daya). 1989;161:1009-11
Menstruation, drug effects
Compliance considerations with estrogen replacement: withdrawal bleeding and other factors (Hahn). 1989;161:1854-8 (Symposium)
The patient's reaction to side effects of oral contraceptives (Hillard). 1989;161:1412-5 (Symposium)
Triphasic Randomized Clinical Trial: comparative frequency of intermenstrual bleeding (Droegemueller et al). 1989;161:1407-11 (Symposium)
2-Mercaptoethanesulfonate
Phase II trial of ifosfamide and mesna in mixed mesodermal tumors of the uterus (A Gynecologic Oncology Group Study) (Sutton et al). 1989;161:309-12
Metastasis; *see* **Neoplasm metastasis**
Methicillin
Methicillin-resistant staphylococcal mastitis (Kalstone). 1989;161:120
Methodology; *see* **Research design**
Methotrexate
Anal submucosal injection: a new route for drug administration in pelvic malignancies. IV. Submucosal anal injection in the treatment of cancer of uterine cervix preliminary study (Shafik). 1989;161:69-72
Local methotrexate injection: a nonsurgical treatment of ectopic pregnancy (Pansky et al). 1989;161:393-6
Successful treatment of a viable cervical pregnancy with methotrexate (Palti et al). 1989;161:1147-8
Methylprednisolone
Dosage and duration differences lead to diverse conclusions (Shulman) (Letter); (Hammond) (Reply). 1989;161:1744-6
Microspheres
Intrauterine growth retardation and the circulatory responses to acute hypoxemia in fetal sheep (Block et al). 1989;161:1576-9
Midtrimester; *see* **Pregnancy trimester, second**
Milk, human
Relaxin in paired samples of serum and milk from women after term and preterm delivery (Eddie et al). 1989;161:970-3
Miscarriage; *see* **Abortion**
Missed abortion; *see* **Abortion, missed**
Mitral valve
Human fetal tricuspid and mitral deceleration time: changes with normal pregnancy and intrauterine growth retardation (Reed et al). 1989;161:1532-5
Mixed Müllerian tumors; *see* **Uterine neoplasms**
Monoclonal antibodies; *see* **Antibodies, monoclonal**
Monosaccharides
Significance of D-mannose as a sperm receptor site on the zona pellucida in human fertilization (Mori et al). 1989;161:207-11
Morbidity
Cocaine abuse during pregnancy: peripartum prevalence and perinatal outcome (Neerhof et al). 1989;161:633-8
Mortality, maternal; *see* **Maternal mortality**
MSH
Fetal intermediate lobe is stimulated by parturition (Facchinetti et al). 1989;161:1267-70
Müllerian tumors, mixed; *see* **Uterine neoplasms**

Muscle, smooth, vascular, animal
Effect of pregnancy on uterine and carotid artery response to norepinephrine, epinephrine, and phenylephrine in vessels with documented functional endothelium (Weiner et al). 1989;161:1605-10
***myc* genes**; *see* **Oncogenes**
Myometrium
Phosphorus 31 nuclear magnetic resonance examination of female reproductive tissues (Noyszewski et al). 1989;161:282-8
Vasopressin receptors in human pregnant myometrium and decidua: interactions with oxytocin and vasopressin agonists and antagonists (Ivanisevic et al). 1989;161:1637-43
Myometrium, animal
The antagonistic effect of oxytocin and relaxin on rat uterine segment contractility (Goldsmith et al). 1989;161:1644-9
Effect of a 48-hour Δ^4-androstenedione infusion on the pregnant rhesus monkey in the last third of gestation: changes in maternal plasma estradiol concentrations and myometrial contractility (Figueroa et al). 1989;161:481-6
Increased myometrial responsiveness to oxytocin during term and preterm labor (Garfield and Beier). 1989;161:454-61
Power spectrum analysis of myometrial electromyogram and intrauterine pressure changes in the pregnant rhesus monkey in late gestation (Hsu et al). 1989;161:467-73
Pregnancy-induced changes in the interaction of guinea pig myometrial β-adrenergic receptors with *l*-isoproterenol (Hatjis and Grogan). 1989;161:1633-6
Treatment of oophorectomized guinea pigs with intrauterine 17β-estradiol pellets may modulate myometrial β-adrenergic receptor binding properties (Hatjis et al). 1989;161:1628-32
Up-regulation of guinea pig myometrial adenylate cyclase activity by intrauterine estradiol and progesterone pellets (Hatjis and Koritnik). 1989;161:1073-8
Myometrium, microbiology
Short course of antibiotic therapy in treatment of postpartum endomyometritis (Morales et al). 1989;161:568-72

N

Narcotics
Fentanyl citrate analgesia during labor (Rayburn et al). 1989;161:202-6
Natriuretic peptides, atrial
Atrial natriuretic factor: the effects of pregnancy and a high-salt diet on atrial levels (Castro et al). 1989;161:1620-3
Atrial natriuretic factor maternal and fetal concentrations in severe preeclampsia (Hatjis et al). 1989;161:1015-9
Atrial natriuretic peptide during the menstrual cycle (Jensen et al). 1989;161:951-2
Atrial natriuretic peptide receptors in guinea pig placentas (Hatjis and Grogan). 1989;161:493-6
Fetal blood volume, urine flow, swallowing, and amniotic fluid volume responses to long-term intravascular infusions of saline (Brace). 1989;161:1049-54
Ovine fetal lung fluid response to atrial natriuretic factor (Castro et al). 1989;161:1337-43
Perinatal factors influencing atrial natriuretic peptide levels in umbilical arterial plasma at the time of delivery (Castro et al). 1989;161:623-7
Perinatal factors influencing atrial natriuretic peptide levels in umbilical arterial plasma at the time of delivery (Castro et al). (1989;161:623-7). 1989;161:1238 (Correction)
Plasma atrial natriuretic peptide in human fetus: response to intravascular blood transfusion (Panos et al). 1989;161:357-61
Neisseria gonorrhoeae
Randomized treatment of mucopurulent cervicitis with doxycycline or amoxicillin (Paavonen et al). 1989;161:128-35
Neonate; *see* **Infant, newborn**
Neoplasm antibodies; *see* **Antibodies, neoplasm**
Neoplasm metastasis
Prognostic significance of steroid receptors measured in primary metastatic and recurrent endometrial carcinoma (Borazjani et al). 1989;161:1253-7
Neoplasms; *see also specific neoplasm*
A clinical-pathologic study of mixed müllerian tumors of the uterus over a 16-year period The Medical College of Georgia experience (Gallup et al). 1989;161:533-9
Decreased β-carotene tissue levels in uterine leiomyomas and cancers of reproductive and nonreproductive organs (Palan et al). 1989;161:1649-52
Neoplasms, epidemiology
The cancer question: an overview of recent epidemiologic and retrospective data (Henderson). 1989;161:1859-64 (Symposium)
Neoplasms, placenta; *see* **Pregnancy complications, neoplastic**
Nerve compression syndromes
Nerve entrapment after Pfannenstiel incision (Piura) (Letter); (Sippo) (Reply). 1989;161:499-500
Neural tube defects
Amniotic fluid acetylcholinesterase ratios in prenatal diagnosis of fetal abnormalities (Kelly et al). 1989;161:703-5
Predictive values, relative risks, and overall benefits of high and low maternal serum α-fetoprotein screening in singleton pregnancies: new epidemiologic data (Milunsky et al). 1989;161:291-7
The role of ultrasonography and amniocentesis in the evaluation of pregnancies at risk for neural tube defects (Hogge et al). 1989;161:520-4
Neurofibromatosis
Neurofibromatosis and pregnancy (Mitterschiffthaler and Fuith) (Letter); (Blickstein) (Reply). 1989;161:501
Newborn; *see* **Infant, newborn**
Nicardipine
Fetal vascular responses to maternal nicardipine administration in the hypertensive ewe (Parisi et al). 1989;161:1035-9
Ovine fetal cardiorespiratory response to nicardipine (Holbrook et al). 1989;161:718-21
Placental vascular responses to nicardipine in the hypertensive ewe (Parisi et al). 1989;161:1039-43
Nicotinamide
Isolation and sequencing of a complementary deoxyribonucleic acid clone encoding human placental 17β-estradiol dehydrogenase: identification of the putative cofactor binding site (Gast et al). 1989;161:1726-31
Nifedipine
Cardiovascular and metabolic effects associated with nifedipine and ritodrine tocolysis (Ferguson et al). 1989;161:788-95
Doppler assessment of the fetal and uteroplacental circulation during nifedipine therapy for preterm labor (Mari et al). 1989;161:1514-8

Nifedipine—cont'd
Neuromuscular blockade with magnesium sulfate and nifedipine (Snyder and Cardwell). 1989;161:35-6
Nifedipine pharmacokinetics during preterm labor tocolysis (Ferguson et al). 1989;161:1485-90
Nomenclature; *see also* **Semantics**
Defining "primiparas" (Gooden) (Letter); (Thomson) (Reply). 1989;161:1417
Missed abortion: Still appropriate terminology? (Pridjian and Moawad). 1989;161:261-2 (Clin. opinion)
Nonstress test
The effect of vibroacoustic stimulation on the nonstress test at gestational ages of thirty-two weeks or less (Druzin et al). 1989;161:1476-8
Norepinephrine
Effect of pregnancy on uterine and carotid artery response to norepinephrine, epinephrine, and phenylephrine in vessels with documented functional endothelium (Weiner et al). 1989;161:1605-10
Nuclear magnetic resonance
Phosphorus 31 nuclear magnetic resonance examination of female reproductive tissues (Noyszewski et al). 1989;161:282-8
Nutrition
Effect of nutrient intake on premenstrual depression (Wurtman et al). 1989;161:1228-34
Nutritional status
Which is better, being small or thin? (Nikliński) (Letter); (Patterson) (Reply). 1989;161:1089-90

O

Obesity
The central role of obesity (hyperinsulinemia) in the pathogenesis of the polycystic ovary syndrome (Nestler et al). 1989;161:1095-7 (Clin. opinion)
Obstetrical forceps
Effect of physician characteristics on the cesarean birth rate (Berkowitz et al). 1989;161:146-9
Occipital bone, growth
In utero diagnosis of benign fetal macrocephaly (DeRosa et al). 1989;161:690-2
Oligomenorrhea
The patient's reaction to side effects of oral contraceptives (Hillard). 1989;161:1412-5 (Symposium)
Oncogenes
Immunocytochemical study of *ras* and *myc* proto-oncogene polypeptide expression in the human menstrual cycle (Odom et al). 1989;161:1663-8
Oncotic pressure; *see* **Cell membrane permeability**
Oocytes
Effects of platelet activating factor on mouse oocyte fertilization in vitro (Minhas et al). 1989;161:1714-7
In vivo administration of allopurinol affects ovulation and early embryonic development in rabbits (Miyazaki et al). 1989;161:1709-14
Semen predictors of in vitro fertilization and embryo cleavage (Daya et al). 1989;161:1284-9
Oophorectomy; *see* **Ovariectomy**
Oral contraceptives; *see* **Contraceptives, oral**
Osteoblasts
Human uterus-derived growth substances for rat bone cells and fibroblasts (Koutsilieris). 1989;161:1313-7
Osteoporosis
Estrogens in the prevention of osteoporosis in postmenopausal women (Genant et al). 1989;161:1842-6 (Symposium)
Outpatients
Outpatient triplet management: a contemporary review (Newman et al). 1989;161:547-5
Ovarian cancer; *see* **Ovarian diseases**
Ovarian cysts
Recurrence of functional ovarian cysts after laparoscopic fenestration (De Wilde). 1989;161:839 (Letter)
Ovarian diseases
Twin pregnancy in premature ovarian failure after estrogen treatment: a case report (Tang and Sawers). 1989;161:172-3
Ovarian diseases, animal
Compensatory ovarian hypertrophy occurs by a mechanism distinct from compensatory growth in the regenerating liver (Alvarez et al). 1989;161:1653-7
Ovarian neoplasms
CA 125 in the epithelium closely related to the embryonic ectoderm: the periderm and the amnion (Nanbu et al). 1989;161:462-7
CA 125 as an independent prognostic factor for survival in patients with epithelial ovarian cancer (Sevelda et al). 1989;161:1213-6
A case-control study of epithelial ovarian cancer (Hartge et al). 1989;161:10-6 (Clin. opinion)
Determination of cellular oncogene rearrangement or amplification in ovarian adenocarcinomas (Smith et al). 1989;161:911-5
The influence of human antimurine antibody on CA 125 levels in patients with ovarian cancer undergoing radioimmunotherapy or immunoscintigraphy with murine monoclonal antibody OC 125 (Muto et al). 1989;161:1206-12
Leiomyoma of the ovary with ascites and hydrothorax (Nicoll and Cox). 1989;161:177-8
Localization of gonadotropin binding sites in human ovarian neoplasms (Nakano et al). 1989;161:905-10
Pelvic and paraaortic lymphocysts after radical surgery because of cervical and ovarian cancer (Petru et al). 1989;161:937-41
Treatment of advanced refractory ovarian carcinoma with a gonadotropin-releasing hormone analogue (Bruckner and Motwani). 1989;161:1216-8
Ovariectomy
Compensatory ovarian hypertrophy occurs by a mechanism distinct from compensatory growth in the regenerating liver (Alvarez et al). 1989;161:1653-7
Treatment of oophorectomized guinea pigs with intrauterine 17β-estradiol pellets may modulate myometrial β-adrenergic receptor binding properties (Hatjis et al). 1989;161:1628-32
Ovary
Ovarian hyperstimulation presenting as acute hydrothorax after in vitro fertilization (Kingsland et al). 1989;161:381-2
Uterine development and endocrine relationships after menarche (Porcu et al). 1989;161:174-7
Vitamin D (soltriol), light, and reproduction (Stumpf and Denny). 1989;161:1375-84 (Curr. dev.)
Ovary, animal
Compensatory ovarian hypertrophy occurs by a mechanism distinct from compensatory growth in the regenerating liver (Alvarez et al). 1989;161:1653-7

Oviducts
Vitamin D (soltriol), light, and reproduction (Stumpf and Denny). 1989;161:1375-84 (Curr. dev.)
Ovulation
Effects of systemic administration of indomethacin on ovulation, luteinization, and steroidogenesis in the rabbit ovary (Katz et al). 1989;161:1361-6
In vivo administration of allopurinol affects ovulation and early embryonic development in rabbits (Miyazaki et al). 1989;161:1709-14
Ovulation induction
Peri-implantation phase endometrial estrogen and progesterone receptors: effect of ovulation induction with clomiphene citrate (Hecht et al). 1989;161:1688-93
Twin pregnancy in premature ovarian failure after estrogen treatment: a case report (Tang and Sawers). 1989;161:172-3
Oximetry
Continuous fetal monitoring with a pulse oximeter: a case of cord compression (Johnson and Johnson). 1989;161:1295-6
Oxygen consumption
Oxygen consumption during treadmill exercise before, during, and after pregnancy (Clapp). 1989;161:1458-64
Predicting maximal oxygen uptake from treadmill testing in trained and untrained women (Martin et al). 1989;161:1127-32
Oxytocin
The antagonistic effect of oxytocin and relaxin on rat uterine segment contractility (Goldsmith et al). 1989;161:1644-9
Increased myometrial responsiveness to oxytocin during term and preterm labor (Garfield and Beier). 1989;161:454-61
Oxytocin may explain neonatal seizures (Goodlin). 1989;161:259 (Letter)
Removing a retained placenta by oxytocin a controlled study (Wilken-Jensen et al). 1989;161:155-6
Vasopressin receptors in human pregnant myometrium and decidua: interactions with oxytocin and vasopressin agonists and antagonists (Ivanisevic et al). 1989;161:1637-43
Oxytocin, antagonists
In vitro uterine response to tetramethylpyrazine, the active constituent of *Chung Chong* (a traditional Chinese medicine) (Tuttle et al). 1989;161:1319-23

P

Papers
Papers of the Society for Gynecologic Investigation. 1989;161:1439-743
Papillomaviruses
Comparison of Cytobrush and cervicovaginal lavage sampling methods for the detection of genital human papillomavirus (Goldberg et al). 1989;161:1669-72
Increased frequency of detection of human papillomavirus deoxyribonucleic acid in exfoliated cervical cells during pregnancy (Rando et al). 1989;161:50-5
Mechanism for human papillomavirus transmission at birth (Sedlacek et al). 1989;161:55-9
Presence of human papillomavirus deoxyribonucleic acid (Beckmann et al) (Letter); (Kaufman et al) (Reply). 1989;161:1419-20
Parathyroid hormones
Erythrocyte cation metabolism in preeclampsia (Sowers et al). 1989;161:441-5
Partial thromboplastin time
The prevalence and biologic significance of lupus anticoagulant and anticardiolipin antibodies in a general obstetric population (Lockwood et al). 1989;161:369-73
Particle size
Absorption of oral progesterone is influenced by vehicle and particle size (Hargrove et al). 1989;161:948-51
Parturition; *see* **Labor**
Paternity
"Possibly" de novo translocations: prenatal risk counseling (Wassman et al). 1989;161:698-702
Pedophilia
Anal and perianal abnormalities in prepubertal victims of sexual abuse (Muram). 1989;161:278-81
Pelvic examination; *see* **Physical examination**
Pelvic neoplasms
Aggressive angiomyxoma first diagnosed as levator hernia (Sutton et al). 1989;161:73-5
Pemphigus
Pemphigus in pregnancy (Kanwar et al). 1989;161:995-6
Peptides
Human α- and β-calcitonin gene-related peptides are vasodilators in human chorionic plate vasculature (Firth and Pipkin). 1989;161:1318-9
Perfusion
Does insulin affect placental glucose metabolism and transfer? (Urbach et al). 1989;161:953-9
Peritoneal cavity
Peritoneal blood painting: an aid in the diagnosis of endometriosis (Redwine). 1989;161:865-6
Peritoneal cavity, cytology
Lymphocyte activity in the presence of peritoneal fluid from fertile women and infertile women with and without endometriosis (Hill and Anderson). 1989;161:861-4
Peritoneal lavage
Release of tumor necrosis factor-α by human peritoneal macrophages in vivo and in vitro (Halme). 1989;161:1718-25
Peritoneoscopy
Recurrence of functional ovarian cysts after laparoscopic fenestration (De Wilde). 1989;161:839 (Letter)
Pessaries
Transfer of prostaglandins to the fetus after prostaglandin E_2 vaginal pessary administration (MacKenzie et al). 1989;161:920-3
Pfannenstiel incision
Nerve entrapment after Pfannenstiel incision (Piura) (Letter); (Sippo) (Reply). 1989;161:499-500
pH; *see* **Hydrogen-ion concentration**
Phenylalanine
Maternal phenylketonuria and hyperphenylalaninemia: implications for medical practice in the United States (Luder and Greene). 1989;161:1102-5 (Clin. opinion)
Phenylephrine
Effect of pregnancy on uterine and carotid artery response to norepinephrine, epinephrine, and phenylephrine in vessels with documented functional endothelium (Weiner et al). 1989;161:1605-10
Phenylketonuria
Maternal phenylketonuria and hyperphenylalaninemia: implications for medical practice in the United States (Luder and Greene). 1989;161:1102-5 (Clin. opinion)

Phenytoin
Clinical experience with phenytoin prophylaxis in severe preeclampsia (Ryan et al). 1989;161:1297-304 (Curr. invest.)
Philosophy, medical
The impact of the acquired immunodeficiency syndrome epidemic on the philosophy of childbirth (Rotmensch et al). 1989;161:855-6 (Clin. opinion)
Phosphatidylglycerols
Uselessness of the phosphatidylglycerol assay for prediction of lung maturity (Egberts and Wijnands) (Letter); (Fadel) (Reply). 1989;161:1417-8
Phospholipid antibodies; *see* **Antibodies**
Phospholipids
Successful treatment of two women with antiphospholipid antibodies and refractory pregnancy losses with intravenous immunoglobulin infusions (Wapner et al). 1989;161:1271-2
Surfactant proteins in the diagnosis of fetal lung maturity. II. The 35 kd protein and phospholipids in complicated pregnancy (Hallman et al). 1989;161:965-9
Physical examination
Pelvic examinations by medical students (Cohen et al). 1989;161:1013-4
Physician's practice patterns
Effect of physician characteristics on the cesarean birth rate (Berkowitz et al). 1989;161:146-9
Pituitary gland
Fetal intermediate lobe is stimulated by parturition (Facchinetti et al). 1989;161:1267-70
Pituitary hormone releasing hormone, analogs
Treatment of advanced refractory ovarian carcinoma with a gonadotropin-releasing hormone analogue (Bruckner and Motwani). 1989;161:1216-8
Placenta
Abscess of allantoic duct remnant (Baill et al). 1989;161:334-6
Aspirin differentially affects thromboxane and prostacyclin production by trophoblast and villous core compartments of human placental villi (Nelson and Walsh). 1989;161:1593-8
Does insulin affect placental glucose metabolism and transfer? (Urbach et al). 1989;161:953-9
Effect of diagnostic and therapeutic cordocentesis on maternal serum α-fetoprotein concentration (Weiner et al). 1989;161:706-8
Effect of placental laterality on uterine artery resistance and development of preeclampsia and intrauterine growth retardation (Kofinas et al). 1989;161:1536-9
High glucose level decreases proliferation of cultured human fetal cells from placenta (Nelson and Curran). 1989;161:1553-8
Human α- and β-calcitonin gene-related peptides are vasodilators in human chorionic plate vasculature (Firth and Pipkin). 1989;161:1318-9
Increased placental progesterone may cause decreased placental prostacyclin production in preeclampsia (Walsh and Coulter). 1989;161:1586-92
A longitudinal study of the relationship between placental and fetal growth as measured by ultrasonography (Wolf et al). 1989;161:1140-5
Phosphorus 31 nuclear magnetic resonance examination of female reproductive tissues (Noyszewski et al). 1989;161:282-8
Removing a retained placenta by oxytocin a controlled study (Wilken-Jensen et al). 1989;161:155-6
Ultrasonographic criteria for the prenatal diagnosis of placental chorionicity in twin gestations (Winn et al). 1989;161:1540-2
Placenta, animal
Atrial natriuretic peptide receptors in guinea pig placentas (Hatjis and Grogan). 1989;161:493-6
Pharmacokinetics of azidothymidine during late pregnancy in Long-Evans rats (Little et al). 1989;161:732-4
Placental vascular responses to nicardipine in the hypertensive ewe (Parisi et al). 1989;161:1039-43
Placenta, ultrastructure
Ultrastructural aspects of preeclampsia. I. Placental bed and uterine boundary vessels (Shanklin and Sibai). 1989;161:735-41
Placenta accreta
Placenta percreta induced uterine rupture and resulted in intraabdominal abortion (Nagy). 1989;161:1185-6
Placenta diseases
Asymptomatic thrombocytopenia associated with chorioangioma of placenta (Limaye and Tchabo). 1989;161:76-7
Doppler velocimetry and placental disease (Bracero et al). 1989;161:388-93
Isolation of human immunodeficiency virus from the placenta or from maternal blood contaminating the placenta? (Greenspoon and Settlage) (Letter); (Hill et al) (Reply). 1989;161:501-2
Twin-to-twin transfusion syndrome: What are appropriate diagnostic criteria? (Danskin and Neilson). 1989;161:365-9
Placenta diseases, animal
Effect of placental embolization on the umbilical arterial velocity waveform in fetal sheep (Morrow et al). 1989;161:1055-60
Placenta neoplasms; *see* **Pregnancy complications, neoplastic**
Placental function tests
Doppler assessment of the fetal and uteroplacental circulation during nifedipine therapy for preterm labor (Mari et al). 1989;161:1514-8
Placental hormones
Aromatase in human fetal tissues (Doody and Carr). 1989;161:1694-7
Isolation and sequencing of a complementary deoxyribonucleic acid clone encoding human placental 17β-estradiol dehydrogenase; identification of the putative cofactor binding site (Gast et al). 1989;161:1726-31
Placental insufficiency
Cordocentesis in the diagnosis of intrauterine starvation (Economides et al). 1989;161:1004-8
Placental proteins; *see* **Pregnancy proteins**
Placental villi; *see* **Chorionic villi**
Plasma
Comparison of in vivo bioavailability of progestational agents into the rat uterus and liver and the effect of plasma protein binding (Van Voorhis et al). 1989;161:1698-703
Platelet activating factor
Effects of platelet activating factor on mouse oocyte fertilization in vitro (Minhas et al). 1989;161:1714-7
Platelet count
Can platelet volume predict progressive hypertensive disease in pregnancy? (Walker et al). 1989;161:676-9
Platelets; *see* **Blood platelets**

Pleural effusion
Noninvasive management of isolated bilateral fetal hydrothorax (Pijpers et al). 1989;161:330-2
Pneumonia
Pneumonia during pregnancy: Has modern technology improved maternal and fetal outcome? (Madinger et al). 1989;161:657-62
Polychondritis, relapsing
Relapsing polychondritis in pregnancy: a case report and review (Gimovsky and Nishiyama). 1989;161:332-4
Polycystic ovary syndrome
Androgen sulfate and glucuronide conjugates in nonhirsute and hirsute women with polycystic ovarian syndrome (Matteri et al). 1989;161:1704-9
The central role of obesity (hyperinsulinemia) in the pathogenesis of the polycystic ovary syndrome (Nestler et al). 1989;161:1095-7 (Clin. opinion)
Polycystic ovary syndrome, animal
Pattern of human chorionic gonadotropin binding in the polycystic ovary (Brawer et al). 1989;161:474-80
Postmature infant; *see* **Infant, postmature**
Postmenopausal bone loss; *see* **Osteoporosis**
Postoperative complications
Nerve entrapment after Pfannenstiel incision (Piura) (Letter); (Sippo) (Reply). 1989;161:499-500
Postpartum period, infection in; *see* **Puerperal infection**
PP14 secretory protein; *see* **Pregnancy proteins**
Predictive value of tests
Flow cytometry of normal, hyperplastic, and malignant human endometrium (Thornton et al). 1989;161:487-92
The predictive value of fetal breathing movement and Bishop score in the diagnosis of "true" preterm labor (Schreyer et al). 1989;161:886-9
Predictive value of increased plasma levels of fibronectin in gestational hypertension (Ballegeer et al). 1989;161:432-6
Predictive values, relative risks, and overall benefits of high and low maternal serum α-fetoprotein screening in singleton pregnancies: new epidemiologic data (Milunsky et al). 1989;161:291-7
Prednisone
Dosage and duration differences lead to diverse conclusions (Shulman) (Letter); (Hammond) (Reply). 1989;161:1744-6
Pre-eclampsia
Atrial natriuretic factor maternal and fetal concentrations in severe preeclampsia (Hatjis et al). 1989;161:1015-9
The central hemodynamics of severe preeclampsia (Mabie et al). 1989;161:1443-8
Clinical experience with phenytoin prophylaxis in severe preeclampsia (Ryan et al). 1989;161:1297-304 (Curr. invest.)
Effect of magnesium and preeclampsia on plasma cholinesterase activity (Marx) (Letter); (Kambam et al) (Reply). 1989;161:1754-5
Effect of placental laterality on uterine artery resistance and development of preeclampsia and intrauterine growth retardation (Kofinas et al). 1989;161:1536-9
Elevated liver enzymes and thrombocytopenia in the third trimester of pregnancy: an unusual case report and a review of the literature (Hannah et al). 1989;161:322-3
Erythrocyte cation metabolism in preeclampsia (Sowers et al). 1989;161:441-5
Increased placental progesterone may cause decreased placental prostacyclin production in preeclampsia (Walsh and Coulter). 1989;161:1586-92
Is central venous pressure monitoring "contraindicated" in patients with severe preeclampsia? (Woodward and Romanoff) (Letter); (Clark and Cotton) (Reply). 1989;161:837-9
Lipid peroxidation in pregnancy: new perspectives on preeclampsia (Hubel et al). 1989;161:1025-34 (Curr. dev.)
Magnesium supplementation during pregnancy: a double-blind randomized controlled clinical trial (Sibai et al). 1989;161:115-9
Predictive value of increased plasma levels of fibronectin in gestational hypertension (Ballegeer et al). 1989;161:432-6
Preeclampsia: an endothelial cell disorder (Roberts et al). 1989;161:1200-4
Prematurity among insulin-requiring diabetic gravid women (Greene et al). 1989;161:106-11
Ultrastructural aspects of preeclampsia. I. Placental bed and uterine boundary vessels (Shanklin and Sibai). 1989;161:735-41
Zinc, cadmium, and hypertension in parturient women (Lazebnik et al). 1989;161:437-40
Pregnancy
Central hemodynamic assessment of normal term pregnancy (Clark et al). 1989;161:1439-42
Exercise prescription in pregnancy: weight-bearing versus non-weight-bearing exercise (Artal et al). 1989;161:1464-9
Measurement of cardiac output in pregnancy by thoracic electrical bioimpedance and thermodilution (Masaki et al). 1989;161:630-4
The measuring of blood pressure during pregnancy (Villar et al). 1989;161:1019-24
Oxygen consumption during treadmill exercise before, during, and after pregnancy (Clapp). 1989;161:1458-64
Vasopressin receptors in human pregnant myometrium and decidua: interactions with oxytocin and vasopressin agonists and antagonists (Ivanisevic et al). 1989;161:1637-43
Pregnancy in adolescence
Maternity shelter care for adolescents: its effect on incidence of low birth weight (LaGuardia et al). 1989;161:303-6
Pregnancy, animal
Atrial natriuretic factor: the effects of pregnancy and a high-salt diet on atrial levels (Castro et al). 1989;161:1620-3
Effect of inhibiting prostaglandin synthesis in pregnant sheep with 4-aminoantipyrine under normothermic and hyperthermic conditions (Andrianakis et al). 1989;161:241-7
Effect of pregnancy on uterine and carotid artery response to norepinephrine, epinephrine, and phenylephrine in vessels with documented functional endothelium (Weiner et al). 1989;161:1605-10
Gestational diabetes in the BHE rat: influence of dietary fat (Bue et al). 1989;161:234-40
In vitro release of endothelium-derived relaxing factor by acetylcholine is increased during the guinea pig pregnancy (Weiner et al). 1989;161:1599-605
Lack of evidence of a vasodepressor role for relaxin in spontaneously hypertensive and normotensive pregnant rats (Ahokas et al). 1989;161:618-22
Lymph flow rate response to angiotensin II is decreased in pregnant sheep (Valenzuela and Longo). 1989;161:1615-9

Pregnancy, animal—cont'd
Pregnancy-induced changes in the interaction of guinea pig myometrial β-adrenergic receptors with *l*-isoproterenol (Hatjis and Grogan). 1989;161:1633-6
Progesterone antagonist lilopristone: a potent abortifacient in the common marmoset (Puri et al). 1989;161:248-53
Uncoupling of excitation from contraction in uterine smooth muscle in near-term ewes (Reid et al). 1989;161:229-33

Pregnancy in diabetes
Do the current standards for glucose tolerance testing in pregnancy represent a valid conversion of O'Sullivan's original criteria? (Sacks et al). 1989;161:638-41
Early pregnancy glycosylated hemoglobin, severity of diabetes, and fetal malformations (Lucas et al). 1989;161:426-31
Glycemic control in gestational diabetes mellitus How tight is tight enough: Small for gestational age versus large for gestational age? (Langer et al). 1989;161:646-53
How reliable is the fifty-gram, one-hour glucose screening test? (Sacks et al). 1989;161:642-5
Management of women with one abnormal oral glucose tolerance test value reduces adverse outcome in pregnancy (Langer et al). 1989;161:593-9
A population-based study of maternal and perinatal outcome in patients with gestational diabetes (Jacobson and Cousins). 1989;161:981-6
Pregnancy after aortofemoral bypass grafting in a diabetic patient (Bradley-Watson). 1989;161:200-1
Prematurity among insulin-requiring diabetic gravid women (Greene et al). 1989;161:106-11
Randomized trial of diet versus diet plus cardiovascular conditioning on glucose levels in gestational diabetes (Jovanovic-Peterson et al). 1989;161:415-9
Screening for gestational diabetes (Hunter) (Letter); (Weiner) (Reply). 1989;161:1423-4
Significant decrease in congenital malformations in newborn infants of an unselected population of diabetic women (Damm and Mølsted-Pedersen). 1989;161:1163-7

Pregnancy in diabetes, animal
Prolonged hyperinsulinemia reduces plasma fatty acid levels in the major lipid groups in fetal sheep (Ogburn et al). 1989;161:728-32

Pregnancy, ectopic
Cervical pregnancy after in vitro fertilization and embryo transfer (Weyerman et al). 1989;161:1145-6
Fertility after ectopic pregnancy (Mitchell et al). 1989;161:576-80
First successful intrauterine pregnancy after treatment of tubal pregnancy with prostaglandin $F_{2\alpha}$ (Egarter and Husslein). 1989;161:904
Local methotrexate injection: a nonsurgical treatment of ectopic pregnancy (Pansky et al). 1989;161:393-6
Low serum progesterone levels and tubal dysfunction a possible cause of ectopic pregnancy (Pulkkinen and Jaakkola). 1989;161:934-7
Maternal mortality in North Carolina: a forty-year experience (May and Greiss). 1989;161:555-61
Serial human chorionic gonadotropin determinations by fluoroimmunoassay for differentiation between intrauterine and ectopic gestation (Lindblom et al). 1989;161:397-400
Spontaneously resorbing ectopic pregnancy: preservation of human chorionic gonadotropin bioactivity despite declining steroid hormone levels (Sauer et al). 1989;161:1673-6
Successful treatment of a viable cervical pregnancy with methotrexate (Palti et al). 1989;161:1147-8
Transvaginal ultrasonography in patients at risk for ectopic pregnancy (Stiller et al). 1989;161:930-3
The use of transvaginal ultrasonography in the diagnosis of ectopic pregnancy (Timor-Tritsch et al). 1989;161:157-61
Vaginosonographic examination Routine procedure? (Furness) (Letter); (Bernaschek and Rudelstorfer) (Reply). 1989;161:499

Pregnancy, multiple
Multiple gestation with intrauterine insemination and menotropin therapy (Corsan et al) (Letter); (Dodson et al) (Reply). 1989;161:1751-2
Successful twin pregnancy after embryo donation to a patient with XY gonadal dysgenesis (Sauer et al). 1989;161:380-1
Twin pregnancy in premature ovarian failure after estrogen treatment: a case report (Tang and Sawers). 1989;161:172-3
Twin-to-twin transfusion syndrome: What are appropriate diagnostic criteria? (Danskin and Neilson). 1989;161:365-9

Pregnancy, prolonged
Macrosomia in the postdate pregnancy: Is routine ultrasonographic screening indicated? (Chervenak et al). 1989;161:753-6
Maternal-fetal outcomes in prolonged pregnancy (Cucco et al). 1989;161:916-20
Postdate fetal surveillance: Is 41 weeks too early? (Guidetti et al). 1989;161:91-3

Pregnancy, tubal; *see also* **Pregnancy, ectopic**
First successful intrauterine pregnancy after treatment of tubal pregnancy with prostaglandin $F_{2\alpha}$ (Egarter and Husslein). 1989;161:904
Local methotrexate injection: a nonsurgical treatment of ectopic pregnancy (Pansky et al). 1989;161:393-6

Pregnancy, unwanted
Attempted suicide and pregnancy (Czeizel and Lendvay). 1989;161:497 (Letter)

Pregnancy-associated alpha plasma protein
Immunoregulatory activity in supernatants from cultures of normal human trophoblast cells of the first trimester (Sanyal et al). 1989;161:446-53

Pregnancy complications
Abscess of allantoic duct remnant (Baill et al). 1989;161:334-6
Aromatase in human fetal tissues (Doody and Carr). 1989;161:1694-7
Candida warrants concern (Elliott) (Letter); (Smith) (Reply). 1989;161:503
The central hemodynamics of severe preeclampsia (Mabie et al). 1989;161:1443-8
Cocaine abuse during pregnancy: peripartum prevalence and perinatal outcome (Neerhof et al). 1989;161:633-8
Elevated liver enzymes and thrombocytopenia in the third trimester of pregnancy: an unusual case report and a review of the literature (Hannah et al). 1989;161:322-3
Erythrocyte cation metabolism in preeclampsia (Sowers et al). 1989;161:441-5
Is a decrease in plasma oncotic pressure enough to explain the edema of pregnancy? (Valenzuela). 1989;161:1624-7

Lipid peroxidation in pregnancy: new perspectives on preeclampsia (Hubel et al). 1989;161:1025-34 (Curr. dev.)
Mechanism for human papillomavirus transmission at birth (Sedlacek et al). 1989;161:55-9
Multidisciplinary team counseling for fetal anomalies (Lorenz and Kuhn). 1989;161:263-6 (Clin. opinion)
Neonatal mortality in normal birth weight babies: Does the level of hospital care make a difference? (Berg et al). 1989;161:86-91
Neurofibromatosis and pregnancy (Mitterschiffthaler and Fuith) (Letter); (Blickstein) (Reply). 1989;161:501
Pemphigus in pregnancy (Kanwar et al). 1989;161:995-6
Phosphorus 31 nuclear magnetic resonance examination of female reproductive tissues (Noyszewski et al). 1989;161:282-8
Pneumonia during pregnancy: Has modern technology improved maternal and fetal outcome? (Madinger et al). 1989;161:657-62
Predictive value of increased plasma levels of fibronectin in gestational hypertension (Ballegeer et al). 1989;161:432-6
Predictive values, relative risks, and overall benefits of high and low maternal serum α-fetoprotein screening in singleton pregnancies: new epidemiologic data (Milunsky et al). 1989;161:291-7
Prematurity among insulin-requiring diabetic gravid women (Greene et al). 1989;161:106-11
The prevalence and biologic significance of lupus anticoagulant and anticardiolipin antibodies in a general obstetric population (Lockwood et al). 1989;161:369-73
Prolactin levels in umbilical cord blood of human infants: relation to gestational age, maternal complications, and neonatal lung function (Parker et al). 1989;161:795-802
Prolongation of pregnancy and survival of twin B after loss of twin A at 21 weeks' gestation (Feichtinger et al). 1989;161:891-3
Relapsing polychondritis in pregnancy: a case report and review (Gimovsky and Nishiyama). 1989;161:332-4
Role of ferritin supported in diagnosis of anemias of pregnancy (Goodlin) (Letter); (Schifman) (Reply). 1989;161:258-9
The role of ultrasonography and amniocentesis in the evaluation of pregnancies at risk for neural tube defects (Hogge et al). 1989;161:520-4
Unexplained elevated maternal serum α-fetoprotein level associated with third-trimester problems (Stephens). 1989;161:500 (Letter)
Usefulness of continuous fetal heart rate monitoring during cesarean section (Petrikovsky et al). 1989;161:36-7
Zinc, cadmium, and hypertension in parturient women (Lazebnik et al). 1989;161:437-40

Pregnancy complications, animal

Effect of a 48-hour Δ^4-androstenedione infusion on the pregnant rhesus monkey in the last third of gestation: changes in maternal plasma estradiol concentrations and myometrial contractility (Figueroa et al). 1989;161:481-6
Gestational diabetes in the BHE rat: influence of dietary fat (Bue et al). 1989;161:234-40
Lack of evidence of a vasodepressor role for relaxin in spontaneously hypertensive and normotensive pregnant rats (Ahokas et al). 1989;161:618-22
Pharmacokinetics of azidothymidine during late pregnancy in Long-Evans rats (Little et al). 1989;161:732-4

Pregnancy complications, cardiovascular

Calcium intake and pregnancy-induced hypertension (Semchyshyn) (Letter); (Belizán and Villar) (Reply). 1989;161:834-5
Can platelet volume predict progressive hypertensive disease in pregnancy? (Walker et al). 1989;161:676-9
Ultrasonographic guidance for central venous access during pregnancy (Lee et al). 1989;161:1012-3

Pregnancy complications, hematologic

Maternal hemoglobin F levels may have an adverse effect on neonatal birth weight in pregnancies with sickle cell disease (Anyaegbunam et al). 1989;161:654-6
Thrombosis prophylaxis in pregnancy with use of subcutaneous heparin adjusted by monitoring heparin concentration in plasma (Dahlman et al). 1989;161:420-5

Pregnancy complications, infectious; *see also* **Amniotic fluid**

Fetal death from chlamydial infection across intact amniotic membranes (Thorp et al). 1989;161:1245-6
Immunosuppression in pregnant women infected with human immunodeficiency virus (Biggar et al). 1989;161:1239-44
Infection and labor. IV. Cachectin–tumor necrosis factor in the amniotic fluid of women with intraamniotic infection and preterm labor (Romero et al). 1989;161:336-41
Risk factors for intraamniotic infection: a prospective epidemiologic study (Soper et al). 1989;161:562-8

Pregnancy complications, neoplastic

Asymptomatic thrombocytopenia associated with chorioangioma of placenta (Limaye and Tchabo). 1989;161:76-7
Sarcoma associated wtih pregnancy (Kyodo et al). 1989;161:94-6

Pregnancy maintenance

Cardiac isoenzymes and electrocardiographic changes during ritodrine tocolysis (Hadi and Albazzaz). 1989;161:318-21
Prolongation of pregnancy and survival of twin B after loss of twin A at 21 weeks' gestation (Feichtinger et al). 1989;161:891-3

Pregnancy outcome

Acid-base status at birth and neurodevelopmental outcome at four and one-half years (Dennis et al). 1989;161:213-20
Antenatal ultrasonographic diagnosis of fetal seizure activity (Landy et al). 1989;161:308
Attempted suicide and pregnancy (Czeizel and Lendvay). 1989;161:497 (Letter)
Breech presentation after cesarean section: always a section (Ophir et al). 1989;161:25-8
Changing outcome of extremely premature infants (≤26 weeks' gestation and ≤750 gm): survival and follow-up at a tertiary center (Ferrara et al). 1989;161:1114-8
Cocaine abuse during pregnancy: peripartum prevalence and perinatal outcome (Neerhof et al). 1989;161:633-8
Data sought to link adverse pregnancy outcome with maternal serum α-fetoprotein levels in black gravid women (Lenke) (Letter); (Macri et al) (Reply). 1989;161:254-5
Doppler velocimetry and placental disease (Bracero et al). 1989;161:388-93
Early pregnancy glycosylated hemoglobin, severity of diabetes, and fetal malformations (Lucas et al). 1989;161:426-31
The effects of maternal exercise on early pregnancy outcome (Clapp). 1989;161:1453-7
Fetal and neonatal cardiovascular complications from β-sympathomimetic therapy for tocolysis (Katz and Seeds). 1989;161:1-9 (Clin. opinion)

Pregnancy outcome—cont'd
In utero cocaine exposure: observations of fetal behavioral state may predict neonatal outcome (Hume et al). 1989;161:685-90
The improving outcome of triplet pregnancies (Lipitz et al). 1989;161:1279-84
Magnesium supplementation during pregnancy: a double-blind randomized controlled clinical trial (Sibai et al). 1989;161:115-9
Management of the nonvertex second twin: primary cesarean section, external version, or primary breech extraction (Gocke et al). 1989;161:111-4
Management of women with one abnormal oral glucose tolerance test value reduces adverse outcome in pregnancy (Langer et al). 1989;161:593-9
Maternal and fetal blood flow velocity waveforms in patients with preterm labor: relationship to outcome (Brar et al). 1989;161:1519-22
Maternal-fetal outcomes in prolonged pregnancy (Cucco et al). 1989;161:916-20
Neonatal mortality in normal birth weight babies: Does the level of hospital care make a difference? (Berg et al). 1989;161:86-91
Noninvasive management of isolated bilateral fetal hydrothorax (Pijpers et al). 1989;161:330-2
Oxygen consumption during treadmill exercise before, during, and after pregnancy (Clapp). 1989;161:1458-64
Perinatal outcome in very preterm births with twin-twin transfusion syndrome (Shan and Chaffin). 1989;161:1111-3
A population-based study of maternal and perinatal outcome in patients with gestational diabetes (Jacobson and Cousins). 1989;161:981-6
Postdate fetal surveillance: Is 41 weeks too early? (Guidetti et al). 1989;161:91-3
Predictive values, relative risks, and overall benefits of high and low maternal serum α-fetoprotein screening in singleton pregnancies: new epidemiologic data (Milunsky et al). 1989;161:291-7
Pregnancy after aortofemoral bypass grafting in a diabetic patient (Bradley-Watson). 1989;161:200-1
Pregnancy in obstetrics residents (Milet). 1989;161:1422 (Letter)
Prematurity among insulin-requiring diabetic gravid women (Greene et al). 1989;161:106-11
The prevalence and biologic significance of lupus anticoagulant and anticardiolipin antibodies in a general obstetric population (Lockwood et al). 1989;161:369-73
Reproductive outcome after anesthesia and operation during pregnancy: registry study of 5405 cases (Mazze and Källén). 1989;161:1178-85
Successful pregnancy after a liver transplant (Sims et al). 1989;161:532-3
Vaginal birth after cesarean delivery in the twin gestation (Strong et al). 1989;161:29-32

Pregnancy outcome, animal
Gestational diabetes in the BHE rat: influence of dietary fat (Bue et al). 1989;161:234-40

Pregnancy proteins
Elevated serum levels of endometrial secretory protein PP14 in patients with advanced endometriosis: suppression by treatment with danazol and high-dose medroxyprogesterone acetate (Telimaa et al). 1989;161:866-71
Immunoregulatory activity in supernatants from cultures of normal human trophoblast cells of the first trimester (Sanyal et al). 1989;161:446-53

Pregnancy tests
Analytical and clinical sensitivity and specificity in pregnancy testing (Norman) (Letter); (Emancipator) (Reply). 1989;161:835-6
Vaginosonographic examination Routine procedure? (Furness) (Letter); (Bernaschek and Rudelstorfer) (Reply). 1989;161:499

Pregnancy trimester, animal, third
Power spectrum analysis of myometrial electromyogram and intrauterine pressure changes in the pregnant rhesus monkey in late gestation (Hsu et al). 1989;161:467-73

Pregnancy trimester, first
Cardiovascular changes in early pregnancy (Capeless and Clapp). 1989;161:1449-53
Dating the early pregnancy by sequential appearance of embryonic structures (Warren et al). 1989;161:747-53
First-trimester midgut herniation: a high-frequency transvaginal sonographic study (Timor-Tritsch et al). 1989;161:831-3
The frequency and management of uterine perforations during first-trimester abortions (Kaali et al). 1989;161:406-8
Immunoregulatory activity in supernatants from cultures of normal human trophoblast cells of the first trimester (Sanyal et al). 1989;161:446-53
Missed abortion: Still appropriate terminology? (Pridjian and Moawad). 1989;161:261-2 (Clin. opinion)

Pregnancy trimester, second
Does midtrimester ΔOD_{450} of amniotic fluid reflect severity of Rh disease? (Ananth and Queenan). 1989;161:47-9
The efficacy of fetal sonographic biometry in Down syndrome screening (Peters et al). 1989;161:297-300
Esterase activity in second- and third-trimester amniotic fluid: an indicator of chorioamnionitis (Hoskins et al). 1989;161:1543-5
Missed abortion: Still appropriate terminology? (Pridjian and Moawad). 1989;161:261-2 (Clin. opinion)

Pregnancy trimester, third
The effect of vibroacoustic stimulation on the nonstress test at gestational ages of thirty-two weeks or less (Druzin et al). 1989;161:1476-8
Effects of maternal hyperoxia on the biophysical assessment of fetuses with suspected intrauterine growth retardation (Ruedrich et al). 1989;161:188-92
Elevated liver enzymes and thrombocytopenia in the third trimester of pregnancy: an unusual case report and a review of the literature (Hannah et al). 1989;161:322-3
Esterase activity in second- and third-trimester amniotic fluid: an indicator of chorioamnionitis (Hoskins et al). 1989;161:1543-5
Individual growth curve standards in twins: prediction of third-trimester growth and birth characteristics (Stefos et al). 1989;161:179-83
Postdate fetal surveillance: Is 41 weeks too early? (Guidetti et al). 1989;161:91-3
Preterm and term fetal cardiac and movement responses to vibratory acoustic stimulation (Thomas et al). 1989;161:141-5
Unexplained elevated maternal serum α-fetoprotein level associated with third-trimester problems (Stephens). 1989;161:500 (Letter)

Pregnancy trimester, third, animal
Effect of a 48-hour Δ^4-androstenedione infusion on the

pregnant rhesus monkey in the last third of gestation: changes in maternal plasma estradiol concentrations and myometrial contractility (Figueroa et al). 1989;161: 481-6

Premature infant; *see* **Infant, premature**

Premature labor; *see* **Labor, premature**

Premature rupture of membranes; *see* **Fetal membranes, premature rupture**

Premenstrual syndrome

Depressive episodes in premenstrual syndrome (Mortola et al). 1989;161:1682-7

Effect of nutrient intake on premenstrual depression (Wurtman et al). 1989;161:1228-34

Prenatal care

Multidisciplinary team counseling for fetal anomalies (Lorenz and Kuhn). 1989;161:263-6 (Clin. opinion)

Prenatal diagnosis

Amniotic fluid acetylcholinesterase ratios in prenatal diagnosis of fetal abnormalities (Kelly et al). 1989;161:703-5

Analysis of fetal loss after transcervical chorionic villus sampling a review of 719 patients (Wade and Young). 1989;161:513-9

Enlarged cisterna magna in trisomy 18: prenatal ultrasonographic diagnosis (Thurmond et al). 1989;161:83-5

Fetal cardiac output in the isoimmunized pregnancy: a pulsed Doppler-echocardiographic study of patients undergoing intravascular intrauterine transfusion (Copel et al). 1989;161:361-5

First-trimester and early second-trimester diagnosis of nuchal cystic hygroma by transvaginal sonography: diverse prognosis of the septated from the nonseptated lesion (Bronshtein et al). 1989;161:78-82

In utero diagnosis of benign fetal macrocephaly (DeRosa et al). 1989;161:690-2

"Possibly" de novo translocations: prenatal risk counseling (Wassman et al). 1989;161:698-702

Pulsed Doppler flow-velocity waveforms in the prediction of fetal hematocrit of the severely isoimmunized pregnancy (Copel et al). 1989;161:341-4

Rapid chromosome analysis with spontaneously dividing cells derived from umbilical cord blood (fetal and neonatal) (Tipton et al). 1989;161:1546-8

Ultrasonographic criteria for the prenatal diagnosis of placental chorionicity in twin gestations (Winn et al). 1989;161:1540-2

Presentation, breech; *see* **Breech presentation**

Presidential address

The challenge of change revisited (Greiss). 1989;161:505-9 (Pres. address)

Preterm delivery; *see* **Infant, premature**

Preterm labor; *see* **Labor, premature**

Prevalence studies

Data sought to link adverse pregnancy outcome with maternal serum α-fetoprotein levels in black gravid women (Lenke) (Letter); (Macri et al) (Reply). 1989;161:254-5

Differences between white and Chinese populations in human leukocyte antigen sharing and gestational trophoblastic tumors (Ho et al). 1989;161:942-8

Prevous cesarean section; *see* **Cesarean section**

Professional practice

The challenge of change revisited (Greiss). 1989;161:505-9 (Pres. address)

Progestational hormones

Comparison of in vivo bioavailability of progestational agents into the rat uterus and liver and the effect of plasma protein binding (Van Voorhis et al). 1989;161:1698-703

Progesterone

Absorption of oral progesterone is influenced by vehicle and particle size (Hargrove et al). 1989;161:948-51

Effects of fetal sex and dexamethasone on preterm maternal serum concentrations of human chorionic gonadotropin, progesterone, estrone, estradiol, and estriol (Haning et al). 1989;161:1549-53

Increased placental progesterone may cause decreased placental prostacyclin production in preeclampsia (Walsh and Coulter). 1939;161:1586-92

Low serum progesterone levels and tubal dysfunction a possible cause of ectopic pregnancy (Pulkkinen and Jaakkola). 1989;161:934-7

Optimal time in the menstrual cycle for serum progesterone measurement to diagnose luteal phase defects (Daya). 1989;161:1009-11

Progesterone antagonist lilopristone: a potent abortifacient in the common marmoset (Puri et al). 1989;161:248-53

Spontaneously resorbing ectopic pregnancy: preservation of human chorionic gonadotropin bioactivity despite declining steroid hormone levels (Sauer et al). 1989;161:1673-6

Up-regulation of guinea pig myometrial adenylate cyclase activity by intrauterine estradiol and progesterone pellets (Hatjis and Koritnik). 1989;161:1073-8

Prognosis

CA 125 as an independent prognostic factor for survival in patients with epithelial ovarian cancer (Sevelda et al). 1989;161:1213-6

Prognostic significance of steroid receptors measured in primary metastatic and recurrent endometrial carcinoma (Borazjani et al). 1989;161:1253-7

Prolactin

Mechanisms that subserve estradiol's induction of increased prolactin concentrations: evidence of amplitude modulation of spontaneous prolactin secretory bursts (Veldhuis et al). 1989;161:1149-58

Prolactin levels in umbilical cord blood of human infants: relation to gestational age, maternal complications, and neonatal lung function (Parker et al). 1989;161:795-802

Proprioception

Effects of low-frequency vibration on human term fetuses (Gagnon et al). 1989;161:1479-85

Prospective studies

Discordant twins—a prospective study of the diagnostic value of real-time ultrasonography combined with umbilical artery velocimetry (Divon et al). 1989;161: 757-60

Primary stress urinary incontinence and pelvic relaxation: prospective randomized comparison of three different operations (Bergman et al). 1989;161:97-101

Prostacyclin; *see* **Prostaglandins**

Prostaglandins

Aspirin differentially affects thromboxane and prostacyclin production by trophoblast and villous core compartments of human placental villi (Nelson and Walsh). 1989;161:1593-8

Decreased in vitro production of 6-keto-prostaglandin $F_{1\alpha}$ by uterine arteries from postmenopausal women (Steinleitner et al). 1989;161:1677-81

Effect of acute intravascular volume expansion on human fetal prostaglandin concentrations (Weiner and Robillard). 1989;161:1494-7

Prostaglandins—cont'd
Effect of inhibiting prostaglandin synthesis in pregnant sheep with 4-aminoantipyrine under normothermic and hyperthermic conditions (Andrianakis et al). 1989;161:241-7
Effects of chronic fetal hyperinsulinemia on plasma arachidonic acid and prostaglandin concentrations (Stonestreet et al). 1989;161:894-9
Increased placental progesterone may cause decreased placental prostacyclin production in preeclampsia (Walsh and Coulter). 1989;161:1586-92
Transfer of prostaglandins to the fetus after prostaglandin E_2 vaginal pessary administration (MacKenzie et al). 1989;161:920-3
Prostaglandins E
Infection and labor. IV. Cachectin–tumor necrosis factor in the amniotic fluid of women with intraamniotic infection and preterm labor (Romero et al). 1989;161:336-41
Prostaglandin E_2 metabolism in the human fetal membranes (Cheung and Challis). 1989;161:1580-5
Transfer of prostaglandins to the fetus after prostaglandin E_2 vaginal pessary administration (MacKenzie et al). 1989;161:920-3
Prostaglandins F
Decreased in vitro production of 6-keto-prostaglandin $F_{1\alpha}$ by uterine arteries from postmenopausal women (Steinleitner et al). 1989;161:1677-81
5-Hydroxyeicosatetraenoic acid, leukotriene C_4, and prostaglandin $F_{2\alpha}$ in amniotic fluid before and during term and preterm labor (Walsh). 1989;161:1352-60
Prostaglandins F, synthetic
First successful intrauterine pregnancy after treatment of tubal pregnancy with prostaglandin $F_{2\alpha}$ (Egarter and Husslein). 1989;161:904
Protein binding
Comparison of in vivo bioavailability of progestational agents into the rat uterus and liver and the effect of plasma protein binding (Van Voorhis et al). 1989;161:1698-703
Proto-oncogenes
Immunocytochemical study of *ras* and *myc* proto-oncogene polypeptide expression in the human menstrual cycle (Odom et al). 1989;161:1663-8
Publications
Criticism of publication of pharmaceutical-supported research results draws response (Ledger) (Letter); (Brewer et al) (Reply). 1989;161:839-40
Puerperal disorders
Thrombosis prophylaxis in pregnancy with use of subcutaneous heparin adjusted by monitoring heparin concentration in plasma (Dahlman et al). 1989;161:420-5
Puerperal infection
Antepartum infection as a result of *Streptococcus pneumoniae* and sepsis in neonate (Andreu et al). 1989;161:1424-5 (Letter)
Methicillin-resistant staphylococcal mastitis (Kalstone). 1989;161:120
Short course of antibiotic therapy in treatment of postpartum endomyometritis (Morales et al). 1989;161:568-72
Pulmonary artery
Central hemodynamic assessment of normal term pregnancy (Clark et al). 1989;161:1439-42
The central hemodynamics of severe preeclampsia (Mabie et al). 1989;161:1443-8
Pulmonary artery catheterization in severe eclampsia (Gibbs). 1989;161:1089 (Letter)
Ultrasonographic guidance for central venous access during pregnancy (Lee et al). 1989;161:1012-3
Pulmonary artery, abnormalities
Pulmonary artery agenesis (Verp). 1989;161:1746-7 (Letter)
Pulmonary hypoplasia; *see* **Lung, abnormalities**
Pulmonary surfactants
Impact of antenatal dexamethasone administration on respiratory distress syndrome in surfactant-treated infants (Farrell et al). 1989;161:628-33
Surfactant proteins in the diagnosis of fetal lung maturity. II. The 35 kd protein and phospholipids in complicated pregnancy (Hallman et al). 1989;161:965-9
Pulsatile flow
Doppler assessment of the pulsatility index of the middle cerebral artery during constriction of the fetal ductus arteriosus after indomethacin therapy (Mari et al). 1989;161:1528-31
Umbilical artery flow velocity waveforms during acute hypoxemia and the relationship with hemodynamic changes in the fetal lamb (van Huisseling et al). 1989;161:1061-4

Q

Quality control
Understanding and eliminating errors in perinatal research (Block). 1989;161:5-9 (Clin. opinion)

R

Radioimmunoassay
The influence of human antimurine antibody on CA 125 levels in patients with ovarian cancer undergoing radioimmunotherapy or immunoscintigraphy with murine monoclonal antibody OC 125 (Muto et al). 1989;161:1206-12
Radiology department, hospital
Breast disease programs in obstetrics and gynecology: a plea for training in mammography (Gleicher). 1989;161:267-70 (Clin. opinion)
Random allocation
Delivery of fetuses with gastroschisis (Weiner). 1989;161:1089 (Letter)
Evaluating triphasic oral contraceptives: the rationale for a randomized trial (Grimes). 1989;161:1390-2 (Symposium)
Study design for the Triphasic Randomized Comparative Trial (Dickerson). 1989;161:1392-6 (Symposium)
Triphasic Randomized Clinical Trial: comparative frequency of intermenstrual bleeding (Droegemueller et al). 1989;161:1407-11 (Symposium)
Triphasic Randomized Clinical Trial: comparison of effects on carbohydrate metabolism (Bowes et al). 1989;161:1402-7 (Symposium)
***ras* genes**
Immunocytochemical study of *ras* and *myc* proto-oncogene polypeptide expression in the human menstrual cycle (Odom et al). 1989;161:1663-8
Receptors, adrenergic, beta, animal
Pregnancy-induced changes in the interaction of guinea pig myometrial β-adrenergic receptors with *l*-isoproterenol (Hatjis and Grogan). 1989;161:1633-6
Pulmonary β-adrenergic receptors and response do not mature precociously in growth-retarded rabbit fetuses (Lee et al). 1989;161:1044-9
Treatment of oophorectomized guinea pigs with intrauter-

ine 17β-estradiol pellets may modulate myometrial β-adrenergic receptor binding properties (Hatjis et al). 1989;161:1628-32

Receptors, androgen, deficiency
Seminoma in pubertal patient with androgen insensitivity syndrome (Hurt et al). 1989;161:530-1

Receptors, epidermal growth factor-urogastrone
Epidermal growth factor receptor expression in normal and malignant endometrium (Berchuck et al). 1989;161:1247-52

Receptors, estrogen
Immunohistochemical expression of TAG-72 in normal and malignant endometrium: correlation of antigen expression with estrogen receptor and progesterone receptor levels (Soisson et al). 1989;161:1258-63
Peri-implantation phase endometrial estrogen and progesterone receptors: effect of ovulation induction with clomiphene citrate (Hecht et al). 1989;161:1688-93

Receptors, insulin
Steroid biosynthesis in the Sertoli-Leydig cell tumor: effects of insulin and luteinizing hormone (Nagamani et al). 1989;161:1738-43

Receptors, pituitary hormone
Vasopressin receptors in human pregnant myometrium and decidua: interactions with oxytocin and vasopressin agonists and antagonists (Ivanisevic et al). 1989;161:1637-43

Receptors, progesterone
Immunohistochemical expression of TAG-72 in normal and malignant endometrium: correlation of antigen expression with estrogen receptor and progesterone receptor levels (Soisson et al). 1989;161:1258-63
Peri-implantation phase endometrial estrogen and progesterone receptors: effect of ovulation induction with clomiphene citrate (Hecht et al). 1989;161:1688-93

Receptors, sensory
Effects of low-frequency vibration on human term fetuses (Gagnon et al). 1989;161:1479-85

Receptors, steroid
Prognostic significance of steroid receptors measured in primary metastatic and recurrent endometrial carcinoma (Borazjani et al). 1989;161:1253-7

Regional medical programs
Neonatal resuscitation in community hospitals: a regional-based, team-oriented training program coordinated by the tertiary center (Moore et al). 1989;161:849-55 (Clin. opinion)

Registries
Reproductive outcome after anesthesia and operation during pregnancy: registry study of 5405 cases (Mazze and Källén). 1989;161:1178-85

Relapsing polychondritis; *see* **Polychondritis, relapsing**

Relaxin
The antagonistic effect of oxytocin and relaxin on rat uterine segment contractility (Goldsmith et al). 1989;161:1644-9
Lack of evidence of a vasodepressor role for relaxin in spontaneously hypertensive and normotensive pregnant rats (Ahokas et al). 1989;161:618-22
Relaxin in paired samples of serum and milk from women after term and preterm delivery (Eddie et al). 1989;161:970-3

Renal artery
Duplex Doppler ultrasonographic evaluation of the fetal renal artery in normal and abnormal fetuses (Veille and Kanaan). 1989;161:1502-7
Renal artery flow-velocity waveforms in normal and hypoxemic fetuses (Vyas et al). 1989;161:168-72

Renin-angiotensin system
The renin-angiotensin system during pregnancy in chronically instrumented, conscious rats (Conrad et al). 1989;161:1065-72

Repeat cesarean section; *see* **Cesarean section**

Reproduction
Gestational trophoblastic disease: a case-control study from the People's Republic of China (Brinton et al). 1989;161:121-7
Vitamin D (soltriol), light, and reproduction (Stumpf and Denny). 1989;161:1375-84 (Curr. dev.)

Research design
Delivery of fetuses with gastroschisis (Weiner). 1989;161:1089 (Letter)
Estrogen replacement therapy: what the future holds (Hammond). 1989;161:1864-8 (Symposium)
Evaluating triphasic oral contraceptives: the rationale for a randomized trial (Grimes). 1989;161:1390-2 (Symposium)
Intrauterine growth retardation: standards for diagnosis (Goldenberg et al). 1989;161:271-7 (Clin. opinion)
Study design for the Triphasic Randomized Comparative Trial (Dickerson). 1989;161:1392-6 (Symposium)
Triphasic Randomized Clinical Trial: comparative frequency of intermenstrual bleeding (Droegemueller et al). 1989;161:1407-11 (Symposium)
Triphasic Randomized Clinical Trial: comparison of effects on carbohydrate metabolism (Bowes et al). 1989;161:1402-7 (Symposium)
Understanding and eliminating errors in perinatal research (Block). 1989;161:5-9 (Clin. opinion)

Research support
Criticism of publication of pharmaceutical-supported research results draws response (Ledger) (Letter); (Brewer et al) (Reply). 1989;161:839-40

Respiratory acidosis
Continuous tissue pH monitoring in the term fetus (Small et al). 1989;161:323-9

Respiratory distress syndrome
Impact of antenatal dexamethasone administration on respiratory distress syndrome in surfactant-treated infants (Farrell et al). 1989;161:628-33
Prolactin levels in umbilical cord blood of human infants: relation to gestational age, maternal complications, and neonatal lung function (Parker et al). 1989;161:795-802
Surfactant proteins in the diagnosis of fetal lung maturity. II. The 35 kd protein and phospholipids in complicated pregnancy (Hallman et al). 1989;161:965-9
Uselessness of the phosphatidylglycerol assay for prediction of lung maturity (Egberts and Wijnands) (Letter); (Fadel) (Reply). 1989;161:1417-8

Respiratory insufficiency
Relapsing polychondritis in pregnancy: a case report and review (Gimovsky and Nishiyama). 1989;161:332-4

Resuscitation
Does terbutaline cause uterine atony and increase intraoperative blood loss? (Brown) (Letter); (Patriarco) (Reply). 1989;161:259
Neonatal resuscitation in community hospitals: a regional-based, team-oriented training program coordinated by

Resuscitation—cont'd
the tertiary center (Moore et al). 1989;161:849-55 (Clin. opinion)
Retrospective studies
The cancer question: an overview of recent epidemiologic and retrospective data (Henderson). 1989;161:1859-64 (Symposium)
Rh isoimmunization
Does midtrimester ΔOD_{450} of amniotic fluid reflect severity of Rh disease? (Ananth and Queenan). 1989;161:47-9
The effect of intravascular transfusion on umbilical venous pressure in anemic fetuses with and without hydrops (Weiner et al). 1989;161:1498-501
Fetal cardiac output in the isoimmunized pregnancy: a pulsed Doppler-echocardiographic study of patients undergoing intravascular intrauterine transfusion (Copel et al). 1989;161:361-5
Fetal heart rate patterns in red blood cell isoimmunized pregnancies (Nicolaides et al). 1989;161:351-6
Fetal liver length in normal and isoimmunized pregnancies (Roberts et al). 1989;161:42-6
Frequency and timing of intravascular intrauterine transfusions reconsidered (Voto and Margulies) (Letter); (Socol et al) (Reply). 1989;161:255-6
Isoimmunization to Cromer antigen in pregnancy (Sacks and Garratty). 1989;161:928-9
Maternal-fetal glucose gradient in normal pregnancies and in pregnancies complicated by alloimmunization and fetal growth retardation (Nicolini et al). 1989;161:924-7
Prediction of hematocrit decline after intravascular fetal transfusion (MacGregor et al). 1989;161:1491-3
Pulsed Doppler flow-velocity waveforms in the prediction of fetal hematocrit of the severely isoimmunized pregnancy (Copel et al). 1989;161:341-4
Two hundred intrauterine exchange transfusions in severe blood incompatibilities (Poissonnier et al). 1989;161:709-13
Risk factors
A case-control study of epithelial ovarian cancer (Hartge et al). 1989;161:10-6 (Clin. opinion)
Maternal mortality in North Carolina: a forty-year experience (May and Greiss). 1989;161:555-61
Neonatal mortality in normal birth weight babies: Does the level of hospital care make a difference? (Berg et al). 1989;161:86-91
"Possibly" de novo translocations: prenatal risk counseling (Wassman et al). 1989;161:698-702
Risk factors for intraamniotic infection: a prospective epidemiologic study (Soper et al). 1989;161:562-8
Risk factors for vulvar lichen sclerosus (Sideri et al). 1989;161:38-42
Vaginal birth after cesarean section: a reappraisal of risk (Yetman and Nolan). 1989;161:1119-23
Risk taking
Basic reference gambles recommended for utility assessment (Thornton and Lilford) (Letter); (Silver and Minogue) (Reply). 1989;161:256-7
Ritodrine
Cardiac isoenzymes and electrocardiographic changes during ritodrine tocolysis (Hadi and Albazzaz). 1989;161:318-21
Cardiovascular and metabolic effects associated with nifedipine and ritodrine tocolysis (Ferguson et al). 1989;161:788-95
Fetal and neonatal cardiovascular complications from β-sympathomimetic therapy for tocolysis (Katz and Seeds). 1989;161:1-9 (Clin. opinion)
Number clarification requested (Hatjis) (Letter); (Wilkins and Lynch) (Reply). 1989;161:1746
Pharmacokinetics of orally administered ritodrine (Caritis et al). 1989;161:32-5
Ruptured membranes; *see* **Fetal membranes, premature rupture**

S

Saline solution, hypertonic
Saline solution amnioinfusion for oligohydramnios after premature rupture of the membranes (Imanaka et al). 1989;161:102-6
Salpingitis
High hepatitis B carrier rate among non-Oriental patients with acute salpingitis (Smith et al). 1989;161:879-80
Sarcoma
Sarcoma associated wtih pregnancy (Kyodo et al). 1989;161:94-6
Scalp
Continuous tissue pH monitoring in the term fetus (Small et al). 1989;161:323-9
Secretary protein PP14; *see* **Pregnancy proteins**
Seizures
Antenatal ultrasonographic diagnosis of fetal seizure activity (Landy et al). 1989;161:308
Neonatal seizures and cesarean section (Boylan) (Letter); (Spellacy) (Reply). 1989;161:836-7
Oxytocin may explain neonatal seizures (Goodlin). 1989;161:259 (Letter)
Semantics; *see also* **Nomenclature**
Semantic changes suggested in hypertensive disorders (Reamy) (Letter); (Davey and MacGillivray) (Reply). 1989;161:1422-3
Semen, analysis
Semen predictors of in vitro fertilization and embryo cleavage (Daya et al). 1989;161:1284-9
Seminoma; *see* **Dysgerminoma**
Sensitivity and specificity (epidemiology)
Specifics of specificity and sensitivity (Kadar) (Letter); (Kolins) (Reply). 1989;161:1416-7
Sensory receptors; *see* **Receptors, sensory**
Serotonin
Effect of nutrient intake on premenstrual depression (Wurtman et al). 1989;161:1228-34
Sertoli cell tumor
Steroid biosynthesis in the Sertoli-Leydig cell tumor: effects of insulin and luteinizing hormone (Nagamani et al). 1989;161:1738-43
Sertoli cells
Molecular scanning of Yq11 (interval 6) in men with Sertoli-cell-only syndrome (Johnson et al). 1989;161:1732-7
Sex determination
Rapid determination of fetal sex by deoxyribonucleic acid amplification of Y chromosome–specific sequences (Pinckert et al). 1989;161:693-8
Sex hormones
Spontaneously resorbing ectopic pregnancy: preservation of human chorionic gonadotropin bioactivity despite declining steroid hormone levels (Sauer et al). 1989;161:1673-6
Treatment of oophorectomized guinea pigs with intrauterine 17β-estradiol pellets may modulate myometrial β-

adrenergic receptor binding properties (Hatjis et al). 1989;161:1628-32
Up-regulation of guinea pig myometrial adenylate cyclase activity by intrauterine estradiol and progesterone pellets (Hatjis and Koritnik). 1989;161:1073-8
Sex steroids, biosynthesis
Steroid biosynthesis in the Sertoli-Leydig cell tumor: effects of insulin and luteinizing hormone (Nagamani et al). 1989;161:1738-43
Sexual abuse, child; *see* **Child abuse, sexual**
Sexually transmitted diseases
High hepatitis B carrier rate among non-Oriental patients with acute salpingitis (Smith et al). 1989;161:879-80
Sickle cell anemia; *see* **Anemia, sickle cell**
Skeleton
Monitoring skeletal response to estrogen (Mazess et al). 1989;161:843-8 (Clin opinion)
Skull, growth
In utero diagnosis of benign fetal macrocephaly (DeRosa et al). 1989;161:690-2
Small for gestational age; *see* **Infant, small for gestational age**
Smoking
Effects of smoking and oral contraception on plasma β-carotene levels in healthy women (Palan et al). 1989;161:881-5
Social change
The challenge of change revisited (Greiss). 1989;161:505-9 (Pres. address)
Social conditions
Perinatal death as a result of social factors (Fribourg) (Letter); (Delke and Minkoff) (Reply). 1989;161:1748
Society for Gynecologic Investigation
Papers of the Society for Gynecologic Investigation. 1989;161:1439-743
Society of Perinatal Obstetricians
Transactions of the ninth annual meeting of the Society of Perinatal Obstetricians. 1989;161:593-833
Sodium, dietary
Atrial natriuretic factor: the effects of pregnancy and a high-salt diet on atrial levels (Castro et al). 1989;161:1620-3
South Atlantic Association of Obstetricians and Gynecologists
Transactions of the fifty-first annual meeting of the South Atlantic Association of Obstetricians and Gynecologists. 1989;161:505-92
Spectroscopy, magnetic resonance; *see* **Nuclear magnetic resonance**
Spectrum analysis
Power spectrum analysis of myometrial electromyogram and intrauterine pressure changes in the pregnant rhesus monkey in late gestation (Hsu et al). 1989;161:467-73
Sperm agglutination
Dosage and duration differences lead to diverse conclusions (Shulman) (Letter); (Hammond) (Reply). 1989;161:1744-6
Sperm-ovum interactions
Significance of D-mannose as a sperm receptor site on the zona pellucida in human fertilization (Mori et al). 1989;161:207-11
Spermatogenesis
Molecular scanning of Yq11 (interval 6) in men with Sertoli-cell-only syndrome (Johnson et al). 1989;161:1732-7
Spermatozoa
Relationship between circulating antisperm antibodies in women and autoantibodies on the ejaculated sperm of their partners (Witkin and Chaudhry). 1989;161:900-3
Significance of D-mannose as a sperm receptor site on the zona pellucida in human fertilization (Mori et al). 1989;161:207-11
Sperm antibodies and infertility (Shulman) (Letter); (Witkin) (Reply). 1989;161:497-9
Stains and staining
Comparison of predelivery versus postdelivery Kleihauer–Betke stains in cases of fetal death (Owen et al). 1989;161:663-6
Staphylococcus aureus
Methicillin-resistant staphylococcal mastitis (Kalstone). 1989;161:120
Starvation
Cordocentesis in the diagnosis of intrauterine starvation (Economides et al). 1989;161:1004-8
Statistics
Understanding and eliminating errors in perinatal research (Block). 1989;161:5-9 (Clin. opinion)
Steroids, biosynthesis
Effects of systemic administration of indomethacin on ovulation, luteinization, and steroidogenesis in the rabbit ovary (Katz et al). 1989;161:1361-6
Stillbirth; *see* **Fetal death**
Streptococcus pneumoniae
Antepartum infection as a result of *Streptococcus pneumoniae* and sepsis in neonate (Andreu et al). 1989;161:1424-5 (Letter)
Stress urinary incontinence; *see* **Urinary incontinence, stress**
Sudden infant death
Perinatal death as a result of social factors (Fribourg) (Letter); (Delke and Minkoff) (Reply). 1989;161:1748
Suicide, attempted
Attempted suicide and pregnancy (Czeizel and Lendvay). 1989;161:497 (Letter)
Suppressor cells; *see also* **Immunosuppression**
Immunoregulatory activity in supernatants from cultures of normal human trophoblast cells of the first trimester (Sanyal et al). 1989;161:446-53
Surfactants; *see* **Pulmonary surfactants**
Surgery, operative
Primary stress urinary incontinence and pelvic relaxation: prospective, randomized comparison of three different operations (Bergman et al). 1989;161:97-101
Surgical flaps
A new surgical technique to treat refractory labial fusion in the elderly (Johnson et al). 1989;161:289-90
Surgical staplers
Evaluation of surgical staples in cesarean section (Burkett et al). 1989;161:540-7
Swallowing; *see* **Deglutition**
Swan-Ganz catheter; *see* **Catheterization, Swan-Ganz**
Sympathomimetics
Effects of terbutaline sulfate on fetal cardiac function (Petersen et al). 1989;161:509-12
Fetal and neonatal cardiovascular complications from β-sympathomimetic therapy for tocolysis (Katz and Seeds). 1989;161:1-9 (Clin. opinion)

T

Tachycardia
Preterm and term fetal cardiac and movement responses to vibratory acoustic stimulation (Thomas et al). 1989;161:141-5

TAG-72; *see* **Antigens, neoplasm**
Terbutaline
Does terbutaline cause uterine atony and increase intraoperative blood loss? (Brown) (Letter); (Patriarco) (Reply). 1989;161:259
Effects of terbutaline sulfate on fetal cardiac function (Petersen et al). 1989;161:509-12
Fetal and neonatal cardiovascular complications from β-sympathomimetic therapy for tocolysis (Katz and Seeds). 1989;161:1-9 (Clin. opinion)
Testicular feminization
Seminoma in pubertal patient with androgen insensitivity syndrome (Hurt et al). 1989;161:530-1
Tetramethylpyrazine
In vitro uterine response to tetramethylpyrazine, the active constituent of *Chung Chong* (a traditional Chinese medicine) (Tuttle et al). 1989;161:1319-23
Thalassemia
Detection of fetal blood (Leiberman et al) (Letter); (Cardwell) (Reply). 1989;161:257-8
Theca cells
Pattern of human chorionic gonadotropin binding in the polycystic ovary (Brawer et al). 1989;161:474-80
Thermodilation
Measurement of cardiac output in pregnancy by thoracic electrical bioimpedance and thermodilution (Masaki et al). 1989;161:680-4
Third trimester, pregnancy; *see* **Pregnancy trimester, third**
Thoracic duct, animal
Effect of whole-body pH changes on thoracic duct lymph flow in anesthetized sheep (Valenzuela et al). 1989;161:1566-70
Thoracic electrical bioimpedance; *see* **Cardiography, impedance**
Thrombocytopenia
Elevated liver enzymes and thrombocytopenia in the third trimester of pregnancy: an unusual case report and a review of the literature (Hannah et al). 1989;161:322-3
Thrombocytopenia and cordocentesis clarifications requested (Weiner). 1989;161:1091-2 (Letter)
Thromboembolism
Thrombosis prophylaxis in pregnancy with use of subcutaneous heparin adjusted by monitoring heparin concentration in plasma (Dahlman et al). 1989;161:420-5
Thromboxane synthetase, antagonists
Effects of thromboxane synthetase inhibition on maternal-fetal homeostasis in gravid ewes with ovine pregnancy-induced hypertension (Keith et al). 1989;161:1305-13
Thromboxanes
Aspirin differentially affects thromboxane and prostacyclin production by trophoblast and villous core compartments of human placental villi (Nelson and Walsh). 1989;161:1593-8
Increased placental progesterone may cause decreased placental prostacyclin production in preeclampsia (Walsh and Coulter). 1989;161:1586-92
Tissue culture
Human uterus-derived growth substances for rat bone cells and fibroblasts (Koutsilieris). 1989;161:1313-7
Tissue pH; *see* **Hydrogen-ion concentration**
Tobramycin
Short course of antibiotic therapy in treatment of postpartum endomyometritis (Morales et al). 1989;161:568-72
Tocolysis
Cardiac isoenzymes and electrocardiographic changes during ritodrine tocolysis (Hadi and Albazzaz). 1989;161:318-21
Cardiovascular and metabolic effects associated with nifedipine and ritodrine tocolysis (Ferguson et al). 1989;161:788-95
Controlled trial of hydration and bed rest versus bed rest alone in the evaluation of preterm uterine contractions (Pircon et al). 1989;161:775-9
The effect of magnesium sulfate tocolysis on the fetal biophysical profile (Peaceman et al). 1989;161:771-4
Fetal and neonatal cardiovascular complications from β-sympathomimetic therapy for tocolysis (Katz and Seeds). 1989;161:1-9 (Clin. opinion)
Impact of residual amniotic fluid volume in patients receiving parenteral tocolysis after premature rupture of the membranes (Silver et al). 1989;161:784-7
Nifedipine pharmacokinetics during preterm labor tocolysis (Ferguson et al). 1989;161:1485-90
Pharmacokinetics of orally administered ritodrine (Caritis et al). 1989;161:32-5
Uncoupling of excitation from contraction in uterine smooth muscle in near-term ewes (Reid et al). 1989;161:229-33
Tocolytic agents
Fetal and neonatal cardiovascular complications from β-sympathomimetic therapy for tocolysis (Katz and Seeds). 1989;161:1-9 (Clin. opinion)
Neuromuscular blockade with magnesium sulfate and nifedipine (Snyder and Cardwell). 1989;161:35-6
Number clarification requested (Hatjis) (Letter); (Wilkins and Lynch) (Reply). 1989;161:1746
Pharmacokinetics of orally administered ritodrine (Caritis et al). 1989;161:32-5
Transactions
Transactions of the fifty-first annual meeting of the South Atlantic Association of Obstetricians and Gynecologists. 1989;161:505-92
Transactions of the ninth annual meeting of the Society of Perinatal Obstetricians. 1989;161:593-833
Translocation
"Possibly" de novo translocations: prenatal risk counseling (Wassman et al). 1989;161:698-702
Transposition of great vessels
Anesthetic management of labor in a patient with dextrocardia, congenitally corrected transposition, Wolff-Parkinson-White syndrome, and congestive heart failure (Sellers et al). 1989;161:1001-3
Treadmill test; *see* **Exercise test**
Trial of labor
Basic reference gambles recommended for utility assessment (Thornton and Lilford) (Letter); (Silver and Minogue) (Reply). 1989;161:256-7
Breech presentation after cesarean section: always a section (Ophir et al). 1989;161:25-8
Vaginal birth after cesarean delivery in the twin gestation (Strong et al). 1989;161:29-32
Tricuspid valve
Human fetal tricuspid and mitral deceleration time: changes with normal pregnancy and intrauterine growth retardation (Reed et al). 1989;161:1532-5
Triplets
The improving outcome of triplet pregnancies (Lipitz et al). 1989;161:1279-84
Outpatient triplet management: a contemporary review (Newman et al). 1989;161:547-5

Trisomy
Enlarged cisterna magna in trisomy 18: prenatal ultrasonographic diagnosis (Thurmond et al). 1989;161:83-5
Trisomy 21; *see* **Down's syndrome**
Trophoblast
Aspirin differentially affects thromboxane and prostacyclin production by trophoblast and villous core compartments of human placental villi (Nelson and Walsh). 1989;161:1593-8
Immunoregulatory activity in supernatants from cultures of normal human trophoblast cells of the first trimester (Sanyal et al). 1989;161:446-53
Trophoblastic tumor
Differences between white and Chinese populations in human leukocyte antigen sharing and gestational trophoblastic tumors (Ho et al). 1989;161:942-8
Gestational trophoblastic disease: a case-control study from the People's Republic of China (Brinton et al). 1989;161:121-7
Gestational trophoblastic disease and use of oral contraceptives (Rosenberg et al). 1989;161:1087-8 (Letter)
Tubal pregnancy; *see* **Pregnancy, tubal**
Tumor antigens; *see* **Antigens, neoplasm**
Tumor cells, cultured
A monoclonal antibody (MSN-1) against a newly established uterine endometrial cancer cell line (SNG-II) and its application to immunohistochemistry and flow cytometry (Nozawa et al). 1989;161:1079-86
Tumor necrosis factor
Infection and labor. IV. Cachectin–tumor necrosis factor in the amniotic fluid of women with intraamniotic infection and preterm labor (Romero et al). 1989;161:336-41
Release of tumor necrosis factor-α by human peritoneal macrophages in vivo and in vitro (Halme). 1989;161:1718-25
Tumors; *see* **Neoplasms**
Twins
Discordant twins—a prospective study of the diagnostic value of real-time ultrasonography combined with umbilical artery velocimetry (Divon et al). 1989;161:757-60
Hemodynamic changes during twin pregnancy: a Doppler and M-mode echocardiographic study (Robson et al). 1989;161:1273-8
Individual growth curve standards in twins: prediction of third-trimester growth and birth characteristics (Stefos et al). 1989;161:179-83
Management of the nonvertex second twin: primary cesarean section, external version, or primary breech extraction (Gocke et al). 1989;161:111-4
Perinatal outcome in very preterm births with twin-twin transfusion syndrome (Shan and Chaffin). 1989;161:1111-3
Prolongation of pregnancy and survival of twin B after loss of twin A at 21 weeks' gestation (Feichtinger et al). 1989;161:891-3
Successful twin pregnancy after embryo donation to a patient with XY gonadal dysgenesis (Sauer et al). 1989;161:380-1
Twin-to-twin transfusion syndrome: What are appropriate diagnostic criteria? (Danskin and Neilson). 1989;161:365-9
Ultrasonographic criteria for the prenatal diagnosis of placental chorionicity in twin gestations (Winn et al). 1989;161:1540-2
Twins, dizygotic
Twin pregnancy in premature ovarian failure after estrogen treatment: a case report (Tang and Sawers). 1989;161:172-3

U

Ultrasonic diagnosis
Antenatal ultrasonographic diagnosis of fetal seizure activity (Landy et al). 1989;161:308
Birth weight prediction from remote ultrasonographic examination (Spinnato et al). 1989;161:742-7
Clinical management of the fetus with markedly diminished umbilical artery end-diastolic flow (Divon et al). 1989;161:1523-7
Comparison of six different ultrasonographic methods for predicting lethal fetal pulmonary hypoplasia (Vintzileos et al). 1989;161:606-12
Concurrence of mouthing movement and rapid eye movement/non–rapid eye movement phases with advance in gestation of the human fetus (Horimoto et al). 1989;161:344-51
Dandy-Walker syndrome: a review of fifteen cases evaluated by prenatal sonography (Russ et al). 1989;161:401-6
Dating the early pregnancy by sequential appearance of embryonic structures (Warren et al). 1989;161:747-53
Discordant twins—a prospective study of the diagnostic value of real-time ultrasonography combined with umbilical artery velocimetry (Divon et al). 1989;161:757-60
Doppler assessment of the fetal and uteroplacental circulation during nifedipine therapy for preterm labor (Mari et al). 1989;161:1514-8
Doppler assessment of the pulsatility index of the middle cerebral artery during constriction of the fetal ductus arteriosus after indomethacin therapy (Mari et al). 1989;161:1528-31
Duplex Doppler ultrasonographic evaluation of the fetal renal artery in normal and abnormal fetuses (Veille and Kanaan). 1989;161:1502-7
Effect of placental embolization on the umbilical arterial velocity waveform in fetal sheep (Morrow et al). 1989;161:1055-60
The efficacy of fetal sonographic biometry in Down syndrome screening (Peters et al). 1989;161:297-300
Empty bladder recommended in initial ultrasonographic examinations (Stephens). 1989;161:1088-9 (Letter)
Enlarged cisterna magna in trisomy 18: prenatal ultrasonographic diagnosis (Thurmond et al). 1989;161:83-5
Estimating gestational age in the term pregnancy with a model based on multiple indices of fetal maturity (Goldstein et al). 1989;161:1235-8
Evaluation of pulsed Doppler echocardiography for measurement of aortic blood flow in the fetal lamb (Veille et al). 1989;161:1610-4
Fetal cardiac output in the isoimmunized pregnancy: a pulsed Doppler-echocardiographic study of patients undergoing intravascular intrauterine transfusion (Copel et al). 1989;161:361-5
Fetal liver length in normal and isoimmunized pregnancies (Roberts et al). 1989;161:42-6
Fetal radius length: a critical evaluation of race as a factor in gestational age assessment (Hill et al). 1989;161:193-9
First-trimester and early second-trimester diagnosis of nuchal cystic hygroma by transvaginal sonography: diverse

Ultrasonic diagnosis—cont'd
prognosis of the septated from the nonseptated lesion (Bronshtein et al). 1989;161:78-82
First-trimester midgut herniation: a high-frequency transvaginal sonographic study (Timor-Tritsch et al). 1989;161:831-3
Hemodynamic changes during twin pregnancy: a Doppler and M-mode echocardiographic study (Robson et al). 1989;161:1273-8
Human fetal tricuspid and mitral deceleration time: changes with normal pregnancy and intrauterine growth retardation (Reed et al). 1989;161:1532-5
The importance of ultrasonography in infertile women with "forgotten" intrauterine contraceptive devices (Ron-El et al). 1989;161:211-2
Intrapartum Doppler velocimetry, amniotic fluid volume, and fetal heart rate as predictors of subsequent fetal distress. I. An initial report (Sarno et al). 1989;161:1508-14
A longitudinal study of the relationship between placental and fetal growth as measured by ultrasonography (Wolf et al). 1989;161:1140-5
Longitudinal ultrasonographic study of the ovarian suppressive activity of a low-dose triphasic oral contraceptive during correct and incorrect pill intake (Hamilton and Hoogland). 1989;161:1159-62
Macrosomia in the postdate pregnancy: Is routine ultrasonographic screening indicated? (Chervenak et al). 1989;161:753-6
Maternal and fetal blood flow velocity waveforms in patients with preterm labor: relationship to outcome (Brar et al). 1989;161:1519-22
Measurement of fetal urine production in normal pregnancy by real-time ultrasonography (Rabinowitz et al). 1989;161:1264-6
Missed abortion: Still appropriate terminology? (Pridjian and Moawad). 1989;161:261-2 (Clin. opinion)
Prenatal informed consent for sonogram: an indication for obstetric ultrasonography (Chervenak et al). 1989;161:857-60 (Clin. opinion)
Pulsed Doppler flow-velocity waveforms in the prediction of fetal hematocrit of the severely isoimmunized pregnancy (Copel et al). 1989;161:341-4
Race and gender factors omitted from results (Ansbacher) (Letter); (Goldstein) (Reply). 1989;161:502-3
The role of ultrasonography and amniocentesis in the evaluation of pregnancies at risk for neural tube defects (Hogge et al). 1989;161:520-4
Screening with Doppler velocimetry in labor (Feinkind et al). 1989;161:765-70
Systolic/diastolic ratios and waveform shapes (Bewley) (Letter); (Schulman) (Reply). 1989;161:1420-1
Techniques differ in use of Doppler ultrasonography (Easterling and Benedetti) (Letter); (Lee and Rokey) (Reply). 1989;161:1748-50
Transvaginal ultrasonography in patients at risk for ectopic pregnancy (Stiller et al). 1989;161:930-3
Twin-to-twin transfusion syndrome: What are appropriate diagnostic criteria? (Danskin and Neilson). 1989;161:365-9
Ultrasonographic criteria for the prenatal diagnosis of placental chorionicity in twin gestations (Winn et al). 1989;161:1540-2
Ultrasonographic guidance for central venous access during pregnancy (Lee et al). 1989;161:1012-3
Ultrasonographic measurement of fetal femur length in growth disturbances (Abramowicz et al). 1989;161:1137-40
Umbilical artery flow velocity waveforms during acute hypoxemia and the relationship with hemodynamic changes in the fetal lamb (van Huisseling et al). 1989;161:1061-4
The use of color Doppler imaging for prenatal diagnosis of umbilical cord anomalies: report of three cases (Jauniaux et al). 1989;161:1195-7
The use of an indwelling Doppler probe to study acute changes in umbilical artery waveforms in the fetal sheep (Abitbol et al). 1989;161:1324-31
The use of transvaginal ultrasonography in the diagnosis of ectopic pregnancy (Timor-Tritsch et al). 1989;161:157-61
Vaginosonographic examination Routine procedure? (Furness) (Letter); (Bernaschek and Rudelstorfer) (Reply). 1989;161:499

Umbilical arteries
Clinical management of the fetus with markedly diminished umbilical artery end-diastolic flow (Divon et al). 1989;161:1523-7
Discordant twins a prospective study of the diagnostic value of real-time ultrasonography combined with umbilical artery velocimetry (Divon et al). 1989;161:757-60
Doppler umbilical artery waveform indices normal values from fourteen to forty-two weeks (Hendricks et al). 1989;161:761-5
Doppler velocimetry and placental disease (Bracero et al). 1989;161:388-93
Perinatal factors influencing atrial natriuretic peptide levels in umbilical arterial plasma at the time of delivery (Castro et al). 1989;161:623-7
Perinatal factors influencing atrial natriuretic peptide levels in umbilical arterial plasma at the time of delivery (Castro et al). (1989;161:623-7). 1989;161:1238 (Correction)

Umbilical arteries, animal
Effect of placental embolization on the umbilical arterial velocity waveform in fetal sheep (Morrow et al). 1989;161:1055-60
Umbilical artery flow velocity waveforms during acute hypoxemia and the relationship with hemodynamic changes in the fetal lamb (van Huisseling et al). 1989;161:1061-4
The use of an indwelling Doppler probe to study acute changes in umbilical artery waveforms in the fetal sheep (Abitbol et al). 1989;161:1324-31

Umbilical cord
Abscess of allantoic duct remnant (Baill et al). 1989;161:334-6
Continuous fetal monitoring with a pulse oximeter: a case of cord compression (Johnson and Johnson). 1989;161:1295-6

Umbilical cord, abnormalities
The use of color Doppler imaging for prenatal diagnosis of umbilical cord anomalies: report of three cases (Jauniaux et al). 1989;161:1195-7

Umbilical cord blood; *see* **Fetal blood**

Umbilical veins
The effect of intravascular transfusion on umbilical venous pressure in anemic fetuses with and without hydrops (Weiner et al). 1989;161:1498-501
Removing a retained placenta by oxytocin a controlled study (Wilken-Jensen et al). 1989;161:155-6

Uncoupling agents
Uncoupling of excitation from contraction in uterine

smooth muscle in near-term ewes (Reid et al). 1989;161:229-33

Urinary incontinence, stress

Primary stress urinary incontinence and pelvic relaxation: prospective randomized comparison of three different operations (Bergman et al). 1989;161:97-101

Urine, animal

Fetal blood volume, urine flow, swallowing, and amniotic fluid volume responses to long-term intravascular infusions of saline (Brace). 1989;161:1049-54

Urodynamics

Primary stress urinary incontinence and pelvic relaxation: prospective randomized comparison of three different operations (Bergman et al). 1989;161:97-101

Uterine contraction

The antagonistic effect of oxytocin and relaxin on rat uterine segment contractility (Goldsmith et al). 1989;161:1644-9

Controlled trial of hydration and bed rest versus bed rest alone in the evaluation of preterm uterine contractions (Pircon et al). 1989;161:775-9

Maternal hemodynamic effects of uterine contractions by M-mode and pulsed-Doppler echocardiography (Lee et al). 1989;161:974-7

Results of uterine activity studies in agreement, but methods differ (Török and Gáti) (Letter); (Nageotte) (Reply). 1989;161:1753-4

Uncoupling of excitation from contraction in uterine smooth muscle in near-term ewes (Reid et al). 1989;161:229-33

Uterine neoplasms

Anal submucosal injection: a new route for drug administration in pelvic malignancies. IV. Submucosal anal injection in the treatment of cancer of uterine cervix—preliminary study (Shafik). 1989;161:69-72

A clinical-pathologic study of mixed müllerian tumors of the uterus over a 16-year period The Medical College of Georgia experience (Gallup et al). 1989;161:533-9

Decreased β-carotene tissue levels in uterine leiomyomas and cancers of reproductive and nonreproductive organs (Palan et al). 1989;161:1649-52

Epidermal growth factor receptor expression in normal and malignant endometrium (Berchuck et al). 1989;161:1247-52

Flow cytometry of normal, hyperplastic, and malignant human endometrium (Thornton et al). 1989;161:487-92

Immunohistochemical expression of TAG-72 in normal and malignant endometrium: correlation of antigen expression with estrogen receptor and progesterone receptor levels (Soisson et al). 1989;161:1258-63

A monoclonal antibody (MSN-1) against a newly established uterine endometrial cancer cell line (SNG-II) and its application to immunohistochemistry and flow cytometry (Nozawa et al). 1989;161:1079-86

Phase II trial of ifosfamide and mesna in mixed mesodermal tumors of the uterus (A Gynecologic Oncology Group Study) (Sutton et al). 1989;161:309-12

Prognostic significance of steroid receptors measured in primary metastatic and recurrent endometrial carcinoma (Borazjani et al). 1989;161:1253-7

Uterine perforation

The frequency and management of uterine perforations during first-trimester abortions (Kaali et al). 1989;161:406-8

Uterine rupture

Placenta percreta induced uterine rupture and resulted in intraabdominal abortion (Nagy). 1989;161:1185-6

Uterine rupture: Are intrauterine pressure catheters useful in the diagnosis? (Rodriguez et al). 1989;161:666-9

Uterus

Uterine development and endocrine relationships after menarche (Porcu et al). 1989;161:174-7

Uterus, animal

Comparison of in vivo bioavailability of progestational agents into the rat uterus and liver and the effect of plasma protein binding (Van Voorhis et al). 1989;161:1698-703

Effect of pregnancy on uterine and carotid artery response to norepinephrine, epinephrine, and phenylephrine in vessels with documented functional endothelium (Weiner et al). 1989;161:1605-10

In vitro release of endothelium-derived relaxing factor by acetylcholine is increased during the guinea pig pregnancy (Weiner et al). 1989;161:1599-605

Power spectrum analysis of myometrial electromyogram and intrauterine pressure changes in the pregnant rhesus monkey in late gestation (Hsu et al). 1989;161:467-73

Uterus, blood supply

Decreased in vitro production of 6-keto-prostaglandin $F_{1\alpha}$ by uterine arteries from postmenopausal women (Steinleitner et al). 1989;161:1677-81

Effect of placental laterality on uterine artery resistance and development of preeclampsia and intrauterine growth retardation (Kofinas et al). 1989;161:1536-9

Uterus, drug effects

Does terbutaline cause uterine atony and increase intraoperative blood loss? (Brown) (Letter); (Patriarco) (Reply). 1989;161:259

In vitro uterine response to tetramethylpyrazine, the active constituent of *Chung Chong* (a traditional Chinese medicine) (Tuttle et al). 1989;161:1319-23

Uterus, histology

Histologic and immunologic study of uterine biopsy tissue of women with incipient abortion (Michel et al). 1989;161:409-14

Uterus, immunology

Histologic and immunologic study of uterine biopsy tissue of women with incipient abortion (Michel et al). 1989;161:409-14

V

Vagina

The use of transvaginal ultrasonography in the diagnosis of ectopic pregnancy (Timor-Tritsch et al). 1989;161:157-61

Vagina, abnormalities

Ileocecal vaginal construction (Bürger et al). 1989;161:162-7

Vagina, microbiology

Evidence relating bacterial vaginosis to intraamniotic infection (Silver et al). 1989;161:808-12

Vagina, surgery

Ileocecal vaginal construction (Bürger et al). 1989;161:162-7

Vaginal birth; *see* **Delivery**

Vaginitis

Allergic vaginitis (Dworetzky) (Letter); (Galland) (Reply). 1989;161:1752-3

Vaginosis; *see* **Vagina, microbiology**
Vancomycin, adverse effects
Vancomycin during pregnancy: Does it cause hearing loss or nephrotoxicity in the infant? (Reyes et al). 1989;161:977-81
Vascular endothelium; *see* **Endothelium, vascular**
Vascular resistance
Cardiovascular changes in early pregnancy (Capeless and Clapp). 1989;161:1449-53
Effect of placental laterality on uterine artery resistance and development of preeclampsia and intrauterine growth retardation (Kofinas et al). 1989;161:1536-9
Vascular smooth muscle, animal; *see* **Muscle, smooth, vascular, animal**
Vasoconstriction, animal
Effect of magnesium on cocaine-induced, catecholamine-mediated platelet and vascular response in term pregnant ewes (Weaver et al). 1989;161:1331-7
Vasodilation
Human α- and β-calcitonin gene-related peptides are vasodilators in human chorionic plate vasculature (Firth and Pipkin). 1989;161:1318-9
Vasopressins, agonists
Vasopressin receptors in human pregnant myometrium and decidua: interactions with oxytocin and vasopressin agonists and antagonists (Ivanisevic et al). 1989;161:1637-43
Vasopressins, antagonists
Vasopressin receptors in human pregnant myometrium and decidua: interactions with oxytocin and vasopressin agonists and antagonists (Ivanisevic et al). 1989;161:1637-43
Vehicle
Absorption of oral progesterone is influenced by vehicle and particle size (Hargrove et al). 1989;161:948-51
Velocimetry, Doppler; *see* **Flowmeters**
Venous pressure
Ovine fetal cardiovascular responses to packed red blood cell transfusions (Brace). 1989;161:1367-74
Vibratory stimulation; *see* **Acoustic stimulation**
Vitamin A
Effects of smoking and oral contraception on plasma β-carotene levels in healthy women (Palan et al). 1989;161:881-5
Vitamin D
Vitamin D (soltriol), light, and reproduction (Stumpf and Denny). 1989;161:1375-84 (Curr. dev.)
Vulvar diseases
Risk factors for vulvar lichen sclerosus (Sideri et al). 1989;161:38-42
Vulvar lichen sclerosus; *see* **Vulvar diseases**
Vulvovaginitis
A new surgical technique to treat refractory labial fusion in the elderly (Johnson et al). 1989;161:289-90

W

Water-electrolyte balance
Is a decrease in plasma oncotic pressure enough to explain the edema of pregnancy? (Valenzuela). 1989;161:1624-7
Lymph flow rate response to angiotensin II is decreased in pregnant sheep (Valenzuela and Longo). 1989;161:1615-9
Withdrawal bleeding; *see* **Menstruation, drug effects**
Wolff-Parkinson-White syndrome
Anesthetic management of labor in a patient with dextrocardia, congenitally corrected transposition, Wolff-Parkinson-White syndrome, and congestive heart failure (Sellers et al). 1989;161:1001-3
Wound healing
Assessment of epidermal growth factor in the healing process of clean full-thickness skin wounds (Jijon et al). 1989;161:1658-62

X

XY gonadal dysgensis; *see* **Gonadal dysgenesis, 46,XY**

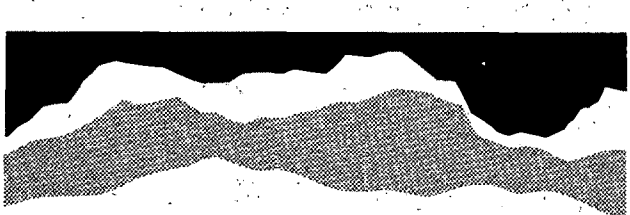
Y

Y chromosome
Molecular scanning of Yq11 (interval 6) in men with Sertoli-cell-only syndrome (Johnson et al). 1989;161:1732-7
Rapid determination of fetal sex by deoxyribonucleic acid amplification of Y chromosome–specific sequences (Pinckert et al). 1989;161:693-8

Z

Zinc
Zinc, cadmium, and hypertension in parturient women (Lazebnik et al). 1989;161:437-40
Zona pellucida
Significance of D-mannose as a sperm receptor site on the zona pellucida in human fertilization (Mori et al). 1989;161:207-11

JOHNS HOPKINS HEALTH SYSTEM medical group needs additional physicians in OB/GYN for full-time and part-time positions at new and established offices throughout the greater Baltimore area. Physicians must be board certified or active candidates, and eligible for Maryland license and DEA certificate. Interviewing now for next summer; some positions available immediately. Equal Employment Opportunity Employer. Send current curriculum vitae to: Recruiting Coordinator; Office of Medical Affairs; Wyman Park Medical Associates; 3100 Wyman Park Drive; Baltimore, Maryland 21211.



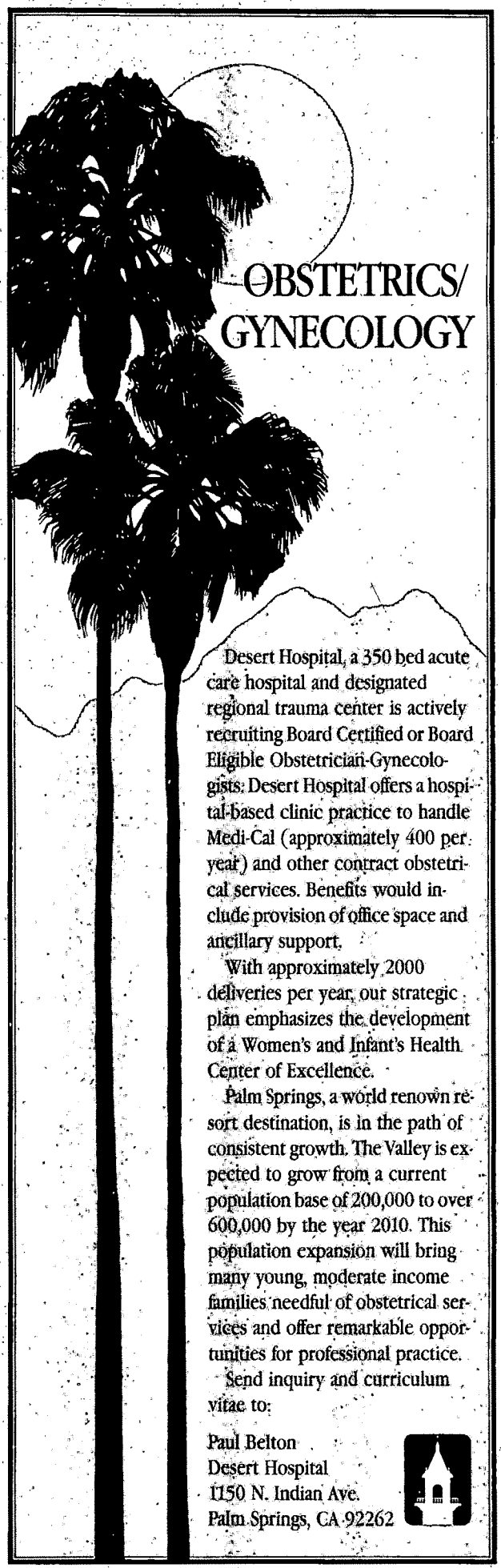
SOUTHERN CALIFORNIA

Los Angeles County

There is now an attractive opportunity to join two young and busy board-certified OB/GYN's practicing in a Los Angeles County community of 116,000. This is a rapidly expanding six-year-old practice and a third member is needed as soon as possible. They are offering generous financial arrangements leading to early partnership.

For more information send CV to: Manager, Professional Relations, Humana Inc., Dept. OB-12, 500 West Main Street, Louisville, KY 40201-1438. Or call TOLL-FREE 1-800-626-1590.





OBSTETRICS/ GYNECOLOGY

Desert Hospital, a 350 bed acute care hospital and designated regional trauma center is actively recruiting Board Certified or Board Eligible Obstetrician-Gynecologists. Desert Hospital offers a hospital-based clinic practice to handle Medi-Cal (approximately 400 per year) and other contract obstetrical services. Benefits would include provision of office space and ancillary support.

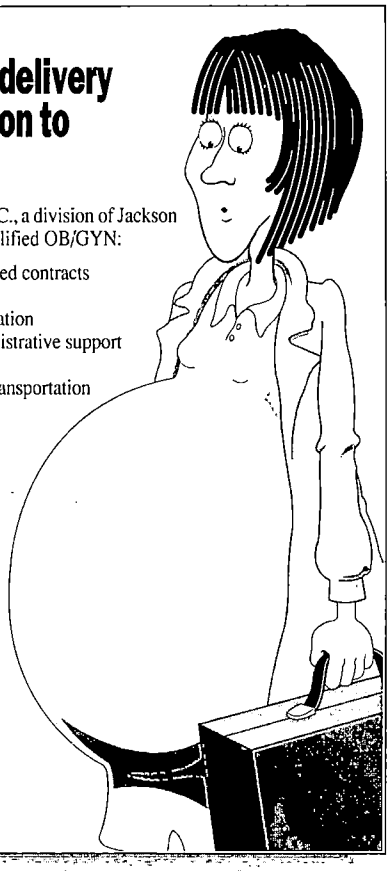
With approximately 2000 deliveries per year, our strategic plan emphasizes the development of a Women's and Infant's Health Center of Excellence.

Palm Springs, a world renown resort destination, is in the path of consistent growth. The Valley is expected to grow from a current population base of 200,000 to over 600,000 by the year 2010. This population expansion will bring many young, moderate income families needful of obstetrical services and offer remarkable opportunities for professional practice.

Send inquiry and curriculum vitae to:

Paul Belton
Desert Hospital
1150 N. Indian Ave.
Palm Springs, CA 92262

Be in on the delivery of the solution to OB staffing.



LOCUM TENENS, INC., a division of Jackson & Coker, offers the qualified OB/GYN:

- 3 to 9 month guaranteed contracts
- competitive daily rate
- licensing and coordination
- knowledgeable administrative support
- round trip airfare
- on-site housing and transportation
- permanent practice opportunities

Call 1-800-544-1987
or write to:
Locum, Tenens, Inc.
400 Perimenter
Center Terrace
Suite 760 OGLT9
Atlanta, Georgia 30346

LOCUM TENENS, INC.
A Division of Jackson and Coker

OB/GYN

WANTED: Obstetrician/Gynecologist, Board Certified or eligible physician to join existing 3 member OB/GYN Department in 22 member multi-specialty group practice in the Lakes Region of central N.H. Position offers an outstanding professional, financial, and life-style opportunity. Respond with C.V. to Alan Awrich,M.D.,Laconia Clinic, 724 Main Street, Laconia, NH 03246.

SPECIAL DELIVERIES. SPECIAL CHALLENGES.

The Southern California Permanente Medical Group invites you to join a unique prepaid health care program. Our multispecialty group practice is a partnership composed of and managed by SCPMG physicians.

Board eligible/certified **PERINATOLOGISTS** are needed for our high-volume obstetric services which handles 3,000-6,000 deliveries per year. Opportunities exist both to join and create high risk services.

There are positions available to practice Perinatology exclusively. Perinatologists who are interested in performing other aspects of obstetrics and gynecology may do so. We also have positions available for Endocrinologists and General Obstetrician-Gynecologists. University affiliation and clinical research are encouraged.

Our compensation is competitive and benefits are outstanding. They include: professional liability, medical and dental coverage, vacation and sick leave, continuing education, life insurance and retirement plans. **After two years full-time employment, physicians are eligible to be considered for partnership.**

For a physician application, please call or write to: William G. McCormick, M.D., Physician Recruitment, Dept. 006, Walnut Center, Pasadena, CA 91188-8013. (714) 829-5365.

Equal Opportunity Employer M/F/H

KAISER PERMANENTE
Good People. Good Medicine.

PRIDE & POTENTIAL

PERINATOLOGIST

Illinois, Chicago—Lutheran General Hospital is seeking a fourth Perinatologist for an excellent opportunity in direct patient care, teaching and clinical research. Lutheran General is a 713-bed teaching hospital affiliated with the University of Illinois College of Medicine in Chicago. It is a designated tertiary care center with 4500 deliveries annually and has an active maternal transport service of over 200 a year. The Obstetrics and Gynecology Department has a residency program, subspecialists in Gynecology/Endocrinology and Gynecology/Oncology. There is a 36-bed neonatal NICU with five neonatologists and a fellowship program. There is also a large referral service for genetic counseling and prenatal diagnoses, and more than 750 genetic amniocentesis are performed annually. We have recently opened a large Perinatal Center office.

The hospital is located in an attractive northwest suburb of Chicago. We offer a competitive salary with a generous incentive plan and an excellent benefit package.

Please send your curriculum vitae to Dr. Haim Elrad, Chief of Obstetrics and Director of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, or call 708/606-6998. Equal Opportunity Employer.

Lutheran General Hospital
Park Ridge
1775 Dempster St.
Park Ridge, IL 60068

Central New Jersey

Freehold Area Hospital, a progressive 248 bed community Hospital, offers an excellent opportunity for a group of two or more Board Eligible obstetricians to assume complete responsibility for our OB clinic. Successful candidates may also establish a private practice, including assistance with office, location and transitional costs.

Located in a rapidly expanding area of Suburban Western Monmouth County, the Hospital is easily accessible to New York City, Philadelphia and the Jersey Shore.

If you are interested in this opportunity, please send your resume and three letters of reference to the address listed below.

For further information, please contact Susan Flaster, RN, BSN, Director of Ambulatory Services at (201) 780-6142.

OB Search Committee
Medical Staff Office
Freehold Area Hospital
West Main Street
Freehold, New Jersey 07728
(201) 780-6072

Join us.

Group Health Cooperative of Puget Sound, a 370,000-member pre-paid group practice, currently has a position for an **OB/GYN Physician.** Referral only practice in a multi-specialty organization.

To inquire, write:
Director of Medical Staff Personnel
Group Health Cooperative
of Puget Sound
521 Wall Street
Seattle, WA 98121

Group Health Cooperative of Puget Sound
Affirmative Action Equal Opportunity Employer

The Medical Center of Delaware, an 1100 bed, tertiary care major teaching affiliate of Thomas Jefferson University, seeks a full-time chairman of the Department of Obstetrics-Gynecology. The Medical Center is the major trauma referral center for the State of Delaware. There are approved programs in Ob-Gyn, surgery, internal medicine, emergency medicine, pediatrics, radiology, and family practice, plus a transitional program and a medicine/pediatric program. Over 6,500 deliveries with a 25% cesarean section rate are done annually and 4,300 gynecologic operations are performed annually with approximately 3,500 of these being major procedures and 800 of these minor. Two thirds of the procedures performed are on an outpatient basis. Approximately 32,078 surgical operations are performed annually with 12,282 inpatient procedures and 19,796 outpatient procedures. Candidates should have proven administrative ability, experience in managing an ob-gyn service; experience in developing, enhancing and/or upgrading clinical services, education programs and research; and the ability to maintain and guide the ob-gyn residency program. The successful candidate will have a senior academic appointment at Jefferson Medical College of the Thomas Jefferson University in Philadelphia, Pennsylvania. Please send curriculum vitae and three references independently to: George Hilty, M.D., Chairman, Search Committee, Medical Center of Delaware, c/o of Patti Falkowski, CMSC, P.O. Box 1668, Wilmington, DE 19899. The Medical Center of Delaware is an affirmative action/equal opportunity employer.

HARTFORD HOSPITAL
DIRECTOR OF AMBULATORY OB/GYN

The Department of Obstetrics and Gynecology at Hartford Hospital is seeking a fulltime salaried board certified/board eligible Director of Ambulatory OB/GYN. We are looking for an individual with innovative ideas in resident and medical student education coupled with an ability to make the Outpatient Department function most efficiently. The OPD presently has approximately 21,000 patient visits annually.

The OB/GYN Department has divisions of Maternal-Fetal Medicine, Gyn-Oncology, and Reproductive Endocrinology. It also includes 49 BC/BE attendings, 16 residents and 6 medical students on two month clinical rotations from the University of Connecticut and Dartmouth.

Located in the capital city of 'Classic Connecticut,' Hartford Hospital is a tertiary care 885 bed institution with approximately 225 house officers. Dr. Stephen L. Curry will become the permanent fulltime Director of the Department on January 1, 1990.

Academic appointment at the University of Connecticut will be commensurate with training and experience.

Please send your curriculum vitae to:

Alan J. Wabrek, M.D. — Acting Director
OB/GYN Department
Hartford Hospital
80 Seymour St.
Hartford, CT 06115

OB/GYN WISCONSIN

Multi-specialty group in suburban Milwaukee seeks BC/BE Obstetrician/Gynecologist to join 6 physician OB/GYN department. Single site, single hospital practice. Competitive salary and fringe benefits. Forward inquiries and CV to:

Administrator
P.O. Box 427
Menomonee Falls, WI
53051

Join a Leader
We're The Ohio Permanente Medical Group, Inc.

and we are looking for board certified/board eligible **Obstetrician/Gynecologists** to help us keep up with rapid growth of the Kaiser Permanente Program in Northeastern Ohio. OPMG is the multispecialty group practice that provides health care services to the more than 185,000 Kaiser members in the Cleveland-Akron area.

Our wealth of experience of over 40 plus years (25 in Ohio) makes Kaiser Permanente a mature, solid, leader in the managed care sector of the health care industry. The rewards of practice with us are substantial—excellent salary and benefit packages, company-paid retirement plan, full malpractice coverage, a stimulating, collegial environment in which to practice quality medicine, and more.

Kaiser Permanente's Ohio Region is located in the heart of the dynamic, resurgent industrial Midwest. The area offers the best of big city sophistication and culture in an affordable, accessible living area.

Please send your resume to: Ronald G. Potts, M.D., Medical Director, Ohio Permanente Medical Group, Inc., 1300 E. 9th Street, Suite 1100, Cleveland, OH 44114. Or you may call us collect at (216) 623-8780.

KAISER PERMANENTE®

Northwestern University Medical School Department of Obstetrics and Gynecology Chicago, Illinois Academic Gynecologist

Responsibilities include development of clinical or basic science research in the field of benign gynecology (interest in the field of urogynecology or infectious diseases is desirable), graduate and undergraduate education, and participation in clinical gynecology service.

Academic rank will be commensurate with qualifiactions and experience. We encourage individuals interested in career development to apply.

Applications should be sent to:

Lane J. Mercer, M.D.
Chief, Section of Gynecology
Department of Obstetrics and Gynecology
333 East Superior Street, Suite 420
Chicago, Illinois 60611

In order to receive full consideration, application must be received by February 15, 1990.

Northwestern University is an affirmative action/equal opportunity employer. Hiring is contingent upon eligibility to work in the United States.

NORTHERN CALIFORNIA

The Kaiser Permanente Medical Care Program of Northern California, San Francisco Bay Area, a federally qualified HMO, seek a board certified eligible physician for the Department of Obstetrics/Gynecology in our Richmond medical office for 1990.

Competitive salary.

Excellent benefits include full malpractice and medical coverage, paid vacation, holiday, education and sick leave, retirement plan and shareholder opportunity.

Direct letter of introduction and curriculum vitae to:

Victor A. David, M.D.
Chief of OB/GYN
Kaiser Permanente Medical Center
901 Nevin Avenue
Richmond, CA 94801

or call collect (415) 231-4823

OBSTETRICIAN GYNECOLOGIST

Expanding Western Massachusetts staff model Health Maintenance Organization is seeking a full-time Board Certified/Board Eligible Obstetrician/Gynecologist to join a staff of eight (8) physicians and six (6) midwives.

Our multi-specialty group practice employs seventy (70) full-time physicians and provides care for a growing membership of over 70,000 from modern suburban facilities, in-patient care is provided at 900-bed Tufts affiliated teaching hospital.

Attractive Pioneer Valley location with fine educational, cultural and recreational opportunities. Excellent salary and comprehensive benefit program. Please send curriculum vitae to: Oded Rencus, M.D., Chief of OB/GYN, 305 Bicentennial Highway, Springfield, MA 01118.

Medical West
Community Health Plan
A BLUE CROSS & BLUE SHIELD HMO

We are an equal opportunity/affirmative action employer

FACULTY POSITION IN OBSTETRICS AND GYNECOLOGY

The Department of Obstetrics and Gynecology, MetroHealth Medical Center (formerly Cleveland Metropolitan General Hospital); Case Western Reserve University is looking for an Obstetrician-Gynecologist interested in pursuing an active research program integrated with part-time clinical practice in a teaching hospital. The current environment provides an excellent opportunity for both independent and collaborative research endeavor. Research interests and productivity should be focused on an area of Reproductive Physiology. Fellowship training in Maternal-Fetal Medicine helpful but clearly not essential.

Interested candidates should send curriculum vitae to:

James F. Clapp, III, M.D.
Department of Obstetrics and Gynecology
MetroHealth Medical Center
3395 Scranton Road
Cleveland, Ohio 44109

State University of New York
Health Science Center
Syracuse

Department of Obstetrics and Gynecology

PELVIC SURGERY FELLOWSHIP

The Department of Obstetrics and Gynecology of S.U.N.Y., Health Science Center at Syracuse is offering a one-year fellowship in Pelvic Surgery for qualified candidates.

The Fellowship will include comprehensive training in benign and malignant pelvic surgery, infertility surgery, laser surgery (abdominal, endoscopic and external genitalia), female urology and endoscopy (laparoscopy, hysteroscopy and colposcopy). The program is designed to provide clinical experience and expertise in gynecologic surgery. Research opportunities are available.

Positions are available beginning July 1, 1990.

Qualifications include ABOG eligible/certified status and eligibility for full NY State licensure.

Requests for applications can be sent to:

MICHAEL S. BAGGISH, M.D.
PROFESSOR AND CHAIRMAN
DEPARTMENT OF OB/GYN
S.U.N.Y., HEALTH SCIENCE CENTER
736 IRVING AVENUE
SUITE 308 WEST TOWER
SYRACUSE, NY 13210

SUNY, Health Science Center is an AA/EEO Employer

KAISER PERMANENTE

OB/GYN

OB/GYN BOARD CERTIFIED OR ELIGIBLE to join large multi-specialty group in San Francisco. Large volume tertiary care obstetrical service; over 3400 deliveries per year; 18 staff OB/GYN; 4 year residency program in OB/GYN. Benefits include excellent vacation and educational leave, medical and dental coverages, malpractice and life insurance, disability, retirement program. Academic appointment and clinical research available.

Send inquiry and C.V. to:

Ira M. Golditch, M.D.
Chief, Dept. of Ob/Gyn
Kaiser Permanente Medical Center
2200 O'Farrell Street
San Francisco, CA 94115

or call
(415) 929-5604

DIRECTOR OF EDUCATION

Baystate Medical Center, a regional referral center for Western Massachusetts and the Western Campus of Tufts University School of Medicine, is seeking a Board Certified physician to direct the educational program for 50 third-year medical students. There is also a free standing resident education program consisting of 20 residents.

The Department of Obstetrics and Gynecology has over 6,000 deliveries, 18,000 outpatient visits and 3,000 operative procedures performed per year. There are 10 full time faculty who support the educational programs within Genetic, Oncology, Maternal Fetal Medicine, Primary Care, Reproductive Endocrinology and Urogynecology.

Salary and academic rank are commensurate with experience and credentials. Interested applicants should send their curriculum vitae in confidence to: Laurence E. Lundy, M.D., Chairman, Department of OB/GYN, BAYSTATE MEDICAL CENTER, 759 Chestnut Street, Springfield, MA 01199, (413) 784-5457. We are an Equal Opportunity Employer.

Baystate Medical Center

The Western Campus of Tufts University School of Medicine.

PERINATOLOGIST

The Department of Obstetrics & Gynecology at Pennsylvania Hospital, Philadelphia, PA is recruiting a Board Certified/Eligible Maternal-Fetal Medicine specialist for a full-time position at the Assistant Professor level. The section now employs eight Maternal-Fetal Medicine specialists and trains two Fellows per year. The position will include half-time consultation at a satellite site and half-time clinical and research responsibilities at the tertiary center. Pennsylvania Hospital is an Affirmative Action, Equal Opportunity Employer. Female and minority applicants are especially welcome.

Interested candidates should send a curriculum vitae to:

Ronald J. Bolognese M.D., Chairman
Department of Obstetrics & Gynecology
Pennsylvania Hospital
8th & Spruce Streets
Philadelphia, PA 19107

Central Minnesota
Group Health Plan

OB/GYN

Rapidly growing, innovative primary care clinic has openings for two board eligible/certified OB/GYN physicians. New clinic facility in the fastest growing urban area in Minnesota. St. Cloud is a community of 45,000 located on the upper Mississippi River 70 miles from Mpls./St. Paul. Be close to lakes, ski areas and excellent schools, including three universities. Join a supportive primary care staff and receive competitive salary, outstanding benefits, liberal vacation and educational leave, malpractice coverage, etc. Contact Physician Services, Central Minnesota Group Health Plan, 1245 15th Street North, St. Cloud, MN 56303. 612/253-5220. EOE.

DEPARTMENT OF OBSTETRICS AND GYNECOLOGY UNIVERSITY OF WISCONSIN MEDICAL SCHOOL, MILWAUKEE CLINICAL CAMPUS AT SINAI SAMARITAN MEDICAL CENTER

Excellent opportunities for **MATERNAL FETAL MEDICINE** and **GENERAL OBSTETRICS AND GYNECOLOGY** with an interest in **AMBULATORY CARE, UROGYNECOLOGY,** or **STATEWIDE FAMILY PLANNING.** Sinai Samaritan Medical Center is the largest obstetrical unit (4,800 deliveries) in Wisconsin and is a tertiary care referral center in downtown Milwaukee. The University of Wisconsin Medical School, Milwaukee Clinical Campus, supports an active ob/gyn department with consultative ultrasound, genetic, infectious disease and reproductive/endocrine services.

Qualifications include board eligibility or certification in Obstetrics and Gynecology and/or subspecialty. Commitment to teaching residents, medical students, consultative services and clinical research.

Salary open and dependent on qualifications and experience. For more information, interested candidates should contact:

Fredrik F. Broekhuizen, M.D.
Chairman and Chief
Department of Obstetrics and Gynecology
University of Wisconsin Medical School
Milwaukee Clinical Campus
Sinai Samaritan Medical Center
Mount Sinai Campus
950 North 12th Street, G405
Milwaukee, WI 53233
(414) 283-7931

BE/BC OB/GYN

St. Louis Missouri Community Health Center looking for a BE/BC OB/GYN to head that department. Center has a busy prenatal, gynecologic and family planning service. Benefits include competitive salary, malpractice, health, disability, pension, CME, and vacation. Possible simultaneous appointment to major university clinical staff. For more information send CV or call:

Family Care Center of Carondelet
6313 Michigan Avenue
St. Louis, MO 63111
(314) 353-5190

Attn:
Dr. Michael Adix, Medical Director

UNIVERSITY OF MIAMI SCHOOL OF MEDICINE DEPARTMENT OF OBSTETRICS & GYNECOLOGY

ASSISTANT PROFESSOR OF CLINICAL OBSTETRICS & GYNECOLOGY

Opportunity available for M.D. who is board certified or board eligible in Maternal Fetal Medicine. Special interest in infectious disease is desirable but not required.

Applicant must be licensed in State of Florida, and have sufficient clinical experience to qualify for a full time faculty appointment.

Salary will be commensurate with training and experience. Compensation includes excellent fringe benefit and financial incentive plan. Apply to:

Mary J. O'Sullivan, M.D.
Professor
Obstetrics & Gynecology, R-136
University of Miami
P.O. Box 016960
Miami, Florida 33101

AN EQUAL OPPORTUNITY/AFFIRMATIVE ACTION EMPLOYER

SUBURBAN PITTSBURGH

A 12-year-old, 18-physician multi-specialty group seeks to expand its services by adding two OB/GYNs to its medical staff. They are affiliated with a 350-bed community hospital and currently have four office locations. The four pediatrician group members will offer a built-in referral base for the OB/GYNs.

Facilities at the offices include a state-licensed lab, mammography, sonography, diagnostic radiology, and much more. Centralized billing is provided for all physicians along with the assistance of a full component of staff which includes clerical support, medical assistants, nurses, and laboratory and radiology technicians.

This opportunity offers a competitive income guarantee, malpractice insurance, pension/profit sharing program, and a highly attractive partnership option. Due to the specialty structure of the group, the partnership buy-in will be minimal for the initial OB/GYNs joining the group. Additional corporate ventures will also be open to new members.

DANIEL STERN & ASSOCIATES

Suite 240 The Medical Center East
211 North Whitfield Street
Pittsburgh, PA 15206
800-438-2476 412-363-9700 FAX 412-363-6032
Tim Maus, Assistant Vice President - OB/GYN

KAISER PERMANENTE

OBSTETRICIAN-GYNECOLOGIST: The Permanente Medical Group, Inc., of San Jose, California is looking for BC/BE physicians for immediate openings in the OB/GYN Department. Positions available either at the main facility in San Jose or at satellite medical offices in Gilroy. The young, dynamic Department is still growing and currently has nineteen physicians and two nurse practitioners. Salary is competitive and includes full coverage malpractice insurance and an outstanding benefits package. San Jose and Gilroy share excellent access to Bay area cultural and recreational activities.

Please contact:

Jerome L. Nehemiah, M.D.
Chief
Department of OB/GYN
The Permanente Medical Group, Inc.
260 International Circle
San Jose, CA 95119

State University of New York
Health Science Center
Syracuse

MATERNAL-FETAL MEDICINE SUBSPECIALIST ASSISTANT PROFESSOR/ ASSOCIATE PROFESSOR

The Department of Obstetrics and Gynecology of the SUNY Healty Science Center at Syracuse is actively recruiting a FOURTH full-time Perinatologist for its Division of Maternal-Fetal Medicine.

The teaching hospital serves as the only Tertiary Care Center for the Regional Perinatal Program encompassing the 15 counties of Central New York with a population of 2.3 million.

Excellent opportunity to participate in teaching and collaborate in ongoing research in clinical Perinatology, Ultrasound, and Prenatal Diagnosis.

TOP SALARY, ATTRACTIVE BENEFITS PACKAGE, AND PROTECTED RESEARCH TIME.

Board eligibility/certification in Maternal-Fetal Medicine is required.

Interested candidates should send curriculum vitae to:

Shiraz Sunderji, M.D. — Director
Division of Maternal-Fetal Medicine
SUNY Health Science Center at Syracuse
Perinatal Center
725 Irving Avenue, Suite 115
Syracuse, NY 13210
(315) 470-7196

SUNY Health Science Center is an equal opportunity, affirmative action employer.

OB-GYN FACULTY IN JAPAN

Ideal for Sabbatical

Univ. of Hawaii Medical Education Program in Okinawa, Japan invites applications for faculties in Ob-Gyn (General, Perinatology, or Oncology) for a period of 3-6 months (between Jan '90 until July '91). Salary commensurate with experience plus 30% overseas allowance, airfare, furnished accommodation with all utilities paid. Additional allowance of approximately Yen 120,000 per month. Applications with C.V. to:

Y. B. Talwalkar, M.D., Program Director
University of Hawaii Program
Okinawa, Chubu Hospital, Gushikawa City
Okinawa, Japan. 904-22
Serious inquiries call collect
Tel. No.: 09897-3-1515

The Department of Obstetrics/Gynecology at the University of Missouri-Kansas City School of Medicine is seeking a board certified or active candidate in obstetrics/gynecology to function as a faculty member. Responsibilities will be split between tertiary level Truman Medical Center-West and Truman Medical Center-East, a Family Practice Residency training hospital. He or she will participate in patient care, education of residents and medical students, and clinical research. Compensation and academic rank are competitive and contingent upon experience.

Please call or send curriculum vitae to:

James Youngblood, M.D.
Chairman Department of Obstetrics/Gynecology
Truman Medical Center
2301 Holmes
Kansas City, MO 64108
(816) 556-3454

or

Bennett Walstatter, M.D., Chief, OB/GYN Services
Truman Medical Center-East
7900 Lee's Summit Road
Kansas City, MO 64139
(816) 373-6228

UNIVERSITY OF ILLINOIS AT CHICAGO ASSISTANT/ASSOCIATE PROFESSOR POSITIONS

The Department of Obstetrics and Gynecology is recruiting a Board eligible/certified general Obstetrician-Gynecologist. This position involved patient care, teaching students and residents and opportunities for research.

Faculty position also available for Board eligible/certified Oncologist. Position involves patient care, teaching medical students, residents and fellows, as well as research opportunities to advance information base of the specialty.

Excellent benefits are offered including liability coverage, medical and dental coverage, and vacation and sick leave. Academic salary and rank will depend on creditials.

Send C.V. to:

Antonio Scommegna, M.D.
Professor and Head
Department of Obstetrics and Gynecology
University of Illinois College of Medicine
840 South Wood Street
Chicago, Illinois 60612

An affirmative action/equal opportunity employer

Practice in beautiful Ithaca, NY

All of the advantages and none of the headaches of a solo practice! BC/BE OB/GYN needed for our new comprehensive Ob/Gyn Service. We have a reputation for quality care and excellent management. You'll be free to practice medicine without the pressures of administrative, personnel and financial worries. Competitive salary, excellent benefits including malpractice, new facility. Beautiful Finger Lakes region, home of Cornell Univ. Begin Fall 1990. Send CV to Executive Director, Planned Parenthood of Tompkins County, 314 W. State St., Ithaca, NY 14850.

Planned Parenthood®

of Tompkins County

Generalist in OB-GYN

We have an opportunity for a generalist in obstetrics and gynecology to join the full time faculty of the University of Rochester Department of Obstetrics and Gynecology at Rochester General Hospital. Rochester General is a 540 bed hospital, located in upstate New York. We serve women from the inner city and suburban areas with a successful mix of privately insured and medicaid patients. Our recently opened Birth Center is the first phase of our master plan to modernize our labor and delivery and surgery facilities. The successful candidate will have a commitment to clinical excellence and patient teaching. The teaching of residents and medical students requires a solid knowledge of general obstetrics and gynecology. Excellent communication and interpersonal skills are essential.

The University of Rochester offers an excellent salary and benefit package commensurate with academic credentials and clinical experience. Board certified or eligible individuals are requested to submit your curriculum vitae in confidence to:

Phyllis C. Leppert, CNM, MD, PhD
Chief of Obstetrics & Gynecology
Rochester General Hospital
1425 Portland Avenue
Rochester, New York 14621

The University of Rochester is an equal opportunity employer.

MATERNAL-FETAL MEDICINE (Board eligible or Certified)

600 Bed University-affilited Level III Hospital with 3,600 deliveries/year, 24-hour in-house Anesthesiology and Neonatology/large NICU, active maternal transfer service, perinatal outreach programs, and fully-approved OB/GYN residency program has immediate opening for a full-time position in Perinatology. Duties to include residency teaching, maternal transfer care, and private practice incentive. Competitive guaranteed salary plus incentive. Excellent public school system, housing, and Lake St. Clair in very close proximity.

CALL COLLECT: 313-343-3495
MINU KASHEF, M.D.
ST. JOHN HOSPITAL
DEPT. OB/GYN
GROSSE POINTE, MI 48236

Academic Position
In Reproductive Endocrinology and Infertility

The University of Calgary Faculty of Medicine invites applications for an academic position in the Department of Obstetrics and Gynaecology. The successful applicant will also receive an appointment at the Foothills Hospital, southern Alberta's major referral and teaching hospital.

Interested applicants should be qualified in obstetrics and gynaecology and hold the Fellowship of the Royal College of Physicians and Surgeons of Canada, or an equivalent recognized certification, and possess proven ability and experience in the area of Reproductive Endocrinology and Infertility.

Responsibilities will include teaching and patient care of the undergraduate and postgraduate level, in addition to directing research within the area and collaborating with an established Reproductive Medicine Research Group. In addition to basic and clinical research, the successful applicant will be involved in an active in-vitro fertilization program. Rank and salary will be commensurate with experience and qualifications.

In accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada. The University of Calgary has an Employment Equity Program and encourages applications from all qualified candidates, including women, aboriginal people, visible minorities, and people with disabilities.

Please submit a curriculum vitae and the names and addresses of three referees in confidence by December 31, 1989 to:

Dr. J.F. Jarrell
Professor and Head
Department of Obstetrics & Gynaecology
1403 - 29 Street N.W.
Calgary, Alberta, Canada T2N 2T9

THE UNIVERSITY OF CALGARY

GYNECOLOGIC ONCOLOGIST

The Department of Obstetrics and Gynecology, Baystate Medical Center, Springfield, Massachusetts, is seeking a third full time Gynecologic Oncologist to join their regional program.

Designated as the Western Campus of Tufts University School of Medicine, there are excellent opportunities for student and resident education, outreach programs and clinical research.

Salary and academic rank will be commensurate with credentials and experience. Please send curriculum vitae to: John L. Powell, M.D., Chief, Gynecologic Oncology, BAYSTATE MEDICAL CENTER, 759Chestnut Street, Springfield, Massachusetts, 01199. Or, call (413) 784-5542. We are an Equal Opportunity Employer.

Baystate Medical Center

The Western Campus of Tufts University School of Medicine.

DIRECTOR OF MATERNAL AND FETAL MEDICINE

University of Illinois
College of Medicine at Chicago and
Michael Reese Hospital and Medical Center

The University of Illinois College of Medicine at Chicago, and Michael Reese Hospital and Medical Center invite applications and nominations for the position of Director of Maternal and Fetal Medicine in the Department of Obstetrics and Gynecology. The Director will lead the Obstetric program of two of Chicago's most important medical institutions, both with a long history of academic excellence and outstanding patient care.

Candidates should be certified in Maternal/Fetal Medicine, have substantial scholarly productivity in the field, and have ability to provide dynamic leadership in administering a comprehensive program of patient care, education, and research in Obstetrics.

Interested individuals should send a curriculum vitae to:

Antonio Scommegna, M.D.
Professor and Head, Department
of Obstetrics and Gynecology
University of Illinois
College of Medicine at Chicago
840 South Wood Street
Chicago, IL 60612

The University of Illinois at Chicago is an affirmative action/equal opportunity employer.

OB/GYN CHIEF
PACIFIC NORTHWEST

The Department of Obstetrics and Gynecology at Emanuel Hospital and Health Center in Portland, Oregon is currently accepting applications for the position of Chief of Obstetrics and Gynecology. This hospital-based position is in a 554 bed tertiary hospital in the beautiful Pacific Northwest, sixty minutes from the Oregon Coast, Cascade Mountains and Columbia River Gorge. Applicants must be board certified in OB/GYN, have administrative, clinical, teaching, and research experience. For confidential consideration, please send CV to:

Lorraine Bivalec, Vice President,
Womens & Childrens Division
Emanuel Hospital & Health Center
2801 N. Gantenbein
Portland, OR 97227

Perinatologist

The Section of Obstetrics-Perinatal Medicine in the Department of Obstetrics and Gynecology at the **University of Wisconsin—Madison**, seeks a board-certified or eligible Perinatologist.

Faculty appointment will include duties such as acting as attending physician on the high-risk pregnancy service. Medical student, resident and outreach instruction are essential duties. Special emphasis is on prenatal diagnosis and genetics. An active research interest in some aspect of perinatal medicine is necessary, with preference given to research in prenatal diagnosis and/or clinical genetics. Send CV and application to: **Chester B. Martin, Jr., M.D., UNIVERSITY OF WISCONSIN—Madison, H4/654 Clinical Science Center, 600 Highland Avenue, Madison, WI 53792.**

UNIVERSITY OF
WISCONSIN-MADISON
MEDICAL SCHOOL

The University of Wisconsin is an Equal Opportunity/Affirmative Action Employer.

Life.

It's our life's work.

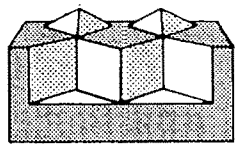
If your mission in life is to practice the ideals of medicine without administrative demands, we invite your inquiry.

Group Health Plan in St. Louis is a staff model HMO that promotes the ideals of private practice while freeing doctors from the financial baggage of the private practitioner.

Group Health Plan isn't for every physician. Just for those few who want to practice medicine without compromise.

For an interview please talk with Tim Walton at (314) 993-5955. Or write Group Health Plan, 11475 Olde Cabin Road, St. Louis, Mo. 63141. An equal opportunity employer.

GROUP HEALTH PLAN



The Mount Sinai Medical Center of New York

Perinatologist

Excellent opportunity for a board-certified perinatologist to join the Department of Obstetrics and Gynecology of the City Hospital at Elmhurst, an affiliate of the Mount Sinai Hospital and School of Medicine. Faculty rank will be granted according to experience. We offer a highly competitive salary and benefit package.

Send curriculum vitae to Richard L. Berkowitz, MD, Professor and Chairman, The Mount Sinai Medical Center and The Mount Sinai School of Medicine, Department of Obstetrics, Gynecology and Reproductive Science, 1176 Fifth Avenue, Box 1170, New York, NY 10029-6574. An Equal Opportunity Employer.

MOUNT SINAI.

TAKE GOOD CARE OF YOUR CAREER.

Michael Reese Health Plan

The **Michael Reese Health Plan,** a large well-established HMO serving its members in 20 Health Centers throughout the Chicago Metro area, is seeking additional Board Certified/Eligible physicians in the following specialty:

Obstetrics Gynecology

Physicians with the Health Plan enjoy a full practice with modern facilities and equipment as well as opportunities for teaching.

A highly-competitive salary and excellent benefits complement a rewarding practice and lifestyle.

Interested applicants should address inquiries to:

Scott P. Smith, M.D.
MICHAEL REESE HEALTH PLAN
2545 Dr. Martin Luther King Dr.,
Chicago 60616
or call (312) 808-4551

an equal opportunity employer m/f/h

TUFTS UNIVERSITY SCHOOL OF MEDICINE

The Department of OB/GYN has the following full-time faculty positions available at the Assistant Professor level:

1. General OB/GYN - Candidate should have interests in teaching at both student and resident levels. Interest in research and administration desirable.
2. Reproductive Endocrinology - Candidate should have both clinical and research interests.

Interested candidates should address inquires to:

Robert Kennison, M.D., Acting Chairman
Department of Obstetrics
and Gynecology
750 Washington Street, Box #324
Boston, MA 02111
(617) 956-6734

FELLOWSHIP

Perinatal Genetics Fellowship Opportunity. The Department of Obstetrics and Gynecology, Section of Genetics, Pennsylvania Hospital, is pleased to announce the establishment of a fellowship in Clinical Genetics. This training program is open to individuals who will have completed a residency in Obstetrics and Gynecology and who wish to pursue either a three year combined fellowship in Clinical Genetics and Maternal-Fetal Medicine or a two year fellowship in Clinical Genetics. This program meets all the requirements for certification by The American Board of Medical Genetics and offers extensive clinical and research experience in obstetrical, neonatal and pediatric genetics as well as metabolism, prenatal diagnosis, fetal therapy, teratology, ultrasonography and cytogenetics. Interested applicants should send their C.V. and the names of three references to:

Alan E. Donnenfeld, M.D.
Section of Genetics
Department of Obstetrics and Gynecology
7th Floor Spruce Building
Pennsylvania Hospital
Eighth and Spruce Streets
Philadelphia, PA 19107
phone (215) 829-5633.

An equal opportunity employer.

OB-GYN

The University of Rochester has expanded its residency program to include Rochester General Hospital. Located in northern Rochester in upstate New York near lakes and other recreational areas. Rochester General is a 540 bed hospital. We currently have a position for a board certified or eligible obstetrician-gynecologist with a major interest in state-of-the-art gyn laparoscopy to join the full time faculty. The position requires strong communication and interpersonal skills and excellent teaching ability. It is anticipated that the successful applicant will provide leadership in the development of ambulatory gyn surgery and contribute to the scientific literature.

The University of Rochester offers an excellent salary and benefits package commensurate with academic credentials and clinical experience.

For consideration please submit your curriculum vitae in confidence to:

Phyllis C. Leppert, CNM, MD, PhD
Chief of Obstetrics & Gynecology
Rochester General Hospital
1425 Portland Avenue
Rochester, New York 14621

The University of Rochester is an equal opportunity employer.

Control Medical Problems More Effectively *And Decrease Risks!*

NEW!

PERIOPERATIVE MANAGEMENT OF THE SURGICAL PATIENT

By Michael J. Breslow, M.D.; Clair F. Miller, M.D.; and Mark C. Rogers, M.D.

Controlling medical problems in surgical patients may well be one of your biggest challenges. Practical and succinct, this new book presents a rational approach to monitoring and managing surgical patients.

- *examines the effects of anesthesia and surgical stress on the patient to keep you informed of potential problems*
- *reviews specific medical problems associated with increased perioperative morbidity and mortality*
- *identifies controversial areas and reviews the appropriate literature to help you know how to best handle the problem*
- *addresses ambulatory care including specific considerations, increased risk factors associated with patient evaluation and medical protocols*

September, 1989. Approx. 400 pages, 150 illus. (Book Code: **05049**) Price, about **$62.95** (U.S.); $82.00 (Can.)

Order by phone for faster service!
Call FREE: 800-221-7700, ext. 15A.
Our ordering line is open 24 hours, 7 days a week. In Canada, call collect: 416-298-2465.

FLR-355-031

The C.V. Mosby Company
11830 Westline Industrial Dr.
St. Louis, MO 63146

The C.V. Mosby Company, Ltd.
5240 Finch Avenue East
Scarborough, Ontario
Canada M1S 5A2

Mosby

YES! Send____copies of **PERIOPERATIVE MANAGEMENT OF THE SURGICAL PATIENT** by Breslow, Miller and Rogers **(05049)** on 30-day approval. Price, about **$62.95** (U.S.); $82.00 (Can.)
SAVE MONEY! Enclose a check or charge to your credit card. Mosby pays the shipping and handling charges on all prepaid orders.
☐ Payment enclosed Charge: ☐ MasterCard ☐ VISA

Card # _____ Exp. date _____
Signature _____
Phone (____) _____

☐ Bill me, plus shipping and handling
☐ Add me to your mailing list (No purchase necessary)

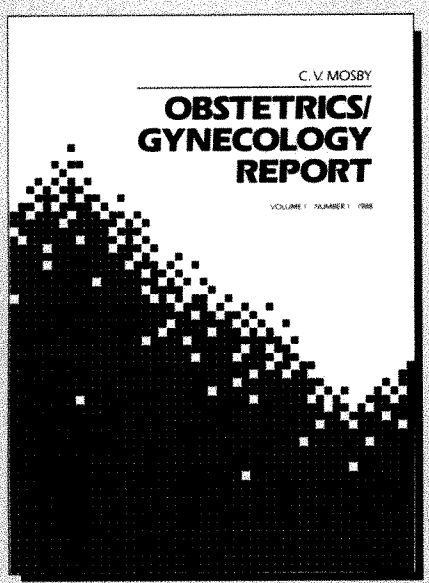
Name _____
Address _____
City _____ State____ Zip _____
Specialty _____

ALL PRICES SUBJECT TO CHANGE. Add applicable sales tax.
30 day approval good in U.S. and Canada. Prices slightly higher outside U.S. Outside the U.S. and Canada, please pay by check or money order in dollar equivalent.
IMPORTANT: If using a Purchase Order, please send it with this coupon to Dennis Carson at the Mosby address for proper processing.

FLR-355-031-03

Current
Comprehensive
Convenient

Now published quarterly!



Editor in Chief:
Frederick P. Zuspan, MD

Editors:
Richard C. Bump, MD
William T. Creasman, MD
Philip J. DiSaia, MD
Patrick Duff, MD
Steven G. Gabbe, MD
Thomas J. Garite, MD
Ronald S. Gibbs, MD
Mary G. Hammond, MD
Glenn Hurt, MD
Mark B. Landon, MD
Edward J. Quilligan, MD
Luther M. Talbert, MD

Detach order form and mail today.
The C.V. Mosby Company
Journal Circulation Department
11830 Westline Industrial Drive
St. Louis, MO 63146 U.S.A.

(314) 872-8370, ext. 394
or 1-800-325-4177, ext. 394

Mosby

OBSTETRICS/GYNECOLOGY REPORT

The depth of texts, the timeliness of journals . . .

OBSTETRICS/GYNECOLOGY REPORT is designed with the **busy practitioner** in mind. It identifies the most important clinical advances and issues in your field and synthesizes **for you** the current, relevant information on these topics.

- Four issues every 12 months.
- Each issue focuses on two topics of current clinical interest.
- Subspecialty areas covered by the REPORT include: infectious disease, reproductive endo-fertility, maternal-fetal medicine, oncology, gynecologic urology, and general obstetrics and gynecology.
- Comprehensive coverage of the selected topics, from new developments to cases of interest.
- Articles written by leaders in the field.
- 450 pages per year; no advertisements.

Our editorial board of prominent physicians is committed to offering you an insightful, reliable, and practical resource that is truly beneficial to clinical practice.

OBSTETRICS/GYNECOLOGY REPORT offers you:

- coverage of clinical advances in the specialty
- opinions and consultations from experts in the specialty
- discussions of controversial issues
- case studies
- information on new techniques, products, and equipment
- reviews of current literature

Risk-free guarantee for all subscribers: Give your first issue of the REPORT a thorough review. If you are not completely satisfied, you may cancel your subscription within 30 days and receive a full refund!

☐ YES! Please start my one-year subscription to OBSTETRICS/GYNECOLOGY REPORT with:
☐ the most recent issue ☐ other (volume, number) _____

Check applicable rate:
☐ $54.00 U.S. ☐ $60.00 Canada and International

Payment Method: OBR-009
☐ Check (U.S. funds, payable to OBSTETRICS/GYNECOLOGY REPORT)
☐ Bill me (U.S. and Canadian orders only)
☐ MasterCard ☐ MC International ☐ VISA
Card # _____ Exp. date _____
Signature _____

Name (print legibly) _____
Address _____ Apt/Suite _____
City _____ State/Prov. _____
ZIP/PC _____ Country _____

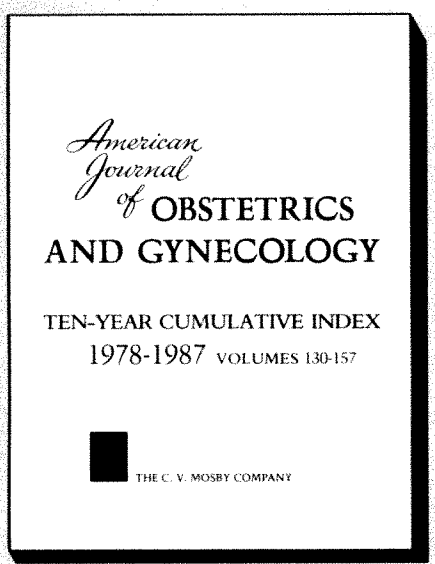


PLEASE!

ASK ABOUT DRUG ABUSE,
BEFORE IT'S TOO LATE.

PARTNERSHIP FOR A DRUG-FREE AMERICA

Your comprehensive reference guide to a decade of valuable clinical information . . .



Ten-Year Cumulative Index (1978-1987) to the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY

Now you have easy access to 10 years of clinical information, indexed in one convenient source — the Ten-Year Cumulative Index to the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY.

This hard-cover index is a comprehensive guide to more than 30,000 pages of original articles, case reports, letters, and editorials published in the Journal from 1978 through 1987 (volumes 130 to 157). These influential, peer-reviewed papers are indexed both by subject and by author in this 800-page volume.

The Cumulative Index features:

- 4,331 subject headings, containing 24,126 references. Each subject entry lists the complete article title, author(s), volume, page, and year of publication.
- 22,456 author entries, listing contributor, respective article title, author-to-author referral, volume, page, and publication date.

The Cumulative Index is a great time-saver for locating information on clinical topics of immediate or historical significance in obstetrics and gynecology. There's no need to search for index issues from each year.

Order this important reference guide for your personal library!

YES! Please send me the Ten-Year Cumulative Index to the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY (1978-1987). I've indicated my method of payment below.

☐ $64.50 U.S. ☐ $69.00 International
(prices include shipping)

Payment must accompany your order.

☐ Check enclosed (U.S. funds, drawn on a U.S. bank, payable to The C.V. Mosby Company)
☐ MasterCard ☐ VISA

Card # _____ Exp. date _____

Signature _____

Name (print legibly) _____

Address _____

Apt/Suite/Dept _____

City _____ State _____

ZIP/PC _____ Country _____

Please allow six weeks for delivery. **ABS-262**

Return to:
The C.V. Mosby Company
Journal Circulation Department
11830 Westline Industrial Drive
St. Louis, MO 63146 U.S.A.

Mosby

INDEX TO ADVERTISERS

Baystate Medical Center
Opportunities Available 48A,52A

Central Minnesota Group Health Plan
Opportunity Available 48A

Columbia Labs
Replens 35A,36A,37A,38A

Daniel Stern & Associates
Opportunity Available 49A

Desert Hospital
Opportunity Available 43A

Emanuel Hospital & Health Center
Opportunity Available 52A

Family Care Center of Carondelet
Opportunity Available 49A

Freehold Area Hospital
Opportunity Available 45A

Group Health Cooperative
Opportunity Available 45A

Group Health Plan
Opportunity Available 53A

Hartford Hospital
Opportunity Available 45A

Humana Inc.
Opportunity Available 43A

Johns Hopkins Health System
Opportunity Available 43A

Johnson & Johnson Patient Care, Inc.
Interceed 39A,40A,41A,42A

Kaiser Permanente
Opportunity Available 44A

Kaiser Permanente Medical Center
Opportunity Available 46A,47A

Laconia Clinic
Opportunity Available 44A

Locum Tenens, Inc.
Opportunity Available 44A

Lutheran General Hospital
Opportunity Available 44A

Medical West
Opportunity Available 47A

MetroHealth Medical Center
Opportunity Available 47A

Medical Center of Delaware
Opportunity Available 45A

Mount Sinai
Opportunity Available 53A

Northwestern University
Opportunity Available 46A

Ohio Permanente Medical Group, Inc.
Opportunity Available 46A

Ortho Pharmaceutical Corporation
All-Flex 4A
Ortho-Novum 7/7/7 ..... Third Cover, Fourth Cover

Pennsylvania Hospital
Opportunity Available 48A,54A

Permanente Medical Group, Inc.
Opportunity Available 49A

Permanente Medical Group, Northern California
Opportunity Available 6A

Planned Parenthood
Opportunity Available 51A

Rochester General Hospital
Opportunity Available 51A,54A

Saratoga Hospital
Opportunity Available 31A

S.U.N.Y Health Science Center
Opportunity Available 47A,50A

Syntex Laboratories, Inc.
Femstat 32A,33A,34A
Synarel 22A,23A
Tri-Norinyl ..... Second Cover,1A,2A

Truman Medical Center-East
Opportunity Available 50A

Tufts University School of Medicine
Opportunity Available 53A

University of Hawaii
Opportunity Available 50A

University of Illinois College of Medicine
Opportunity Available 50A

University of Illinois College of Medicine at Chicago
Opportunity Available 52A

University of Miami
Opportunity Available 49A

University of Wisconsin Medical School
Opportunity Available 48A

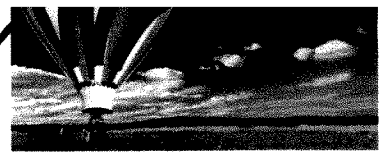
University of Wisconsin-Madison Medical School
Opportunity Available 52A

Wyeth-Ayerst Laboratories
Premarin 18A,19A,20A

While every precaution is taken to insure accuracy, we cannot guarantee against the possibility of an occasional change or omission in the preparation of this index.

21- and 28-day regimens

ORTHO-NOVUM® 777 Tablets Spectacular!



norethindrone/ethinyl estradiol

Each white tablet contains 0.5 mg norethindrone and 0.035 mg ethinyl estradiol. Each light peach tablet contains 0.75 mg norethindrone and 0.035 mg ethinyl estradiol. Each peach tablet contains 1 mg norethindrone and 0.035 mg ethinyl estradiol. Each green tablet in 28 day regimen contains inert ingredients.

IMPORTANT NOTE—This information is a BRIEF SUMMARY of the complete prescribing information provided with the product and therefore should not be used as the basis for prescribing the product. This summary was prepared by deleting from the complete prescribing information certain text, tables and references. The physician should be thoroughly familiar with the complete prescribing information before prescribing the product.

INDICATIONS AND USAGE: PREVENTION OF PREGNANCY.

CONTRAINDICATIONS: Oral contraceptives should not be used in women who currently have the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Carcinoma of the endometrium or other known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Cholestatic jaundice of pregnancy or jaundice with prior pill use. 8. Hepatic adenomas or carcinomas. 9. Known or suspected pregnancy.

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia, and gallbladder disease, although the risk of serious morbidity or mortality is very small in healthy women without underlying risk factors. The risk of morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, obesity and diabetes. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks. The information contained in this brief summary is principally based on studies carried out in patients who used oral contraceptives with higher formulations of estrogens and progestogens than those in common use today. The effect of long term use of the oral contraceptives with lower formulations of both estrogens and progestogens remains to be determined. Throughout this brief summary epidemiological studies reported are of two types: retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of a disease, namely a *ratio* of the incidence of a disease among oral contraceptive users to that among nonusers. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide a measure of attributable risk, which is the *difference* in the incidence of disease between oral contraceptive users and nonusers. The attributable risk does provide information about the actual occurrence of a disease in the population. For further information, the reader is referred to a text on epidemiological methods. 1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. a. Myocardial Infarction. An increased risk of myocardial infarction has been associated with oral contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary artery disease such as hypertension, hypercholesterolemia, morbid obesity, and diabetes. The relative risk of heart attack for current oral contraceptive users has been estimated to be two to six. The risk is very low under the age of 30. Smoking in combination with oral contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women in their mid-thirties or older with smoking accounting for the majority of excess cases. Mortality rates associated with circulatory disease have been shown to increase substantially in smokers, especially in those 35 years of age and older among women who use oral contraceptives. Oral contraceptives may compound the effects of well-known risk factors, such as hypertension, diabetes, hyperlipidemias, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and cause glucose intolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see section 9 in WARNINGS). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors. b. Thromboembolism. An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to non-users to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. Cohort studies have shown the relative risk to be somewhat lower, about 3 for new cases and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease associated with oral contraceptives is not related to length of use and disappears after pill use is stopped. A two- to four-fold increase in relative risk of post-operative thromboembolic complications has been reported with the use of oral contraceptives. The relative risk of venous thrombosis in women who have predisposing conditions is twice that of women without such medical conditions. If feasible, oral contraceptives should be discontinued at least four weeks prior to and for two weeks after elective surgery of a type associated with an increase in risk of thromboembolism and during and following prolonged immobilization. Since the immediate postpartum period is also associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than four weeks after delivery in women who elect not to breast feed. c. Cerebrovascular diseases. Oral contraceptives have been shown to increase both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes), although, in general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and non-users, for both types of strokes, and smoking interacted to increase the risk of stroke. In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for non-smokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk is also greater in older women. d. Dose-related risk of vascular disease from oral contraceptives. A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high density lipoproteins (HDL) has been reported with many progestational agents. A decline in serum high density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the activity of the progestogen used in the contraceptive. The activity and amount of both hormones should be considered in the choice of an oral contraceptive. Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral contraceptive agents should be started on preparations containing 0.035 mg or less of estrogen. e. Persistence of risk of vascular disease. There are two studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40-49 years who had used oral contraceptives for five or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. However, both studies were performed with oral contraceptive formulations containing 50 micrograms or higher of estrogens. 2. ESTIMATES OF MORTALITY FROM CONTRACEPTIVE USE. One study gathered data from a variety of sources which have estimated the mortality rate associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is low and below that associated with childbirth. However, smokers 35 and older and non-smokers 40 and older who use oral contraceptives have a significant increase in mortality higher than those using other methods of birth control. These facts must be weighed in conjunction with failure rates for other methods and the risk associated with subsequent pregnancy. 3. CARCINOMA OF THE REPRODUCTIVE ORGANS. Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. While there are conflicting reports most studies suggest that use of oral contraceptives is not associated with an overall increase in the risk of developing breast cancer. Some studies have reported an increased relative risk of developing breast cancer, particularly at a younger age. This increased relative risk appears to be related to duration of use. Some studies suggest that oral contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors. 4. HEPATIC NEOPLASIA. Benign hepatic adenomas are associated with oral contraceptive use, although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases/100,000 for users, a risk that increases after four or more years of use especially with oral contraceptives of higher dose. Rupture of benign, hepatic adenomas may cause death through intra-abdominal hemorrhage. Studies from Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (>8 years) oral contraceptive users. However, these cancers are rare in the U.S. and the attributable risk (the excess incidence) of liver cancers in oral contraceptive users approaches less than one per million users. 5. OCULAR LESIONS. There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately. 6. ORAL CONTRACEPTIVE USE BEFORE OR DURING EARLY PREGNANCY. Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. The majority of recent studies also do not indicate a teratogenic effect, particularly in so far as cardiac anomalies and limb reduction defects are concerned, when taken inadvertently during early pregnancy. The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion. It is recommended that for any patient who has missed two consecutive periods pregnancy should be ruled out before continuing oral contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral contraceptive use should be discontinued until pregnancy is ruled out. 7. GALLBLADDER DISEASE. Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral contraceptive formulations containing lower hormonal doses of estrogens and progestogens. 8. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. Oral contraceptives have been shown to cause a decrease in glucose tolerance in a significant percentage of users. This effect has been shown to be directly related to estrogen dose. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the non-diabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects prediabetic and diabetic women in particular should be carefully monitored while taking oral contraceptives. A small proportion of women will have persistent hypertriglyceridemia while on the pill. As discussed earlier (see WARNINGS 1a and 1d), changes in serum triglycerides and lipoprotein levels have been reported in oral contraceptive users. 9. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in women taking oral contraceptives, and this increase is more likely in older oral contraceptive users and with extended duration of use. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing progestational activity. Women with a history of hypertension or hypertension-related diseases, or renal disease, should be encouraged to use another method of contraception. If women elect to use oral contraceptives, they should be monitored closely and if significant elevation of blood pressure occurs, oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives, and there is no difference in the occurrence of hypertension between former and never users. 10. HEADACHE. The onset or exacerbation of migraine or development of headache with a new pattern which is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause. 11. BLEEDING IRREGULARITIES. Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first three months of use. Non-hormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out. Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was preexistent. 12. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. **PRECAUTIONS:** 1. PHYSICAL EXAMINATION AND FOLLOW UP. A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology, and relevant laboratory tests. In case of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care. 2. LIPID DISORDERS. Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult. 3. LIVER FUNCTION. If jaundice develops in any woman receiving such drugs, the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function. 4. FLUID RETENTION. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention. 5. EMOTIONAL DISORDERS. Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 6. CONTACT LENSES. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist. 7. DRUG INTERACTIONS. Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar association, though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines. 8. INTERACTIONS WITH LABORATORY TESTS. Certain endocrine and liver function tests and blood components may be affected by oral contraceptives: a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. b. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radio-immunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. c. Other binding proteins may be elevated in serum. d. Sex-binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged. e. Triglycerides may be increased. f. Glucose tolerance may be decreased. g. Serum folate levels may be depressed by oral contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives. 9. CARCINOGENESIS. See WARNINGS section 10. PREGNANCY. Pregnancy Category X. See CONTRAINDICATIONS and WARNINGS sections. 11. NURSING MOTHERS. Small amounts of oral contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child. **INFORMATION FOR THE PATIENT: See Patient Package Insert. ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS section). Thrombophlebitis and venous thrombosis with or without embolism. Arterial thromboembolism. Pulmonary embolism. Myocardial infarction. Cerebral hemorrhage. Cerebral thrombosis. Hypertension. Gallbladder disease. Hepatic adenomas or benign liver tumors. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea. Vomiting. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Amenorrhea. Temporary infertility after discontinuation of treatment. Edema. Melasma which may persist. Breast changes: tenderness, enlargement, secretion. Change in weight (increase or decrease). Change in cervical erosion and secretion. Diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted: Pre-menstrual syndrome. Cataracts. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. Acne. Changes in libido. Colitis. **OVERDOSAGE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea and withdrawal bleeding may occur in females.

Revised 9/89

ORTHO PHARMACEUTICAL CORPORATION
Raritan, NJ 08869-0602

ORTHO


Spectacular!
777
†Serious as well as minor side effects have been reported with the use of oral contraceptives. The physician should remain alert to the earliest manifestations of any symptoms of serious disease and discontinue oral contraceptive therapy when appropriate.
Please see complete Prescribing Information, a brief summary of which appears on the preceding page.
© OPC 1989
OJ-709R
Low breakthrough bleeding†
Low-impact norethindrone—no significant change in mean values for total cholesterol, HDL, and LDL
For OC patients of all ages
21- and 28-day regimen
ORTHO-NOVUM® 777
norethindrone/ethinyl estradiol
Table
ORTHO
ORTHO PHARMACEUTICAL CORPORATION
Raritan, New Jersey 08869-0602

11.12.90

December 1989
in two parts, part 2
volume 161, *number* 6

SUPPLEMENT TO

American Journal of OBSTETRICS AND GYNECOLOGY

Copyright © 1989 by The C.V. Mosby Company

INTERDISCIPLINARY REVIEW OF ESTROGEN REPLACEMENT THERAPY

Guest Editor
Daniel R. Mishell, Jr., MD

Supported by an educational grant from
ABBOTT LABORATORIES

SUNY HEALTH SCIENCE CENTER



Published by
THE C.V. MOSBY COMPANY
St. Louis, Missouri 63146

ISSN 0002-9378

American Journal of OBSTETRICS AND GYNECOLOGY

Copyright © 1989 by The C.V. Mosby Company

INTERDISCIPLINARY REVIEW OF ESTROGEN REPLACEMENT THERAPY

Guest Editor
Daniel R. Mishell, Jr., MD

Supported by an educational grant from
ABBOTT LABORATORIES

Mosby

American Journal of Obstetrics and Gynecology

Founded in 1920

Copyright © 1989 by The C.V. Mosby Company

Contents

December
Part 2
1989

Estrogen replacement therapy: An overview 1825
Daniel R. Mishell, Jr., MD
Los Angeles, California

Because supplementation with exogenous estrogen mitigates or prevents the undesirable and often life-limiting sequelae of estrogen deficiency, nearly all postmenopausal women should receive estrogen replacement therapy.

Biosynthesis and physiologic effects of estrogen and pathophysiologic effects of estrogen deficiency: A review 1828
Wulf H. Utian, MD, PhD
Cleveland, Ohio

Postmenopausal women experience various health problems, such as cardiovascular disease, osteoporosis, neuroendocrine changes, and urogenital atrophy because of lack of estrogen production.

Estrogen replacement therapy: Indications, contraindications, and agent selection 1832
Morris Notelovitz, MD
Gainesville, Florida

Estrogens relieve deficiency-related symptoms, decrease the rate of fractures, and confer cardioprotection for postmenopausal women and have therefore proved beneficial to selected postmenopausal women.

Estrogens in the prevention of osteoporosis in postmenopausal women 1842
Harry K. Genant, MD, David J. Baylink, MD, and J. C. Gallagher, MD
San Francisco and Loma Linda, California, and Omaha, Nebraska

Estrogen replacement therapy started at menopause prevents bone loss and reduces the risk of fractures associated with menopause.

(Continued on page 4A)

Vol. 161, No. 6, Part 2, December 1989. The American Journal of Obstetrics and Gynecology (ISSN 0002-9378) is published monthly (six issues per volume, two volumes per year) by The C.V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318. Second-class postage paid at St. Louis, Missouri, and additional mailing offices. POSTMASTER: Send address changes to The C.V. Mosby Company, 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318, (314) 872-8370, ext. 351. Annual subscription rates for 1990: domestic, $71.50 for individuals and $130.00 for institutions. Printed in the U.S.A. Copyright © 1989 by The C.V. Mosby Company.

Contents continued from page 3A

Estrogen replacement and cardiovascular disease: Serum lipids and blood pressure effects 1847
William R. Hazzard, MD
Winston-Salem, North Carolina

Estrogen use by postmenopausal women significantly improves lipoprotein profiles and has been associated with lower mobidity and all-cause mortality rates.

Compliance considerations with estrogen replacement: Withdrawal bleeding and other factors 1854
Ricardo G. Hahn, MD
Memphis, Tennessee

Education is critical to the postmenopausal woman's balanced view of the benefits and risks associated with her use of estrogen and her compliance with such a regimen.

The cancer question: An overview of recent epidemiologic and retrospective data 1859
Brian E. Henderson, MD
Los Angeles, California

Despite the potential risk of malignancies associated with postmenopausal estrogen use, survival in this population is prolonged by estrogen replacement therapy.

Estrogen replacement therapy: What the future holds 1864
Charles B. Hammond, MD
Durham, North Carolina

Several areas of research remain to refine our understanding and optimal use of hormonal replacement therapy in postmenopausal women.

1990 Annual subscription rates	*U.S.A.*	*International (surface mail)*	*International (airmail)°*				
		All regions	*Canada*	*Mexico*	*Region 1*	*Region 2*	*Region 3*
Institutions†	$130.00	$156.00	$182.00	$188.25	$200.50	$242.25	$284.00
Individuals‡	$ 71.50	$ 97.50	$123.50	$129.75	$142.00	$183.75	$225.50
Students, residents‡	$ 38.00	$ 64.00	$ 90.00	$ 96.25	$108.50	$150.25	$192.00

Subscription rates include supplements. Single copies are $7.00. Remittances should be made by check, draft, or post office or express money order, in U.S. funds drawn through a U.S. bank, payable to this JOURNAL. Claims for missing issues will be serviced only within six months of cover date. Single copy prices will be charged on missing issue claims older than six months from cover date. Back issues generally are available for the previous five years. Contact the Publisher to confirm availability of specific issues.

°*Airmail breakdown—Domestic:* First-class and Priority rates for the U.S. and possessions are available upon request. *Canada:* All provinces of Canada. *Mexico:* All localities within Mexico. *Region 1:* Colombia, Venezuela, Central America, Caribbean Islands, Bahamas, Bermuda, and St. Pierre and Miquelon (also from American Samoa to Western Samoa and from Guam to the Philippines). *Region 2:* South America (except Colombia and Venezuela), Europe (except Estonia, Latvia, Lithuania, and U.S.S.R), and North Africa (Morocco, Algeria, Tunisia, Libya, and Egypt). *Region 3:* Estonia, Latvia, Lithuania, U.S.S.R., Asia, Australia, New Zealand, Pacific Ocean Islands, Africa (other than North Africa), Indian Ocean Islands, and the Middle East.

†Institutional (multiple-reader) subscriptions are available to public and private libraries, schools, hospitals, and clinics; city, county, state, provincial, and national government bureaus and departments; and all commercial and private institutions and organizations.

‡Individual subscriptions and all student-rate subscriptions must be in the names of, billed to, and paid by individuals. All student-rate requests must indicate training status and name of institution.

Subscriptions may begin at any time.

American Journal of Obstetrics and Gynecology

Founded in 1920

volume 161 *number* 6 *part* 2 DECEMBER 1989

Estrogen replacement therapy: An overview

Daniel R. Mishell, Jr., MD

Los Angeles, California

Levels of circulating estrogen fall during the perimenopausal period, resulting in changes in the postmenopausal woman's genitourinary tract, central nervous and cardiovascular systems, skin, and bone. Exogenous estrogen minimizes the benign symptoms and prevents the increased incidence of osteoporosis and atherosclerotic heart disease that accompany estrogen deficiency. Thus to avoid the recognized problems of estrogen deficiency, nearly all women should begin a lifelong course of estrogen replacement therapy during the perimenopausal period. (AM J OBSTET GYNECOL 1989;161:1825-7.)

Key words: Estrogen replacement therapy

During menopause the ovarian follicles stop secreting estradiol into the circulation. Except in obese women, sufficient amounts of estrone will not be converted from androstenedione in peripheral adipose tissue to maintain premenopausal, physiologic levels of circulating estrogen. Therefore, untreated women may experience the consequences of estrogen deficiency (as summarized below), including changes in their genitourinary tracts, central nervous and cardiovascular systems, skin, and bone, during approximately one third of their average life spans. Because supplementation with exogenous estrogen mitigates or prevents the undesirable and often life-limiting sequelae of estrogen deficiency,[1-5] nearly all postmenopausal women should receive estrogen replacement therapy. Given estrogen's high benefit-to-risk ratio in this setting, the real therapeutic challenge is to identify the few inappropriate candidates for estrogen replacement therapy who are at increased risk for unwanted, adverse effects. Once these women are identified, the remainder can use estrogen replacement therapy so that they can continue to enjoy the same quality of life they experienced before menopause.

From the Department of Obstetrics and Gynecology, University of Southern California School of Medicine.

Reprint requests: Daniel R. Mishell, Jr., MD, Department of Obstetrics and Gynecology, Womens Hospital Room 1013, 1200 N. State St., Los Angeles, CA 90033.

6/0/16681

End organ changes due to estrogen deficiency during menopause

Estrogen deficiency may manifest as changes in a woman's genital tract. The postmenopausal woman may complain of itching, bleeding, or dyspareunia, which are symptoms of atrophic vaginitis. She may also experience pruritus and pain from atrophic changes of her vulva (kraurosis vulvae). Loss of tone of supporting ligaments and elastic tissue can lead to uterine prolapse and hernias in the anterior (cystocele) and posterior (rectocele) vaginal wall. Estrogen replacement therapy can prevent the vaginal and vulvar atrophy associated with menopause and maintain the normal tone of supporting structures to the uterus and vagina.

The untreated postmenopausal woman may also experience dysuria and other symptoms associated with senile urethritis,[6] a syndrome caused by atrophy of the bladder and urethral mucosa. Subsequent alterations in urethral and bladder pressure can cause urgency and stress incontinence and urinary frequency.[7] Using exogenous estrogens either prevents or relieves the urinary tract symptoms of menopause.[8-10]

Hot flashes, the pathognomonic symptom of menopause and the one that most frequently prompts the postmenopausal woman to seek medical care, are usually acute, short-lived events that initially occur several times daily, often during sleep.[11,12] The rate of decrease in estrogen levels during the menopausal period is directly related to the severity of hot flashes, which are the body's homeostatic response to estrogen deficiency-

induced changes in central nervous system thermoregulatory centers (i.e., peripheral blood flow increases and perspiration occurs in an attempt to dissipate heat). Thus women commonly experience hot flashes during the first 2 years of menopause, after which symptoms gradually subside.

Women may experience symptoms of anxiety and depression more frequently during the menopausal period. In placebo-controlled trials, estrogen replacement therapy has proved effective in relieving hot flashes[1, 2] and symptoms of anxiety and depression.[3-5]

The absence of estrogen may cause the skin to become thinner and more wrinkled due to less collagen in the dermis. Estrogen stimulates the synthesis of collagen, a protein that makes up 90% of the dermis. In postmenopausal women receiving estrogen replacement therapy, the collagen in their dermis is maintained at premenopausal levels.[13]

Declining levels of estrogen during menopause lead to an increased rate of bone resorption and greater urinary excretion of calcium,[14] resulting in reduced bone density. Women may remain asymptomatic for about 10 years. By this time they have typically lost 15% of their bone mass,[15] setting the stage for fractures. Trabecular bone in the spine and distal radius are affected more rapidly than cortical bone in the axial skeleton. Therefore, osteoporotic hip fractures usually begin to occur around the seventh decade of life, increasing exponentially after the age of 80 years.

Postmenopausal osteoporosis affects approximately 25% (5 million) of white and Oriental women. It occurs less frequently in obese women and in blacks. Wasserman and Barzel[16] estimated that in 1987 about 300,000 hip fractures occurred in the American population, predominantly in women. The 6-month mortality rate associated with hip fractures approached 10%, and acute health care costs were estimated at $4 billion annually. One third of the patients with hip fractures required skilled nursing home care once discharged from the hospital, accounting for additional costs of $2 billion per year. Estrogen replacement therapy not only prevents loss of bone mass,[17, 18] it also reduces the risk of bone fractures[15, 19] to a rate comparable to that in men matched for age.

Oral estrogen has a cardioprotective effect, most likely because it increases high-density lipoprotein cholesterol and reduces low-density lipoprotein cholesterol levels.[20] Estrogen replacement therapy reduces the incidence of myocardial infarction in postmenopausal women and prolonges their life span.[21] In numerous case-control and cohort studies, estrogen users have a crude and adjusted relative risk of cardiovascular disease approximately half that of their untreated counterparts. These data have been further corroborated in a multicenter trial in which the use of estrogens significantly reduced the cardiovascular disease-associated death rate per 10,000 women for each decade between the ages of 50 and 80 years; the age-adjusted relative risk of death due to cardiovascular disease in estrogen users was 0.34.[22] The relative risk of death due to stroke was also reduced by about 50% in postmenopausal women using estrogen.[23]

Estrogen replacement therapy: Benefit versus risk

The benefits of estrogen replacement therapy are clear, improving both the quality and the quantity of the postmenopausal woman's life. Several studies have shown that estrogen users are less likely to die from all causes compared with nonusers.[24, 25] Although estrogen replacement therapy's benefit is greatest among women who have had oophorectomies, those who have intact uteri also benefit.

Before we could universally recommend using this therapy in postmenopausal women, we had to resolve the question of an association between estrogen replacement therapy and cancer. Most case-control studies show that exogenous postmenopausal estrogen replacement neither increases nor reduces the risk of breast cancer.[26] Furthermore, although unopposed estrogen may cause endometrial cancer, the concomitant, appropriate use of a progestational agent not only prevents this risk but may also lower it to below that of untreated women.[27]

Contraindications to estrogen replacement therapy include acute liver disease, previously diagnosed breast or endometrial cancer, and, probably, thromboembolic disorders. Chronic liver disease, obesity, and, possibly, a history of thromboembolism are relative contraindications.

We now generally consider that the benefits of estrogen replacement therapy far outweigh its risks. To avoid the problems associated with estrogen deficiency, women should begin a lifelong course of estrogen replacement therapy during the perimenopausal period.

Summary

Estrogen replacement therapy should be considered for all postmenopausal women. The goals of estrogen replacement therapy are to minimize the benign symptoms and to reverse the predictable osteoporosis and atherosclerotic heart disease that can accompany estrogen deficiency. In this way we hope to reduce the high morbidity, mortality, and health care costs associated with the sequelae of untreated menopause.

REFERENCES

1. Tataryn IV, Lomax P, Meldrum DR, et al. Objective techniques for the assessment of postmenopausal hot flashes. Obstet Gynecol 1981;57:340-4.

2. Coope J. Double blind crossover study of estrogen replacement therapy. In: Campbell S, ed. The management of the menopause and postmenopausal years. Lancaster, England: MTP Press, 1976:159-68.
3. Campbell S, Whitehead M. Oestrogen therapy and the menopause syndrome. Clin Obstet Gynecol 1977;4:31-47.
4. Dennerstein L, Burrows GD, Hyman GJ, et al. Hormone therapy and affect. Maturitas 1979;1:247-59.
5. Sherwin BB. Affective changes with estrogen and androgen replacement therapy in surgically menopausal women. J Affect Dis 1988;14:177-87.
6. Youngblood VH, Tomlin EM, Davis JB. Senile urethritis in women. J Urol 1957;78:150-2.
7. Rud T. Urethral pressure profile in continent women from childhood to old age. Acta Obstet Gynecol 1982; 59:331-5.
8. Rud T. The effect of estrogens and gestagens on the urethral pressure profile in urinary continent and stress incontinent women. Acta Obstet Gynecol Scand 1980; 59:265-70.
9. Hilton P, Stanton SL. The use of intravaginal estrogen cream in genuine stress incontinence. Br J Obstet Gynaecol 1983;90:940-4.
10. Smith P. Postmenopausal urinary symptoms and hormonal replacement therapy. Br Med J 1976;2:941.
11. Mashchak CA, Kletzky OA, Artal R, et al. The relation of physiological changes to subjective symptoms in postmenopausal women with and without hot flushes. Maturitas 1985;6:301-8.
12. Erlik Y, Tataryn IV, Meldrum DR, et al. Association of waking episodes with menopausal hot flushes. JAMA 1981;245:1741-4.
13. Brincat M, Moniz CF, Studd JWW, et al. The long term effects of the menopause and of administration of sex hormones on skin collagen and skin thickness. Br J Obstet Gynaecol 1985;92:256-9.
14. Riggs BL, Jowsey J, Kelley PJ, et al. Effect of sex hormones on bone in primary osteoporosis. J Clin Invest 1969; 48;1065-72.
15. Ettinger B, Genant HK, Cann CE. Long-term estrogen replacement therapy prevents bone loss and fractures. Ann Intern Med 1985;102:319-24.
16. Wasserman SHS, Barzel US. Osteoporosis: the state of the art in 1987: a review. Semin Nucl Med 1987;17; 283-92.
17. Lindsay R, Hart DM, Forrest C, et al. Prevention of spinal osteoporosis in oophorectomized women. Lancet 1980; 2:1151-4.
18. Al-Azzawi, Hart DM, Lindsay R. Long term effect of estrogen replacement therapy on bone mass as measured by dual absorptiometry. Br Med J 1987;294:1261-2.
19. Weiss NS, Ure CL, Ballard JH, et al. Decreased risk of fractures of the hip and lower forearm with postmenopausal use of estrogen. N Engl J Med 1980;303:1195-8.
20. Wahl P, Walden C, Knopp R, et al. Effect of estrogen/progestin potency on lipid/lipoprotein cholesterol. N Engl J Med 1983;308:862-7.
21. Bush TL, Cowan LD, Barrett-Connor E, et al. Estrogen use and all-cause mortality. Preliminary results from the Lipid Research Clinics Program follow-up study. JAMA 1983;249:903-6.
22. Bush TL, Barrett-Connor E, Cowan LD, et al. Cardiovascular mortality and noncontraceptive use of estrogen in women: results from the Lipid Research Clinics Program follow-up study. Circulation 1988;75:1102-9.
23. Paganini-Hill A, Ross RK, Henderson BE. Postmenopausal oestrogen treatment and stroke: a prospective study. Br Med J 1988;297:519-22.
24. Mashchak CA, Lobo RA, Dozono-Takano R, et al. Comparison of pharmcodynamic properties of various formulations. Am J Obstet Gynecol 1982;144:511-8.
25. Hunt K, Vessey M, McPherson K, et al. Long-term surveillance of mortality and cancer incidence in women receiving hormone replacement therapy. Br J Obstet Gynaecol 1987;94:620-35.
26. Armstrong BK. Oestrogen therapy after the menopause—boon or bane? Med J Aust 1988;148:213-9.
27. Gambrell RD Jr, Massey FM, Castaneda TA, Boddie AW. Estrogen therapy in breast cancer in postmenopausal women. J Am Geriatr Soc 1980;28:251-7.

Biosynthesis and physiologic effects of estrogen and pathophysiologic effects of estrogen deficiency: A review

Wulf H. Utian, MD, PhD
Cleveland, Ohio

Women are increasingly spending more years of their lives beyond the menopause, which places them at risk for various health problems due to estrogen deficiency. Tissues and organs with estrogen receptors such as the ovaries, endometrium, vaginal epithelium, hypothalmus, urinary tract, and skin are directly affected by a lack of estrogen production. Other tissues in which estrogen receptors have not been consistently identified, such as bone, are also affected by waning levels of estrogen. The postmenopausal woman frequently experiences neuroendocrine changes (hot flashes) that often dissipate over time and a steady rise in her risk of cardiovascular disease, which approaches that in men of comparable age. (AM J OBSTET GYNECOL 1989;161:1828-31.)

Key words: Estrogen deficiency, menopause

In 1980, men and women aged 65 years or older represented 11.3% of the population, a percentage that is expected to increase.[1] As the average life span of the American population continues to increase, the number of women who live beyond the age of menopause is growing significantly. Currently there are about 230 million people in the United States, of whom 118 million (51%) are women. About one third of these (39 million) are 45 years old or older, and almost 13.5% (16 million) are 65 years old or older.

What do all these statistics mean? Simply stated, compared with their ancestors, more women will live a significantly increasing number of years beyond menopause, placing them at risk for various health problems that can be caused by ovarian failure and a lack of estrogen production.

The current median age of menopause is 50 years. However, approximately 8% of women undergo premature menopause before the age of 40 years.[2] During the time of the climacteric (the phase in the aging of women that marks the transition from the reproductive to the nonreproductive stage of life),[3] several months may pass between menstrual periods. This unpredictable perimenopausal menstrual pattern makes identifying the onset of menopause difficult. Therefore, the diagnosis of menopause is usually made retrospectively after a women experiences her last menstrual period.

From the Departments of Obstetrics and Gynecology and Reproductive Biology, Case Western Reserve University and University Hospitals of Cleveland.
Reprint requests: Wulf Utian, MD, PhD, Department of Obstetrics and Gynecology, University Hospitals of Cleveland, University Circle, Cleveland, OH 44106.
6/0/16773

Table I. World Health Organization definitions[4]

Menopause:	Permanent cessation of menstruation resulting from loss of ovarian function
Perimenopausal:	Climacteric; the period immediately before menopause and at least 5 years after menopause
Postmenopausal:	The period dating from the menopause, which can only be assessed in retrospect

Table I provides the World Health Organization standard definitions of the terms menopausal, perimenopausal, and postmenopausal.

Research has looked for factors that may influence the age at which menopause occurs. For instance, women who smoke may have lower serum estrogen levels than nonsmokers and, on average, may also experience menopause at an earlier age. For the most part, directly associating specific factors as a cause for early or late menopause is difficult because so many variables exist.

Physiology of menopause

As a woman ages, ovarian function begins to fail. The number of follicles in the ovaries decreases, and less estrogen is synthesized. A deficiency of follicular inhibin and estrogen leads to decreased negative feedback on the anterior pituitary gland, which results in an increase in levels of follicle-stimulating hormone and luteinizing hormone. In postmenopausal women, the amount of follicle-stimulating hormone present is greater than the amount of luteinizing hormone, which is the opposite of what is found in premenopausal women. Feedback sensitivity on the hypothala-

Table II. Potential problems of the untreated climacteric[5]

Target Organ	*Possible symptom or problem*
Vulva	Atrophy, dystrophy, pruritus vulvae
Vagina	Dyspareunia, blood-stained discharge, vaginitis
Bladder and urethra	Cystourethritis, ectropion, frequency and/or urgency, stress incontinence
Uterus and pelvic floor	Uterovaginal prolapse
Skin and mucous membranes	Atrophy, dryness, or pruritus; easily traumatized; loss of resilience and pliability; dry hair or loss of hair; minor hirsutism of face; dry mouth
Vocal cords	Voice changes (reduction in upper register)
Cardiovascular system	Atherosclerosis, angina, and coronary heart disease
Skeleton	Osteoporosis with related fractures, backache
Breasts	Reduced size, softer consistency, drooping
Neuroendocrine system	Hot flashes, psychologic disturbances

mus and anterior pituitary glands may also decrease (Fig. 1).

The predominant estrogen in premenopausal women is estradiol, which is produced primarily by the ovary. Estrone, a less active form of estrogen, is the predominant form in postmenopausal women. Most postmenopausal estrone is synthesized in the periphery, predominantly in adipose tissue, by the conversion of androstenedione to estrone (Fig. 2)[5].

As these hormonal changes occur, the amount of total circulating estrogen may be reduced, with a potential decline in menstruation usually around the age of 40 years. The decline manifests as irregular cycles with a lighter menstrual flow, less frequent menses, and finally a total absence of menstruation. If anovulatory cycles occur, the absence of progesterone can result in irregularly heavy menstrual periods.

The tissues or organs directly affected by this relative estrogen deficiency are those with specific estrogen receptors, such as the ovaries, endometrium, vaginal epithelium, hypothalamus, urinary tract, and skin. Other tissues in which estrogen receptors have not been inconsistently identified, such as bone,[6-7] are nevertheless affected by a lack of estrogen (Fig. 3). Table II[5] summarizes the resultant symptoms or problems that may occur when these various tissues and organs become estrogen deficient.

Urogenital atrophy manifests as a loss of soft tissue elasticity and a decrease in muscle tone and secretions. Usually the uterus shrinks and the vagina undergoes progressive thinning and shortening, with a loss of the rugal pattern. These vaginal changes are a common cause of pruritis in postmenopausal women and they may be responsible for complaints of dyspareunia. Vaginal atrophy also puts the woman at increased risk of vaginal trauma and infection. The increased risk of infection is probably due, in part, to the slight increase in vaginal pH associated with changes in the bacterial flora.

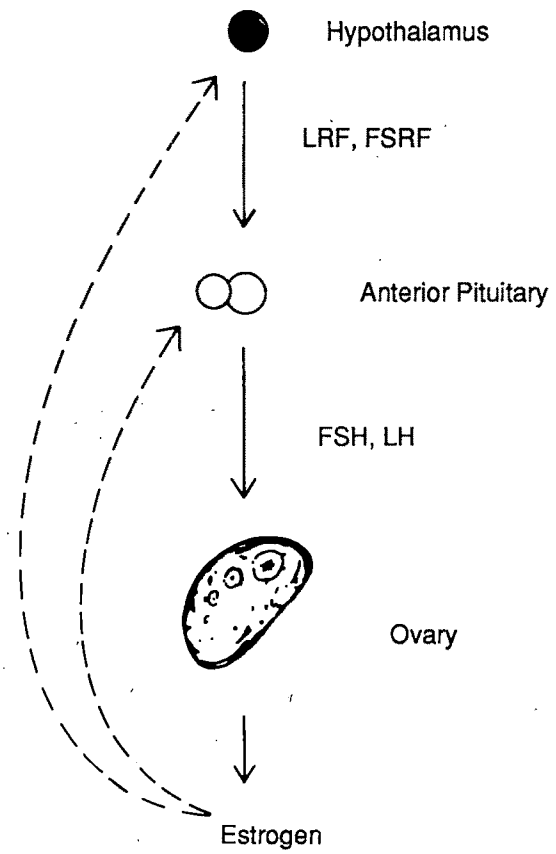


Fig. 1. Feedback control of the anterior pituitary and ovary by the hypothalmus. *LRF,* Luteotropin (or luteinizing hormone [LH]) releasing factor; *FSRF,* follicle stimulating releasing factor; *FSH,* follicle stimulating hormone.

Similar changes also occur in the urethra and the trigone area of the bladder. Urethral atrophy may lead to the "urethral syndrome," in which women have a recurrent abacterial urethritis characterized by symptoms of dysuria, frequency and urgency of urination, nocturia, and postvoid dribbling.

Other atrophic changes may also occur. The amount of collagen in the skin decreases, and the skin becomes thinner. The breasts also diminish in size, become softer, and may droop.

Systemic problems related to menopause

The risk of cardiovascular disease is higher in men compared with age-matched women before the age of

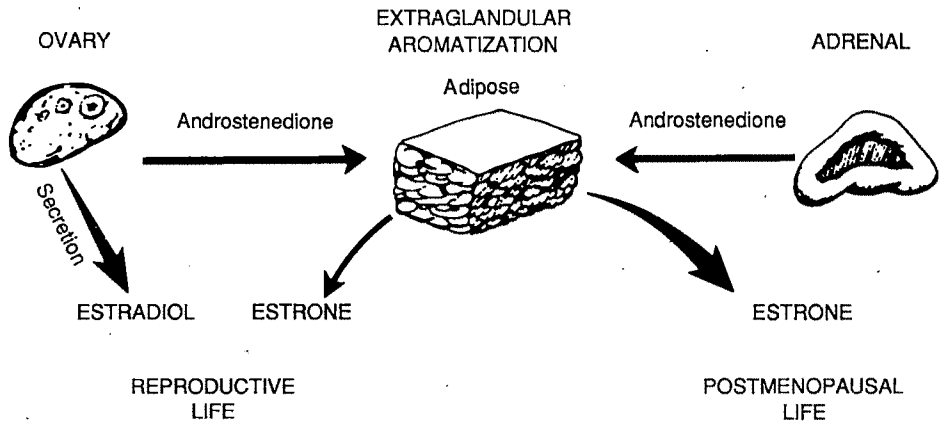


Fig. 2. Sources of estrogen during reproductive and postmenopausal life. (Reprinted with permission from Carr BR, MacDonald PC. In: Stollerman GH, Harrington WJ, Lamont JT, eds. Advances in internal medicine. Chicago: Year Book Medical Publishers, 1983.)

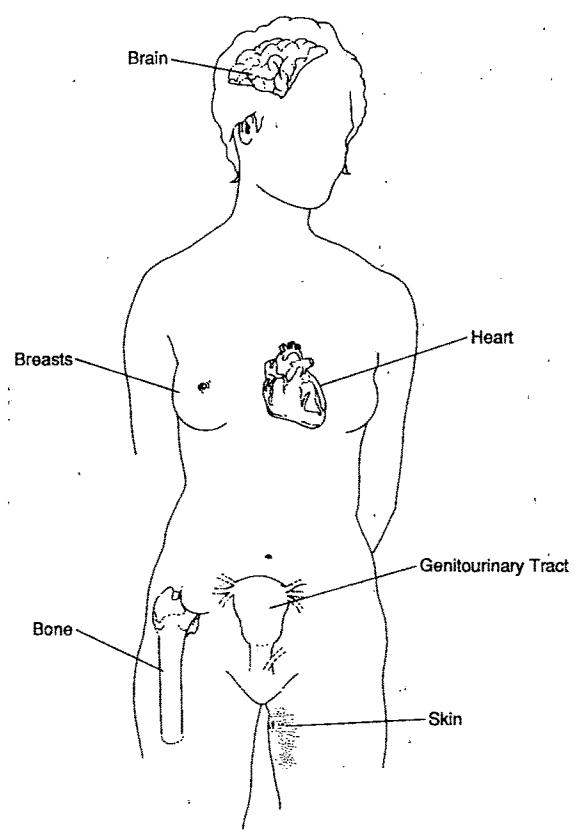


Fig. 3. Tissues and organs affected by estrogen deficiency.

40 to 45 years. During the perimenopausal period, the risk of cardiovascular disease in women begins to increase to a rate similar to that in men (Fig. 4).[8] The decrease in estrogen production is thought to be responsible for this acceleration of risk.

After cardiovascular disease, the most significant problem related to estrogen deficiency is osteoporosis. By the age of 80 years, about 25% of all women have sustained one or more fractures of the proximal femur, vertebrae, or distal radius. Hip fractures are associated with high morbidity and mortality rates. The management of hip fractures constitutes a large public health care expense and carries with it about a 20% mortality rate within the first year.

Table III. Psychologic complaints of perimenopausal and postmenopausal women

Decreased energy and drive	Mood fluctuations
Difficulty concentrating	Tension
Irritability	Depression
Aggressiveness	Introversion
Nervous exhaustion	Sense of internal frustration and inadequacy
Intolerance of loneliness	Anxiety
Marital troubles	Headache
Antisocial behavior patterns	Insomnia

Neuroendocrine problems are also relatively common in perimenopausal and postmenopausal women. Hot flashes, which can begin several years before the actual menopause, occur in 75% to 85% of climacteric women. One postulated mechanism is that the temperature-regulating set point of the hypothalamus may be reset downward. Most women describe a sudden onset of warmth and vasodilation, often visible as a red flush that is most apparent over the face and chest. The flush usually lasts from 30 seconds to 5 minutes and may occur every few minutes or only once or twice a week. Other vasomotor symptoms include nausea, dizziness, weakness, headaches, palpitations, diaphoresis, and night sweats. Women who experience hot flashes may also complain of sleep disturbances, characterized by an increased number of awakenings and increased fatigue.

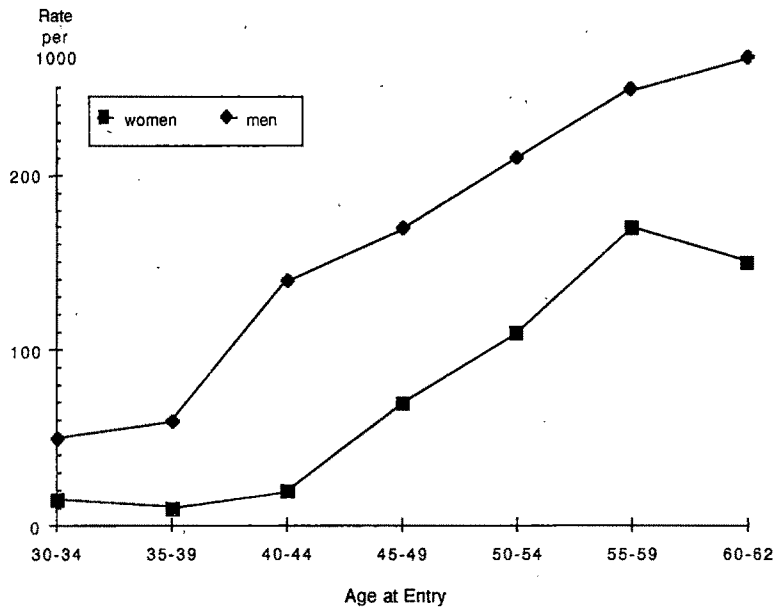


Fig. 4. Incidence of coronary heart disease based on age and sex. (Reprinted with permission from Castelli WP. Am J Med 1984;76(2A):4-12.)

Many other psychologic changes associated with menopause have been reported and studied. These changes are probably due to the interactive effects of endocrine changes, sociocultural effects, and psychologic factors in each individual. The complaints are usually multiple, vague, and nonspecific. They may be related to vasomotor symptoms and a lack of sleep. Table III lists a variety of complaints that perimenopausal and postmenopausal women report. The severity of psychologic complaints during the menopausal years are usually mild, not continuous, and tend to fluctuate.

Women may associate menopause with old age and feelings of asexuality. Hopefully, women's attitudes, as well as society's, are changing today to shatter the myth that women past the age of menopause lose their sexuality.

Summary

A woman who is 50 years old today can expect to live to 89 years of age. By the year 2000, more women will be spending a larger percentage of their life beyond the age of 40 years. The numbers alone dictate addressing the issues that surround menopause. There are still many myths to dispel and facts to discover that could promote a healthier, more productive life-style for women in this age group. Meanwhile, what we do know is that the benefits of estrogen replacement therapy can improve the quality of life for postmenopausal women.

REFERENCES

1. Schneider J. Forward. Med Clin North Am 1987;71:ix-xi.
2. Coulam CB, Anderson SC, Annegen JF. Incidence of premature ovarian failure. Obstet Gynecol 1986;67:604-6.
3. Utian WH. Menopause in modern perspective. New York: Appleton-Century-Crofts, 1980.
4. Research on the menopause. Geneva: WHO Technical Report Series, 1981:670.
5. Carr BR, MacDonald PC. Estrogen treatment of postmenopausal women. In: Stollerman GH, Harrington WJ, Lamont JT, eds. Advances in internal medicine. Chicago: Year Book Medical Publishers, 1983.
6. Komm BS, Terpening CM, Benz DJ, et al. Receptor mRNA, and biologic response in osteoblast-like osteosarcoma cells. Science 1988;241:81-4.
7. Eriksen EF, Colvard DS, Berg NJ, et al. Evidence of estrogen receptors in normal osteoblast-like cells. Science 1988; 241:84-6.
8. Castelli WP. Epidemiology of coronary heart disease: the Framingham Study. Am J Med 1984;76(2A):4-12.

SUGGESTED READING

An additional list of references is provided for those interested in a review on pathophysiology.

Barbo DM, ed. The postmenopausal woman. Med Clin North Am 1987;71:1.
Gambrell RD Jr, ed. The menopause. Obstet Gynecol Clin North Am 1987;14:1.
Mishell DR. Menopause: physiology and pharmacology. Chicago: Year Book Medical, 1987.
Studd, JW, Whitehead MI, eds. The menopause. Oxford: Blackwell Scientific, 1988.
Utian WH. Menopause in modern perspective: a guide to clinical practice. New York: Appleton-Century-Crofts, 1980.
Utian WH. Overview on menopause. Am J Obstet Gynecol 1987;156:1280.

Estrogen replacement therapy: Indications, contraindications, and agent selection

Morris Notelovitz, MD
Gainesville, Florida

Three groups of indications exist for postmenopausal estrogen use: relief of symptoms related to estrogen deficiency, osteoporosis prophylaxis and treatment, and cardioprotection. Estrogen replacement therapy enhances a woman's sense of well-being and reduces the morbidity, mortality, and health care costs associated with osteoporosis and atherosclerotic heart disease. There are a few absolute contraindications to estrogen replacement therapy. Many estrogen preparations are currently available in the United States. Establishing equivalencies among the different preparations is complicated by the many physiologic and pharmacologic effects of estrogens and the variety of treatment end points used. Most estrogens have the same biologic effect provided equivalent blood levels are achieved. Estrogen replacement therapy has proved beneficial to selected postmenopausal women. (Am J Obstet Gynecol 1989;161:1832-41.)

Key words: Estrogen, menopause, indications, contraindications

The average age at which menopause occurs is 50 years. More than 30 million women in the United States are more than 50 years old, with an average life expectancy of 81 years. Therefore, a women in the United States can expect to live more than one third of her life in the postmenopausal state.[1] Ovarian function diminishes gradually; the climacteric (reproductive senescence) begins at about the age of 35 years and ends some 15 years after menopause.[2] In this context, menopause per se is a relatively inconsequential event. It does, however, herald the onset of changes that can have far-reaching consequences on future health, primarily cardiovascular disease and osteoporosis. This review will summarize only a small fraction of the extensive research on the use of hormone-additive[2] or estrogen replacement therapy.

Indications

Three groups of indications exist for the use of postmenopausal estrogens: symptoms related to estrogen deficiency such as vasomotor symptoms, atrophic changes of the vagina and other genitourinary tissues, and certain psychologic/mood changes; osteoporosis prophylaxis and treatment; and prevention of cardiovascular morbidity and death.

The symptomatic menopause

Hot flashes. Vasomotor "hot flashes" occur in 85% of perimenopausal women, 25% of whom will experience symptoms sufficiently severe to prompt them to seek medical care.[3] In 82% of women the symptoms persist for more than 1 year.[2] Most women do not require exogenous estrogens to control vasomotor symptoms for more than 3 to 5 years, although in some women symptoms persist for many years.[2-4] Estrogen replacement therapy for control of vasomotor symptoms should use the lowest possible dose that controls symptoms; therapy can be tapered over a few years and then withdrawn.

Vaginal atrophy. Atrophic changes of the vagina, urethra, and bladder base occur in virtually all women 4 to 5 years after menopause. Symptoms include dyspareunia, repeated vaginal infections, and dysuria. Twenty-five percent of women will seek medical attention because of these symptoms.[3] Unfortunately, symptoms often recur when estrogen replacement therapy is discontinued, indicating that long-term or indefinite therapy may be needed.[3] Again, the lowest effective dose for control of symptoms should be used.

Psychologic changes. Mood changes such as irritability, depression, insomnia, impaired memory, and crying jags are common "hormone-related" symptoms that frequently precede and postdate menopause. In many instances these changes are estrogen dependent. Utian coined the phrase "mental tonic" effect to describe the sense of well-being that most women experience when taking exogenous estrogen. Depression may be due to numerous nonhormonal conditions; care must be taken to exclude those women with endogenous depression. A simple test is to give a trial dose of estrogen to patients who have no other stigmata of a central nervous system problem. If the patient improves, the therapy is continued; if not, an appropriate referral to a psychiatrist is warranted. Clinicians should remember that certain

From the Women's Medical and Diagnostic Center, The Climacteric Clinic Inc.
Reprint requests: Morris Notelovitz, MD, PhD, Women's Medical and Diagnostic Center, The Climacteric Clinic Inc., 222 S.W. 36th Terrace, Suite C, Gainesville, FL 32607.
6/0/16682

progestins, especially medroxyprogesterone acetate, may "exacerbate" depression in susceptible women.

Osteoporosis. A great deal of attention has focused on using estrogen replacement therapy for prophylaxis and treatment of osteoporosis. This interest is appropriate given the significant impact this preventable disease has on the health of countless menopausal women.

Osteoporosis affects 15 to 20 million Americans and accounts for 1.3 million fractures per year in the United States.[5,6] The risk of vertebral fractures in white women is 25% by the age of 50 years; by the age of 75 years the risk increases to 50%.[7] Hip fractures represent the most serious complication of osteoporosis. Each year more than 120,000 elderly women sustain hip fractures; 15% to 17% die from associated complications.[8,9] Osteoporosis-associated health care costs are estimated to be $6 billion annually.[6]

Normal bone metabolism and risk factors for osteoporosis. Put simplistically, two cell types influence the skeletal bone mass volume, the primary determinant of osteoporotic fractures. Osteoclasts break down bone mineral and collagen, whereas osteoblasts lay down new bone tissue (osteoid). Both men and women reach maximal trabecular (vertebral) bone mass between the ages of 25 to 30 years and maximal cortical (compact) bone mass by the age of 35 years. Thereafter, cortical bone mass undergoes a net demineralization of 0.3% to 0.5% per year. After menopause, however, women lose 2% to 3% of their cortical bone mass and 5% to 8% of their trabecular bone mass annually.[10-13] An accelerated rate of bone loss (perimenopausal/climacteric) occurs 3 to 6 years after menopause; the rate of bone loss decreases after the age of 65 years.[14] A more detailed overview of osteogenesis is available elsewhere.[15]

After 15 to 20 years of bone loss, about 50% of trabecular and 30% of cortical bone mass will have eroded. As a result of this accelerated breakdown in bone mass, postmenopausal women are at high risk for the development of involutional osteoporosis. A number of traditional risk factors for the development of osteoporosis are listed in Table I.[14-19] We[14] and others have shown that these "risk factors" are rather insensitive, and that measuring bone mass is the only realistic way of identifying women at risk and quantifying their degree of bone mineral deficiency (osteopenia). This allows for appropriate and specific intervention, both hormonal and nonhormonal.

Efficacy of estrogen replacement therapy in postmenopausal osteoporosis prophylaxis. It is clear that estrogen deficiency contributes to bone loss,[20] and that estrogen replacement therapy reduces loss of bone mineral.[21-26] Most importantly, this therapy reduces the risk of bone fractures.[21,27-31]

Several reviews have recently addressed the value of estrogen replacement therapy in treating postmenopausal women.[5,6,13-15,32-35] For example, Weiss et al.[28] demonstrated a 50% to 60% reduction in the risk of fractures with the use of 0.625 to 1.25 mg of conjugated estrogens daily. Concomitant use of calcium may decrease the minimum effective dose of estrogen necessary to prevent bone loss (Table II).[13] In a study investigating the effects of several pharmacologic treatments, estrogen replacement therapy in combination with vitamin D and calcium and/or fluoride produced the greatest reduction in the rate of vertebral fractures compared with calcium and vitamin D alone or a calcium and fluoride combination (Table III).[31] A consensus panel has recommended that estrogen replacement therapy be used in postmenopausal women at risk for osteoporosis.[36]

If the impact of estrogen replacement therapy is clearly beneficial, why aren't all women receiving such therapy? There are two main reasons. First, 75% of all postmenopausal women will not develop osteoporosis, so the clinical indication is not present. Also significant are women's attitudes toward this therapy. In a recent survey, only 27% of postmenopausal women not receiving estrogen replacement therapy knew that estrogen deficiency was a risk factor for osteoporosis, compared with 89% of those taking estrogen replacement therapy, suggesting the need for further patient education. A physician's recommendation of estrogen replacement therapy was highly associated with its use, suggesting that prescribers have a powerful role in influencing decisions. Also, a woman's concern about estrogen's side effects, an issue with a high degree of visibility in the lay press, may lead her to decide not to

Table I. Risk factors for osteoporosis

Primary risk factors	*Secondary risk factors*
White or Oriental race	Chronic renal failure
Women of Northern Europe	Gastrectomy and intestinal bypass
Family history of osteoporosis	Malabsorption syndromes
Petite, small frame	Multiple myeloma
Poor nutrition	Hyperparathyroidism
Low calcium intake	Hyperthyroidism
Lack of physical activity	Adrenocortical overactivity
Decreased weight and muscle mass	Diabetes mellitus
Nulliparity	Anticonvulsants (phenytoin)
Premature or surgical menopause	Aluminum antacids
Cigarette smoking	Thyroid extracts (>180 mg/day)
Excessive alcohol intake	
High-fiber diets	
High-caffeine intake	
High-protein diet	
High-sodium diet	
High-phosphate diet	

Table II. Minimum effective estrogen dosages for preventing bone loss

	Estrogen (mg)	*Serum estradiol level (pg/ml)*	*Estrogen (mg) plus calcium**
17 β-Estradiol cream	1.5	50-60	—
Micronized estradiol	2.0	50-60	1.0
Conjugated estrogens	0.625	50-60	0.3
Estrone sulfate	0.625	50-60	—

Adapted with permission from Ettinger B. Obstet Gynecol 1988;72(suppl):125-75; and Notelovitz M. Clin Obstet Gynecol 1987;30:871-2.

*Minimum amount of estrogen needed when given with calcium to prevent bone loss (not assessed for estradiol cream).

Table III. Rate of vertebral fractures in postmenopausal women

Treatment	*No.*	*Fracture rate per 1000 woman-years*
Placebo	45	834
Calcium and vitamin D	27	419
Calcium, fluoride, and vitamin D	33	304
Calcium, estrogen, and vitamin D	32	181
Calcium, estrogen, fluoride, and vitamin D	28	53

Reprinted with permission from Riggs BL, Seeman E, Hodgson SF, et al. N Engl J Med 1982;306:446-50, by permission of *The New England Journal of Medicine.*

use or to discontinue use of estrogen replacement therapy.[37] Simply put, the following clinical guidelines should be followed: All women at high risk (especially low bone mass) should be treated with estrogen replacement therapy; the remainder should be regularly monitored and treated if their rate or degree of "natural" bone loss is excessive.

Cardiovascular disease. Concern about estrogen use and an increased cardiovascular risk has been tainted by the association with oral contraceptives. The association is, at best, controversial; more likely estrogen replacement therapy has a beneficial effect on cardiovascular disease risk. The use of synthetic estrogens in men (e.g., in treating prostatic carcinoma[38]) and case-control trials of birth control pills in women[39-42] reveal a positive correlation between exogenous estrogens and risk of cardiovascular disease. A recent report from the Framingham study suggested a relationship between postmenopausal estrogen use and an increased cardiovascular risk.[43] In contrast, most studies examining postmenopausal estrogen use show a reduction in cardiovascular risk or no effect.[44-48] Indeed, animal data demonstrate that estrogen treatment may retard the development of atherosclerotic coronary lesions.[49]

Recent studies of estrogen replacement therapy have demonstrated the potential benefit of estrogens on the risk of cardiovascular disease. In three important trials, a risk reduction for stroke,[50] myocardial infarction,[51] and cardiovascular death[52] was associated with this therapy's use. Estrogen replacement therapy was associated with a relative risk for death from stroke of 0.53; recent users (those using estrogens at the time of the questionnaire) had a relative risk of 0.21.[50] In another analysis therapy was associated with a 41% reduction in deaths and a 29% reduction in morbidity due to acute myocardial infarctions.[51] Finally, in an 8.5-year mean follow-up period, estrogen replacement therapy was associated with a relative risk of cardiovascular death of 0.54.[52]

Several postulates have been advanced to explain this decrease in cardiovascular death. By far, the leading hypothesis is an estrogen replacement therapy–induced reduction in low-density lipoprotein (LDL) cholesterol and an increase in high-density lipoprotein (HDL) cholesterol.[53-55] Estrogens increase HDL_2 cholesterol and its associated apolipoproteins, A1 and A2.[56] This effect occurs with both oral and nonoral ethinyl estradiol,[57] but with only oral (not vaginal) conjugated estrogens.[58] Percutaneous estradiol cream reduced total and LDL cholesterol, but did not change HDL cholesterol.[59] To date, the transdermal patch has not produced as favorable an effect on lipids and lipoproteins.[56, 60, 61]

Synthetic progestins have an effect opposite to that of estrogens on lipoproteins: HDL cholesterol is decreased and LDL cholesterol is increased.[62] At conventional doses, progestins appear to ameliorate or abolish estrogen's beneficial effect on lipoproteins, a critical predictor of coronary heart disease risk.[63-65] In one study the addition of a "naturalized" oral micronized progesterone at a dose of 200 mg/day to percutaneous estradiol for 12 days per cycle resulted in no further change in total and LDL cholesterol and an increase in HDL cholesterol.[59] The same was noted in an 18-month study involving naturally menopausal women using cyclic conjugated equine estrogen (both 0.625 and 1.25 mg) and a 7-day course of 10 mg medroxyprogesterone acetate for week. A practical point: Patients with an intact uterus who are at risk for cardiovascular disease should be given relatively "high-dose" daily estrogen replacement therapy (e.g., 1.25 mg conjugated equine estrogen) and cyclic progestins (e.g., 5 mg medroxyprogesterone acetate for the first 2 weeks of each

Table IV. Cautions and contraindications to estrogen replacement therapy

Disease	*Absolute contraindication*	*Relative contraindication*	*Not a contraindication*
Cardiovascular disease			
Recent myocardial infarction	X		
Recent cerebrovascular accident or transient ischemic attack	X		
Established ischemic heart disease		X	
Hypertension		X	
Liver disease			
Acute, marked disease	X		
Chronic disease		X	
Gall bladder disease		X	
Estrogen-dependent tumors			
History of or active malignant breast cancer	X		
History of benign breast tumor			X
Endometrial cancer, poorly differentiated	X		
Endometrial cancer, early, well differentiated	X		
Thromboembolic disease			
Recurrent, active, or spontaneous	X		
Single episode, recent		X	
Postoperative, several years past			X
Diabetes mellitus		X	
Hyperlipidemia		X	
Pancreatitis		X	
Migraine headaches		X	
Epilepsy		X	
Leiomyomata		X	

month).[64] A recent study has shown that a regimen similar to this results in an overall increase in plasma HDL-cholesterol. Most hysterectomized menopausal women do not need added progestins.

We have generally believed that hypertension, by far the most significant risk factor for stroke, is a relative contraindication for estrogen replacement therapy.[66, 67] However, Paganini-Hill et al.[50] reported a reduction in the incidence of mortality from stroke in estrogen replacement therapy users with and without histories of hypertension. Indeed, although some women may experience an elevation in blood pressure when given estrogens, most studies report a reduction in blood pressure with this therapy.[68-70] Thus some experts do not feel that hypertension is a contraindication to therapy.[71] There may be a difference between the "natural estrogens" and their more potent synthetic counterparts, because ethinyl estradiol stimulates renin substrate and thus may pose a risk.[56] Progestins, on the other hand, can produce a dose-related elevation in blood pressure.[72]

Another possible mechanism for estrogens' beneficial effect on cardiovascular disease is enhanced prostacyclin production, resulting in vasodilatation. This effect is also opposed by progestins.[73]

Contraindications

There are few absolute contraindications to estrogen replacement therapy: recent myocardial infarction, cerebrovascular accident or transient ischemic attack, and recurrent, active, or spontaneous thromboembolic disease (Table IV). Acute liver disease, history of or active breast cancer, and poorly differentiated endometrial cancer are other, noncardiovascular-related contraindications.

Breast cancer. Disagreement exists regarding the possible association of estrogen replacement therapy and breast cancer. Some investigators have demonstrated a small but significant increase in risk of breast cancer,[74] whereas others have concluded that estrogen therapy does not increase the risk of breast cancer and may, in fact, improve the prognosis of those in whom breast cancer develops during therapy.[75, 76] Others feel that whereas high doses of estrogens may be associated with a slight increase in risk of breast cancer, low doses (<0.625 mg/day of conjugated estrogens) may not be associated with such a risk.[35, 74, 77] However, most believe that estrogen replacement therapy is absolutely contraindicated in patients with breast cancer.[35, 78] Although some feel that only women with surgically removed benign breast tumors are at increased risk,[71] all women taking this therapy should be encouraged to practice monthly breast self-examination, to be examined yearly by a physician, and to have regular mammograms. The potential protective role of added progestins is still not clear.

Endometrial cancer. In 1987 there were 35,000 new cases of endometrial cancer in the United States and 2900 deaths from endometrial cancer. The disease is usually diagnosed at an early stage; those with stage 1 disease have a 92% 5-year survival rate and those with stage 2 disease have an 84% 5-year survival rate.[33] The first reports of an association between endometrial cancer and estrogen use were published in 1975.[79, 80] This

Table V. Estimated changes in mortality rates associated with high- or moderate-dose estrogen replacement therapy in women 50 to 75 years old

Condition	*High-dose ERT*		*Moderate-dose ERT*	
	RR	*Change in mortality/100,000*	*RR*	*Change in mortality/100,000*
Osteoporotic fractures	0.4	−563	0.4	−563
Gallbladder disease	2.0	+3	1.5	+2
Endometrial cancer	7.0	+378*	2.0	+63*
Breast cancer	1.5	+938	1.1	+187
Ischemic heart disease	1.0†	0	1.0†	0
Net change		+756		−311

Reprinted with permission from Ross RK, Paganini Hill A, Mack TM, Henderson BE. In: Mishell, DR, Jr, ed. Menopause: physiology and pharmacology. Chicago: Year Book Medical Publishers, 1987:209-23.

RR, Relative risk.

*Case-fatality rate for estrogen-induced endometrial cancer estimated at 0.05.

†More recent studies have demonstrated a risk reduction for ischemic heart disease.

Table VI. Estimated annual mortality rate changes due to estrogen replacement therapy with low-dose (0.625 mg/day conjugated estrogens) alone or with 10 days of progestin per month (therapy given for 10 years)

Condition	*Annual mortality rate/100,000, ages 65-74 yr*	*Estrogen replacement therapy*		*Estrogen replacement therapy + progestin*	
		RR	*Change in mortality/100,000*	*RR*	*Change in mortality/100,000*
Osteoporotic hip fractures	55	0.40	−33	0.28*	−40
Endometrial cancer	42†	6.00	+26‡	1.00	0
Breast cancer	102	1.60	+61	1.60	+61
Ischemic heart disease	592	0.52	−284	0.69	−184
Net change in mortality rate					
Nonhysterectomized			−230		−163
Hysterectomized			−256		−163

From Henderson BE, Ross RK, Lobo RA, et al. Fertil Steril 1988;49(suppl):9S-15S. Reproduced with permission of the publisher, The American Fertility Society.

RR, Relative risk for 10 years of use.

*Estimated on the assumption that hip fracture does not increase with age when hormonal replacement is used.

†Nonhysterectomized women.

‡Case-fatality rate for exogenous estrogen-induced endometrial cancer estimated at one eighth the normal rate.

risk has been well established: Estrogen users have a four- to eightfold increase (range two- to twenty-fold)[33, 35, 79-82] in risk of developing endometrial cancer compared with those not taking estrogens.

The risk is related to many factors, including duration of use, dosage (Table V)[83], method of administration, drug-free interval, and concomitant progestin use. In one evaluation, a dose of 0.625 mg/day of conjugated estrogens was associated with a 1.8-fold increased risk, whereas a dose of 1.25 mg/day was associated with a 12.7-fold increased risk.[84] These same investigators also determined that duration of therapy correlated with risk: Those using estrogens for less than 5 years had no increase in risk, those with a 5- to 9-year usage history had a 4.1-fold increase in risk, and those with more than 10 years of use had an 11.6-fold increased risk. A drug-free interval of 4 days per month was associated with a reduction in risk compared with continuous use,[81, 84] although other investigators have noted no difference in the rate of endometrial hyperplasia with cyclic (3 weeks on, 1 week off) estrogen therapy compared with continuous therapy.[85] An increased risk of endometrial cancer persists after discontinuation of therapy.[81]

Two studies published in 1979 determined that using a progestin with the estrogen was associated with a risk of endometrial cancer comparable to that of postmenopausal women not receiving hormonal therapy.[76, 86] In one study a high dose of estrogen was used (2.5 mg/day conjugated estrogen) with 10 mg/day medroxyprogesterone acetate for 7 days per cycle.[76] When unopposed by progestins, estrogen promoted endometrial hyperplasia. Because progestins confer protection, concurrent progestin therapy with estrogen replacement therapy, either for a few days per month or continuously, is generally recommended and prac-

Table VII. Estrogen preparations

Generic name	*Brand name*	*Components*	*Available doses*
Natural			
Micronized estradiol	Estrace	100% 17β-Estradiol*	1 mg, 2 mg
Estropipate (piperazine estrone sulfate)	Ogen	100% Estrone sulfate	0.625 mg, 1.25 mg, 2.5 mg, 5 mg
Equine estrogen			
Conjugated estrogens	Premarin	50%-60% Estrone sulfates; 22.5%-32.5% sodium equilin sulfate; 7.5%-20% unspecified conjugated estrogens	0.3 mg, 0.625 mg, 0.9 mg, 1.25 mg, 2.5 mg
Synthetic			
Ethinyl estradiol	Estinyl	100% Ethinyl estradiol	0.02 mg, 0.05 mg, 0.5 mg
Diethylstilbestrol	DES	100% Diethylstilbestrol	0.1 mg, 0.5 mg (higher doses used for other indications)

*Converted to estrone sulfate during absorption.

ticed.[35, 75, 76] Others oppose the addition of a progestin because endometrial cancer is relatively rare and is nonfatal in most cases and because progestins oppose the beneficial lipoprotein effects of estrogens. A nonclinical study comparing a low-dose estrogen regimen with a combination regimen showed a higher relative risk of endometrial cancer but a greater net reduction in deaths (based on cardioprotective effects) for the group taking estrogen alone (Table VI).[73] The question of progestin supplementation remains controversial,[33, 73] but most physicians prescribe a 10-day cyclic course of progestins with long-term estrogen replacement therapy in women with an intact uterus.

Coagulation and thrombosis. Estrogens produce several effects on coagulation, complicating determinations of their exact net effect. Estrogen tends to reduce endothelial prostacyclin production.[87] Synthetic estrogens may specifically or preferentially increase prostacyclin activity, but this area requires further investigation.[88] Progestins may increase[89] or reduce[90] prostacyclin activity. Because thromboxane has the opposite effect and in addition increases platelet adhesiveness, women taking estrogen replacement therapy (especially those with high-risk profiles) should take 60 to 80 mg of aspirin every other day. This low dose inhibits thromboxane synthesis more than that of prostaglandin, allowing the latter to exert its preventive effect on blood flow and platelet adhesion.

Platelet aggregation is generally unchanged by estrogens,[91] although one study demonstrated an increase.[92] Virtually all clotting factors have been shown to be elevated in women receiving synthetic estrogens (ethinyl estradiol, diethylstilbestrol), with factors II, VII, IX, X, XII, and fibrinogen being elevated most frequently. This phenomenon is primarily related to the potency of the estrogen used. The elevations are more marked with ethinyl estradiol and less marked with the estriol-containing agents.[91] Clotting factor elevations were not observed in women receiving estrogen via patches[60] or ointment.[61]

Table VIII. Serum estrogen levels 24 hours after 25 days of treatment with estrone sulfate, conjugated estrogens, or estradiol

	Total estradiol (pg/ml)	*Total estrone (pg/ml)*
Baseline	10	37
Estrone sulfate	34	125
Conjugated estrogens	40	153
Micronized estradiol	40	150

From Lobo RA. Obstet Gynecol Clin North Am 1987; 14:143-67.

No clinically significant increase in coagulation factors has been observed with the use of the natural estrogens.[56, 93, 94] For example, the alterations in clotting factors seen with 30 μg/day of ethinyl estradiol did not occur with 3 mg/day of estrone sulfate.[56, 94] Despite their effect on clotting factors, estrogens do not lead to a hypercoagulable state; the observed increase in clotting factors occurs because proenzymes remain inactive until exposed to injured endothelium. Antithrombin III is not reduced to clinically relevant levels by either oral estrogen replacement therapy or transdermal estrogens.[91] Clinical trials have not shown any increase in risk of thromboembolism in postmenopausal women receiving estrogen replacement therapy.[75]

Plasminogen, a major factor in the fibrinolytic pathway, and especially plasminogen activity are elevated by estrogen administration.[95] This effect, which is enhanced by combination estrogen/progestin therapy,[95] is seen even with low-dose oral contraceptives.[96] This, combined with the persistence of normal antithrombin III activity, ensures against inappropriate venous thrombosis.

Neurologic effects. Estrogens may potentiate headaches and migraines. In one trial of 87 women who experienced migraine headaches while receiving estrogens, 58% experienced a reduction in symptoms with a dose reduction. The mean latency period was ap-

Table IX. Relative potencies of five estrogens according to four parameters

Estrogen	*Serum FSH*	*Serum CBG*	*Serum SHBG*	*Serum angiotensinogen*
Piperazine estrone sulfate*	1.1	1.0	1.0	1.0
Estradiol	1.3	1.9	1.0	0.7
Conjugated estrogens	1.4	2.5	3.2	3.5
Diethylstilbestrol	3.8	70	28	13
Ethinyl estradiol	(80-200)†	(1000)†	614	232

Reprinted with permission from Mashchak CA, Lobo RA, Dozono-Takano, R, et al. AM J OBSTET GYNECOL 1982;144:511-8.
FSH, Follicle-stimulating hormone; *CBG,* corticosteroid-binding globulin; *SHBG,* sex hormone–binding globulin.
*Used as a standard.
†Estimate in the absence of parallelism.

proximately 4 weeks.[97] Thus reducing the dose may relieve migraine headaches, a relative contraindication to the use of estrogens. Parenteral testosterone administration often alleviates this infrequent, but troublesome, side effect (Gambrell RD, personal communication, 1989).

Agent selection: differences between preparations

Alternate routes of administration. Estrogens are well absorbed transdermally and from deposition in subcutaneous fat. These routes circumvent a first-pass hepatic effect. Intravaginal administration of conjugated estrogens, when corrected for dose, results in less stimulation of hepatic proteins;[56] with ethinyl estradiol, circumventing the portal pathway does not affect hepatic protein production.[57] Estrogens' stimulation of hepatic proteins, however, results in no apparent clinical disadvantage. The proved decrease in cardiovascular risk is in fact based on the use of oral estrogen, possibly (in part) by its pharmacologic effect on the liver and its ability to synthesize the cardioprotective lipoprotein HDL cholesterol.

Estradiol pellet implants may be useful for relatively noncompliant patients, as these deliver steady levels of estradiol for 4 to 6 months.[98] In one study, 25 mg of 17β-estradiol (delivering an estradiol level of 60 pg/ml) was as effective as a 50 mg dose (delivering estradiol levels of 80 to 100 pg/ml) in maintaining bone mass.[14]

Estrogen preparations. Three classes of estrogens are used for estrogen replacement therapy in the U.S. (Table VII). Natural estrogens include estradiol and estrone. 17β-Estradiol is not well absorbed except in a micronized form. Adequate estrogen levels may be attained when other forms of micronized estradiol (Estrace) are administered. However, because of the enterohepatic conversion of estradiol to estrone, the primary estrogen in the peripheral circulation is estrone. Estrone is administered as estrone sulfate (Ogen). Conjugated estrogens (Premarin) contain up to 33% equine estrogens, primarily equilin sulfate. The synthetic estrogens are ethinyl estradiol (Estinyl) and diethylstilbestrol. Because of a potent first-pass effect, a reduction in bioavailability of up to 30% occurs with any oral estrogen. Except for piperazine estrone sulfate (Ogen), all oral estrogens are converted primarily to estrone.[56] When Lobo[56] measured free and total serum estradiol levels and total estrone levels after 25 days of oral administration of 0.625 mg of conjugated estrogens or piperazine estrone sulfate or 1 mg of estradiol, values for the three products were similar (Table VIII).[56]

Establishing equivalencies among different estrogen preparations is complicated by the many physiologic and pharmacologic effects of estrogens and the various end points of treatment measured. Estrogen potency can be measured in terms of gonadotropin suppression (luteinizing hormone and follicle-stimulating hormone) and hepatic protein production (angiotensinogen, corticosteroid-binding globulin, and sex hormone–binding globulin).

Potency can also be measured in terms of a reduction of urinary calcium corrected for urinary creatinine, a measure of prevention of bone demineralization. Additionally, estrogenic potency can be assessed in terms of maturation of vaginal epithelial cells. Investigators have attempted to quantify estrogenic potency using one or more of these parameters and to compare different agents using these measures.[56, 99-102]

In a study involving 23 healthy postmenopausal women, Mashchak et al.[102] found that the potencies of five estrogen preparations differed depending on which parameter was used to measure potency (Table IX). At doses that produced comparable effects on serum follicle-stimulating hormone, piperazine estrone sulfate and micronized estradiol had less of an effect on hepatic protein stimulation than the other preparations. For example, conjugated estrogens had a 2.5- to 3.5-fold greater stimulatory effect on serum corticosteroid-binding globulin, sex hormone–binding globulin, and angiotensinogen compared with estrone sulfate and micronized estradiol.[102] No dose-response relationship was found for leutenizing hormone suppression in this study; this effect may likely have been obscured by interpatient variation.

Most estrogens have the same biologic effect, irrespective of the type or route of administration, provided equivalent blood levels are reached. The route of administration may alter the patient's response.

Combination therapy. The scope of this article precludes appropriate discussion of combined therapy with estrogen; only two of the commonly prescribed preparations (calcium and progestins) will be discussed.

Combination of estrogens with calcium and bone loss. The coadministration of 1500 mg/day of elemental calcium with 0.3 mg of conjugated equine estrogen was as effective as 0.625 mg of estrogen without calcium in maintaining bone mass.[9, 103, 104] Thus when calcium is added to the regimen, lower doses of estrogens may be effective. Given the decreased likelihood of low-dose estrogens to stimulate the endometrium, this approach may be more acceptable to elderly women who are averse to menstruation (induced by the need for progestins when higher doses of estrogen are used).

Combination of estrogens with progestins. Progestins may exert antiresorptive effects on bone[25] and may act synergistically with estrogens to increase bone mass.[105] Megestrol acetate, 80 to 160 mg/day, slows the rate of bone loss and may be useful when estrogens are contraindicated.[14]

Cyclic therapy with estrogen and progestin can result in resumption of menses, a "side effect" few women relish. Making menses regular and predictable or achieving amenorrhea improves acceptance. To regulate menses, one can administer estrogen on a daily basis with progestin added for the first 2 weeks of each month[14] or give medroxyprogesterone acetate, 10 mg/day, for the last 10 days of each cycle.[106] Alternatively, estrogen and progestin can be given continuously (e.g., estrone sulfate or conjugated estrogen, 0.625 mg/day, plus medroxyprogesterone acetate, 2.5 mg/day).[14] This regimen will cause some withdrawal bleeding over the first 6 months, usually followed by amenorrhea.[107] Restricting the number of endometrial biopsies also helps. Provided the patient bleeds at appropriate times (e.g., from day 12 or after when taking cyclic progestins), some experts advise against biopsy. However, taking two biopsies may be prudent, with one 3 months after starting estrogen replacement therapy and the other 1 year later.[14] Women taking estrogen replacement therapy alone require yearly biopsies. All patients with "abnormal" bleeding need further testing through curettage with or without hysteroscopy.

Conclusion

Estrogen replacement therapy enhances a woman's feeling of well-being and reduces the morbidity, mortality, and health care costs associated with osteoporosis and atherosclerotic heart disease. Menopause is often referred to as an endocrinopathy, thus justifying the "routine" use of estrogen. Postmenopausal women do not, however, have a "disease." Many nonhormonal interventions—primarily nutrition and exercise—impact positively and equally well on well-being, bone metabolism, and cardiovascular fitness and can (and should) be used as substitutes for or additions to medical treatment.

It is only appropriate and fair that both the patient and the physician be more fully aware of this fact, and that hormonal therapy (estrogen alone or in combination with a progestin) be used on an individual and selective basis in postmenopausal women.

REFERENCES

1. Judd HL, Meldrum DR, Defios LJ, et al. Estrogen replacement therapy: indications and complications. Ann Intern Med 1983;98:195-205.
2. Notelovitz M. Climacteric medicine: cornerstone for mid-life health and wellness. Public Health Rep 1986; (suppl):114-24.
3. Hammond CB. Indications and protocols for estrogen replacement therapy. Hosp Pract 1986;(suppl):9-15.
4. Yen SS. The biology of menopause. J Reprod Med 1977;18:287-96.
5. Lindsay R. Osteoporosis. Clin Geriatr Med 1988;4:411-30.
6. Sarrel PM. Estrogen replacement therapy. Obstet Gynecol 1988;72(suppl):2S-5S.
7. Anonymous. Oestrogen replacement treatment in old age. Br Med J 1980;573:572-3.
8. American College of Gynecologists. Estrogen replacement therapy. Technical bulletin no 70. Washington, DC: American College of Gynecologists, 1983.
9. Helgason S. Estrogen replacement therapy after the menopause. Acta Obstet Gynecol Scand Suppl 1982;107: 1-29.
10. Riggs BL, Wahner HW, Dunn WL, et al. Differential changes in bone mineral density of the appendicular and axial skeleton with aging: relationship to spinal osteoporosis. J Clin Invest 1981;67:328-35.
11. Cann CE, Genant HK, Kobb FO, et al. Quantitative computed tomography for prediction of vertebral fracture risk. Bone 1985;6:1-7.
12. Eirkson EF, Mosekilde L, Melsen F. Trabecular bone resorption depth decreases with age: differences between normal males and females. Bone 1985;6:141-6.
13. Ettinger B. Prevention of osteoporosis: treatment of estradiol deficiency. Obstet Gynecol 1988;72(suppl):12S-7S.
14. Notelovitz M. The role of the gynecologist in osteoporosis prevention: a clinical approach. Clin Obstet Gynecol 1987;30:871-82.
15. Notelovitz M. Post-menopausal osteoporosis. A practical approach to its prevention. Acta Obstet Gynecol Scand Suppl 1986;134:67-80.
16. Labor BC, France MW, Powell D, et al. Bone and mineral metabolism and chronic alcohol abuse. Q J Med 1985; 59:497-9.
17. Parfitt AM. Dietary risk factors for age related bone loss and fractures. Lancet 1983;2:1181-2.
18. Seeman E, Melton LJ, O'Fallon WM, et al. Risk factors for spinal osteoporosis in men. Am J Med 1983;75:977-83.
19. Daniell HW. Osteoporosis of the slender smoker. Arch Intern Med 1976;136:298-304.
20. Richelson LS, Wahner HW, Melton LJ, Riggs BL. Relative contributions of aging and estrogen to postmenopausal bone loss. N Engl J Med 1984;311:1273-5.

21. Ettinger B, Genant HK, Cann CE. Long-term oestrogen replacement therapy prevents bone loss and fractures. Ann Intern Med 1985;102:319-24.
22. Weinstein MC. Estrogen use in postmenopausal women—costs, risks, and benefits. N Engl J Med 1980; 303:308-16.
23. Munk-Jensen N, Nielsen SP, Obel EB, et al. Reversal of postmenopausal vertebral bone loss by oestrogen and progestogen: a double-blind placebo controlled study. Br Med J 1988;296:1150-2.
24. Aitken JM, Hart DM, Lindsay R. Oestrogen replacement therapy for prevention of osteoporosis after oophorectomy. Br Med J 1973;3:1436-40.
25. Lindsay R, Hart DM, Purdie D, et al. Comparative effects of estrogens and progestogen on bone loss in postmenopausal women. Clin Sci 1978;54:193-5.
26. Civitelli R, Agnusdei D, Nardi P, et al. Effects of one-year treatment with estrogens on bone mass, intestinal calcium absorption, and 25-hydroxyvitamin D-1-alpha-hydroxylase reserve in postmenopausal osteoporosis. Calcif Tissue Int 1988;42:77-86.
27. Hutchinson TA, Polansky SM, Feinstein AR. Postmenopausal oestrogens protect against fractures of the hip and distal radius. Lancet 1979;2:705-9.
28. Weiss NS, Ure CC, Ballard JH, et al. Decreased risk of fractures of the hip and lower forearm with postmenopausal use of estrogen. N Engl J Med 1980;303:1195-8.
29. Lindsay R, Hart DM, Forrest C, Baird C. Prevention of spinal osteoporosis in oophorectomized women. Lancet 1980;2:1151-3.
30. Hillner BE, Hollenberg JP, Pauker SG. Postmenopausal estrogens in prevention of osteoporosis. Am J Med 1986;80:1115-27.
31. Riggs BL, Seeman E, Hodgson SF, et al. Effect of the fluoride-calcium regimen on vertebral fracture occurrence in postmenopausal osteoporosis: comparison with conventional therapy. N Engl J Med 1982;306:446-50.
32. Barzel US. Estrogens in the prevention and treatment of postmenopausal osteoporosis: a review. Ann Intern Med 1988;85:847-50.
33. Ernster VL, Bush TL, Huggins GR, et al. Benefits and risks of menopausal estrogen and/or progestin hormone use. Prevent Med 1988;17:201-23.
34. Bellantoni MF, Blackman MR. Osteoporosis: diagnostic screening and its place in current practice. Geriatrics 1988;43:63-70.
35. Lufkin EG, Carpenter PC, Ory SJ, et al. Estrogen replacement therapy: current recommendations. Mayo Clin Proc 1988;63:453-60.
36. Anonymous. Consensus conference: osteoporosis. JAMA 1984;252:799-802.
37. Ferguson KJ, Hoegh C, Johnson S. Estrogen replacement therapy. A survey of women's knowledge and attitudes. Arch Intern Med 1989;149:133-6.
38. Byar DP. The Veterans Administration Cooperative Urological Research Group's studies of cancer of the prostate. Cancer 1973;32:1126-30.
39. Vessey MP, Doll R. Investigation of relation between use of oral contraceptives and thromboembolic disease. Br Med J 1968;2:199-205.
40. Sartwell PE, Masi AT, Arthes FG. Thromboembolism and oral contraceptives: an epidemiologic case-control study. Am J Epidemiol 1969;90:365-80.
41. Drill VA. Oral contraceptives and thromboembolic disease. JAMA 1972;219:583-96.
42. Inman WHW, Vessey MP, Westerholm B. Thromboembolic disease and the steroidal content of oral contraceptives—a report to the committee on safety of drugs. Br Med J 1970;2:203-9.
43. Wilson PWF, Garrison RJ, Castelli WP. Postmenopausal estrogen use, cigarette smoking, and cardiovascular morbidity in women over 50: the Framingham Study. N Engl J Med 1985;313:1038-43.
44. Stampfer MJ, Willett WC, Colditz GA, et al. A prospective study of postmenopausal use of estrogen therapy and coronary heart disease. N Engl J Med 1985; 313:1044-9.
45. Henderson BE, Ross RK, Paganini-Hill A, et al. Estrogen use and cardiovascular disease. Am J Obstet Gynecol 1986;154:1181-6.
46. Pfeffer RI, Whipple GH, Kurosaki TT, et al. Coronary risk and estrogen use in postmenopausal women. Am J Epidemiol 1978;107:479-87.
47. Rosenberg L, Armstrong B, Phil D, et al. Myocardial infarction and estrogen therapy in postmenopausal women. N Engl J Med 1976;294:1256-9.
48. Ross RK, Paganini-Hill A, Mack TM, et al. Menopausal oestrogen therapy and protection from death from ischaemic heart disease. Lancet 1981;1:858-60.
49. Pick R, Stamler J, Rodbard S, et al. Estrogen-induced regression of coronary atherosclerosis in cholesterol-fed chicks. Circulation 1952;6:858-61.
50. Paganini-Hill A, Ross RK, Henderson BE. Postmenopausal oestrogen treatment and stroke: a prospective study. Br Med J 1988;297:519-22.
51. Henderson BE, Paganini-Hill A, Ross RK. Estrogen replacement therapy and protection from acute myocardial infarction. Am J Obstet Gynecol 1988;159:312-7.
52. Bush TL, Barrett-Connor E, Cowan LD, et al. Cardiovascular mortality and noncontraceptive use of estrogens in women: results from the Lipid Research Clinics Program follow-up study. Circulation 1987;75:1102-9.
53. Wahl P, Walden C, Knopp R. Effect of oestrogen/progestin potency on lipid lipoprotein cholesterol. N Engl J Med 1983;308:862-7.
54. Wallentin L, Larsson-Cohn U. Metabolic and hormonal effects of postmenopausal oestrogen replacement treatment. II. Plasma lipids. Acta Endocrinol 1977;86:597-607.
55. Silfverstolpe G, Gustafson A, Samsioe G, et al. Lipid metabolic studies in oophorectomized women: effects induced by two different estrogens in serum lipids and lipoproteins. Gynecol Obstet Invest 1980;11:161-9.
56. Lobo RA. Absorption and metabolic effects of different types of estrogens and progestogens. Obstet Gynecol Clin North Am 1987;14:143-67.
57. Goebelsmann U, Mashchak CA, Mishell DR. Comparison of hepatic impact of oral and vaginal administration of ethinyl estradiol. Am J Obstet Gynecol 1985;151: 868-77.
58. Mandel FP, Geola FL, Meldrum DR, et al. Biological effects of various doses of vaginally administered conjugated equine estrogens in postmenopausal women. J Clin Endocrinol Metabol 1983;57:133-9.
59. Jensen J, Riis BJ, Strom V, et al. Long-term effects of percutaneous estrogens and oral progesterone on serum lipoproteins in postmenopausal women. Am J Obstet Gynecol 1987;156:66-71.
60. Chetkowski RJ, Meldrum DR, Steingold KA, et al. Biologic effects of transdermal estradiol. N Engl J Med 1986;314:1615-20.
61. Elkik F, Gompel A, Mercier-Bodard C, et al. Effects of percutaneous estradiol and conjugated estrogens on the level of plasma proteins and triglycerides in postmenopausal women. Am J Obstet Gynecol 1982;143:888-92.
62. King RJB, Whitehead MI. Assessment of the potency of orally administered progestins in women. Fertil Steril 1986;46:1062-6.
63. Hirvonen E, Malkonen N, Manninen V. Effects of different progestogens on lipoproteins during postmenopausal replacement therapy. N Engl J Med 1981; 304:560-3.
64. Jensen J, Nilas L, Christiansen C. Cyclic changes in serum cholesterol and lipoproteins following different doses of combined postmenopausal hormone replacement therapy. Br J Obstet Gynecol 1986;93:613-8.
65. Ottosson UB, Johansson BG, von Schoultz B. Subfractions of high-density lipoprotein cholesterol during es-

trogen replacement therapy: a comparison between progestogens and natural progesterone. Am J Obstet Gynecol 1985;151:746-51.
66. Pfeffer RI, Kunasaki TT, Charlton SK. Estrogen use and blood pressure in later life. Am J Epidemiol 1979; 110:469-78.
67. Utian WH. Effect of postmenopausal estrogen therapy on diastolic blood pressure and body weight. Maturitas 1978;1:3-8.
68. Wren BG, Routledge DA. The effect of type and dose of oestrogen on the blood pressure of post-menopausal women. Maturitas 1983;5:135-42.
69. Lund T, Cameron EC, Hunter WM, et al. A prospective, controlled trial of six forms of hormone replacement therapy given to postmenopausal women. Br J Obstet Gynecol 1979;86(suppl 3):1-29.
70. Luotola H. Blood pressure and hemodynamics in postmenopausal women during estradiol-17-beta substitution. Ann Clin Res 1983;15(suppl 38):9-121.
71. Kaplan NM, Mishell DR, Hammond CB, et al. Management of the postmenopausal woman with hypertension. Case presentation and panel discussion. J Reprod Med 1985;30(suppl):821-6.
72. Kay CR. Progestogens and arterial disease—evidence from the Royal College of General Practitioner's study. Am J Obstet Gynecol 1982;142:762-5.
73. Henderson BE, Ross RK, Lobo RA, et al. Re-evaluating the role of progestogen therapy after the menopause. Fertil Steril 1988;49(suppl):9S-15S.
74. Ross RK, Paganini-Hill A, Gerkins VR, et al. A case-control study of menopausal estrogen therapy and breast cancer. JAMA 1980;243:1635-9.
75. Gambrell RD. The menopause: benefits and risks of estrogen-progestogen replacement therapy. Fertil Steril 1982;37:457-74.
76. Nachtigall LE, Nachtigall RH, Nachtigall RD, et al. Estrogen replacement therapy. II. A prospective study in the relationship of carcinoma and cardiovascular and metabolic problems. Obstet Gynecol 1979;54:74-9.
77. Hammond CB, Jeloesk FR, Lee KL, et al. Effects of long-term estrogen therapy. Am J Obstet Gynecol 1977; 133:537-47.
78. Notelovitz M. When and how to use estrogen therapy in women over 60. Geriatrics 1980;35:113-24.
79. Ziel HK, Finkle WD. Increased risk of endometrial carcinoma among users of conjugated estrogens. N Engl J Med 1975;293:1167-70.
80. Smith DC, Prentice R, Thompson DJ, et al. Association of exogenous estrogens and endometrial carcinoma. N Engl J Med 1975;293:1164-7.
81. Mack TM, Pike MC, Henderson BE, et al. Estrogens and endometrial cancer in a retirement community. N Engl J Med 1976;294:1262-7.
82. McDonald TW, Annegers JF, O'Fallon WM, et al. Exogenous estrogen and endometrial carcinoma: case-control and incidence study. Am J Obstet Gynecol 1977;127:572-80.
83. Ross RK, Paganini Hill A, Mack TM, Henderson BE. Estrogen use and cardiovascular disease. In: Mishell DR Jr, ed. Menopause: physiology and pharmacology. Chicago: Year Book, 1987:209-23.
84. Gray LA, Christropherson WM, Hoover RN. Estrogens and endometrial carcinoma. Obstet Gynecol 1977;49: 385-9.
85. Schiff I, Sela HK, Cramer D, et al. Endometrial hyperplasia in women on cyclic or continuous estrogen regimens. Fertil Steril 1982;37:79-82.
86. Thom MH, White PJ. Prevention and treatment of endometrial diseases in climacteric women receiving oestrogen therapy. Lancet 1979;2:455-7.
87. Elam MB, Lipscomb GE, Chesney CM, et al. Effect of synthetic estrogen on platelet aggregation and vascular release of PGI_2-like material in the rabbit. Prostaglandins 1980;20:1037-51.
88. Silfverstolpe GL, Enk L, Kallfet B, et al. Effects of exogenous oestrogens on the prostacyclin/thromboxane balance in oophorectomized women. Maturitas 1984; 6:184-90.
89. Roncaglioni MC, DiMinno G, Reyers I, et al. Increased prostacyclin-like activity in vascular tissue from rats on long-term treatment with an oestrogen-progestin combination. Thromb Res 1979;14:793-7.
90. Makila U-M, Wahlberg L, Viinikka L, et al. Regulation of prostacyclin and thromboxane production by human umbilical vessels: the effect of estradiol and progesterone in a superfusion model. Prostaglandins Leukotrienes Med 1982;8:115-24.
91. Notelovitz M. Exercise, nutrition, and the coagulation effects of estrogen replacement on cardiovascular health. Obstet Gynecol Clin North Am 1987;14:121-41.
92. Poller L, Thompson JM, Thomas W. Conjugated equine oestrogens and blood clotting: a follow-up report. Br Med J 1977;1:935-6.
93. Notelovitz M, Kitchens CS, Ware MD. Combination estrogen and progestogen replacement therapy does not adversely affect coagulation. Obstet Gynecol 1983; 62:596-600.
94. Aylward M, Maddock J, Rees P. Natural oestrogen replacement therapy and blood clotting. Br Med J 1976;1:220.
95. Notelovitz M, Kitchens CS, Ware MD. Coagulation and fibrinolysis in estrogen-treated surgically menopausal women. Obstet Gynecol 1984;63:621-5.
96. Notelovitz M, Zauner C, McKenzie L, et al. The effect of low-dose oral contraceptives on cardiorespiratory function, coagulation, and lipids in exercising young women: a preliminary report. Am J Obstet Gynecol 1987;156:591-8.
97. Kudrow L. The relationship of headache frequency to hormone use in migraine. Headache 1975;15:36-40.
98. Lobo RA, March CM, Goebelsmann U. Subdermal estradiol pellets following hysterectomy and oophorectomy. Am J Obstet Gynecol 1980;138:714-9.
99. Geola FL, Frumar AM, Tataryn IV, et al. Biological effects of various doses of conjugated equine estrogens in postmenopausal women. J Clin Endocrinol Metab 1980; 51:620-5.
100. Mandel FP, Geola FL, Lu JKH. Biologic effects of various doses of ethinyl estradiol in postmenopausal women. Obstet Gynecol 1982;59:673-9.
101. Horsman A, Jones M, Francis R, et al. The effect of estrogen dose on postmenopausal bone loss. N Engl J Med 1983;309:1404-7.
102. Mashchak CA, Lobo RA, Dozono-Takano R, et al. Comparison of pharmacodynamic properties of various estrogen formulations. Am J Obstet Gynecol 1982; 144:511-8.
103. Riis B, Thomsen K, Christiansen C. Does calcium supplementation prevent postmenopausal bone loss? N Engl J Med 1987;316:173-7.
104. Ettinger B, Genant HK, Cann CE. Postmenopausal bone loss is prevented by treatment with low-dose estrogen with calcium. Ann Intern Med 1987;106:40-5.
105. Christiansen C, Christiansen MS, Transbol I. Bone mass in postmenopausal women after withdrawal of oestrogen/gestogen replacement therapy. Lancet 1981;1:459-61.
106. American College of Gynecologists. Estrogen replacement therapy. Technical bulletin no 93. Washington, DC: American College of Gynecologists, 1986.
107. Magos AL, Brincat M, Studd JWW, et al. Amenorrhea and endometrial atrophy with continuous oral estrogen and progestogen therapy in postmenopausal women. Obstet Gynecol 1985;65:496-9.

Estrogens in the prevention of osteoporosis in postmenopausal women

Harry K. Genant, MD,[a] David J. Baylink, MD,[b] and J. C. Gallagher, MD[c]
San Francisco and Loma Linda, California, and Omaha, Nebraska

Menopause-related bone loss leaves a woman at high risk for fractures. Estrogen use by postmenopausal women, especially when started within 3 years of the last menstrual period, prevents bone loss and reduces the risk of osteoporotic fractures. Estrogens do not restore lost bone. Withdrawal of estrogen therapy is followed by significant bone loss, thus suggesting that long-term therapy is needed. Concomitant progesterone therapy does not impair estrogen's bone-preserving activity. (AM J OBSTET GYNECOL 1989;161:1842-6.)

Key words: Estrogen, menopause, osteoporosis

Osteoporosis currently affects between 15 to 20 million individuals in the United States. About 1.2 million fractures occur annually as a result of osteoporosis, disabling many elderly adults.[1] Vertebral crush fractures are the most frequent fractures, accounting for 50% of all fractures. The risk of sustaining a crush fracture is high, with 25% of individuals over 70 years old showing radiographic evidence of these fractures. About 5% to 10% of these patients remain asymptomatic.[2] Osteoporosis may also account for 150,000 to 200,000 hip fractures annually in the United States, with about 80% resulting from minor trauma. Approximately 10% of patients with hip fracture die within 6 months of the fracture due to surgical complications; another 100,000 to 150,000 patients require institutionalization for rehabilitation after the hip fracture.[2] The total health care costs for fractures in the United States are estimated to be about $6 billion annually.

Bone turnover and postmenopausal loss

The skeleton consists of two bone types: cortical bone, which is responsible for 80% of the total bone mass, and trabecular bone, whose honeycomb structure provides greater surface area per unit volume than does cortical bone. Because the kinetic process of bone resorption and formation (i.e., turnover) occurs on bone surfaces, trabecular bone turns over more rapidly, perhaps four to eight times as fast as cortical bone.[1]

From the [a]Departments of Radiology, Medicine and Orthopedic Surgery, University of California, San Francisco; the [b]Department of Medicine, Loma Linda University and the Mineral Metabolism Section, Jerry L. Pettis Veterans Administration Hospital, Loma Linda; and the [c]Section of Endocrinology and Metabolism, Department of Medicine, Creighton University, Omaha.
Reprint requests: Harry K. Genant, MD, Professor of Radiology, Medicine and Orthopedic Surgery, Department of Radiology, UCSF, Box 0628, San Francisco, CA 94143.
6/0/16772

Table I. Minimum dosages of estrogen to prevent postmenopausal bone loss

Estrogen preparation	*Effective dose (mg)*
Conjugated equine estrogens	0.625
Ethinyl estradiol	0.02
Estrone sulfate	0.625

During the years the skeleton is maturing, and before the perimenopausal period, the amounts of bone resorbed and formed remain approximately equal. Once a woman reaches about 40 years of age, resorption begins to exceed formation by about 0.5% per year. When menopause begins, the amount of bone being resorbed increases sharply, while the amount being formed increases only moderately.[3] Consequently, each year losses of 1% to 3% of cortical bone and up to 5% of trabecular bone occur. The accelerated erosion of bone lasts about 10 to 15 years, after which bone loss is considerably diminished. Bone loss may actually stop after the eighth decade.[3]

Menopause-related bone loss results in a 15% to 30% decrease in trabecular bone and a 10% to 15% reduction in cortical bone.[4] Therefore, estrogen deficiency is responsible for one third to one half the bone lost during a woman's lifetime. Over 20 years, 50% of trabecular and 30% of cortical bone mass will be lost. This kind of reduction in total bone mass leaves a woman at high risk for fracture. By the age of 70 years, 15% of women will experience a wrist fracture, 25% a vertebral fracture, and 15% a hip fracture.

Mechanism of estrogen's actions

The precise mechanism by which estrogen prevents bone loss is not known. Exogenous estrogen administration reverses the biochemical changes associated with estrogen withdrawal, including decreases in serum cal-

Table II. Studies demonstrating beneficial effect of estrogens in oophorectomized women

Study	*Patients*	*Daily estrogen dose*	*Study design*	*Study duration (yr)*	*Bone measurement*	*Comments*
Aitken et al.[10] (1973)	50 Control 53 Treated	Mestranol average dose: 23 μg	Prospective, randomized, double-blind, controlled	1	Radiographic morphometry of hand; photon absorptiometry of third metacarpal	Sixteen patients in treated group and 15 in control group completed <1 year of the study
Meema et al.[11] (1975)	29 Control 53 Treated	0.625 or 1.25 mg CEE	Prospective, nonrandomized, uncontrolled	4+	Radiographic densitometry of third metacarpal	No breakdown of patients receiving 0.625 mg vs. 1.25 mg
Lindsay et al.[6] (1976)	57 Control 63 Treated	Mestranol mean daily dose: 24.8 μg	Prospective, double-blind	5	Photon absorptiometry: midpoint of third metacarpal, right hand	Midradius photon absorptiometry was performed in 46 of 63 treated patients 3 years after entering the study
Lindsay et al.[12] (1978)	14 Control 27 Treated	Mestranol mean daily dose: 27.6 μg	Prospective, double-blind, controlled	8	Same as above	Patients studied were part of original sample described in reference 6
Lindsay et al.[13] (1980)	42 Control 58 Treated	Mestranol mean daily dose: 23.3 μg; range 1 to 30 μg	Prospective, double-blind, controlled	9	Same as above; also photon absorptiometry of the midradius	Same as above
Genant et al.[14] (1982)	9 Control 27 Treated	CEE, 0.15 to 0.6 mg	Prospective, double-blind, controlled	2	Radiogrammetry of second metacarpal midshaft; photon absorptiometry of distal radius; CT of spine	Several CEE doses were used: 0.15 mg (n = 8), 0.3 mg (n = 6), 0.45 mg (n = 8), 0.6 mg (n = 6); determined a dose-response curve: doses <0.6 mg were adequate to prevent vertebral bone loss

CEE, Conjugated equine estrogen; *CT*, computed tomography.

cium, phosphorus, alkaline phosphatase, and osteocalcin levels and urinary output of calcium and hydroxyproline.[2] Conflicting changes are seen in the regulatory hormones that control calcium homeostasis. Levels of parathyroid hormone have been shown to fall, although data on this issue are conflicting.

All estrogens are probably not equally effective against postmenopausal bone loss (Table I) because they have different potencies depending on the end organ being studied.[5] Lindsay et al.[6] found that 25 μg of mestranol was needed to prevent bone loss; 0.625 mg is the accepted dose of conjugated estrogens.

As previously mentioned, estrogen deficiency increases the rate of bone resorption, resulting in more remodeling sites. Estrogen use by a postmenopausal woman stabilizes bone density by preventing further loss.[7,8] This phenomenon can best be explained by considering what occurs during the remodeling cycle: Estrogen reduces the rate of bone resorption, allowing the normal mineralization of the remodeling unit. Once resorption and formation return to equilibrium, usually in 6 to 24 months, bone mass stabilizes due to a slower turnover rate and an improvement in the coupling of bone formation to resorption. However, despite their preventative effects, estrogens do not restore lost bone; thus a woman with poor skeletal density at menopause may still be at risk for fractures even with estrogen replacement.

Prevention of bone loss after oophorectomy

Some 50 years have passed since Albright et al.[9] first described osteoporosis as a consequence of ovarian failure or removal. Estrogen deficiency's major role in the pathogenesis of postmenopausal osteoporosis is now well appreciated. Oral estrogen replacement therapy is the only consistently effective modality currently available to prevent bone loss after either oophorectomy or menopause.

Table III. Studies showing positive effects of estrogens on bone loss in postmenopausal women

Study	*Patients*	*Daily estrogen dose*	*Study design*	*Study duration (yr)*	*Bone measurement*	*Comments*
Recker et al.[15] (1977)	18 Treated 20 Control	0.625 mg CEE + 5 mg methyltestosterone	Randomized, uncontrolled	2	Radiogrammetry; photon absorptiometry of distal radius	
Horsman et al.[16] (1977)	19 Treated 18 Control	Ethinyl estradiol 25 or 50 μg		2	Photon absorptiometry of midpoint of third metacarpal	
Nachtigall et al.[17] (1979)	67 Treated 62 Control	CEE 2.5 mg + medroxyprogesterone	Prospective, randomized, double-blind	10	Photon absorptiometry of midpoint of third metacarpal	
Christiansen et al.[18] (1981)	Part I: 43 Treated 51 Control Part II: 35 Treated 42 Control	Trisequens forte	Prospective, randomized, double-blind	2	Photon absorptiometry	All patients also received 500 mg elemental calcium
Lindsay et al.[19] (1984)	120 Treated 30 Control	CEE	Prospective, single-blind, controlled	2	Photon absorptiometry and radiogrammetry of third right metacarpal	30 patients each received CEE 0.15 mg, 0.3 mg, 0.625 mg, 1.25 mg
Ettinger et al.[20] (1987)	15 Treated 25 Control	CEE 0.3 mg	Nonblinded, uncontrolled	2	Photon absorptiometry of distal radius; dual photon absorptiometry of L2-L4	All treated patients also received 1500 mg elemental calcium
Riis et al.[21] (1987)	11 Treated 11 Control	3 mg 17-β-estradiol percutaneously	Randomized, double-blind, controlled	2	Same as above	
Genant et al.[22] (1989)	94 Treated 28 Control	Estrone sulfate 0.3125 mg, 0.625 mg, 1.25 mg	Double-blind, randomized, controlled	1	CT of spine	All patients also received 1000 mg of elemental calcium

CEE, Conjugated equine estrogen; *CT*, computed tomography.

In several studies estrogens have been shown to reduce cortical and vertebral bone loss significantly in oophorectomized women (Table II).[6, 10-14] Of particular significance was the finding of a relationship between estrogen dose and vertebral response.[14] Specifically, doses of conjugated equine estrogens less than 0.6 mg/day were inadequate to prevent vertebral bone loss consistently.

Various forms of estrogen are also effective in preventing bone loss in postmenopausal women. Table III[15-22] lists several studies that reported consistent benefit from the use of estrogens. Nachtigall et al.[17] were the first to show long-term benefits with estrogens in a prospective, double-blind study. They found that conjugated equine estrogens with progesterone protected against postmenopausal bone mass loss for at least 10 years. Furthermore, estrogen prevented bone loss most effectively when begun within 3 years of the last menstrual period, during which interval the rate of bone loss is greatest. Lindsay et al.[19] and Christiansen et al.[18] independently demonstrated that estrogens were effective in preventing bone loss and that withdrawal of therapy was followed by significant bone loss, suggesting that long-term therapy is needed. Ettinger et al.[20] showed that a reduced dose of conjugated estrogen (0.3 mg) combined with 1500 mg of total calcium offered the same level of protection against bone loss as the 0.625 mg estrogen dose alone. More recently, another form of estrogen, estrone sulfate, protected against vertebral bone loss after a 1-year trial at doses of 0.625 and 1.25 mg ($p < 0.05$ versus placebo).[22]

Effect of estrogens on fracture incidence

Although data establishing estrogens' effectiveness against bone loss after oophorectomy and menopause are convincing, the real measure of estrogen therapy's efficacy is its effect on the risk of fracture. Numerous studies (Table IV)[23-29] support the efficacy of estrogen therapy in reducing fractures. Although estrogen therapy does not completely eliminate osteoporotic fractures, it does significantly reduce their occurrence.

Studies have consistently demonstrated that estrogens reduce the incidence of wrist fractures and, most importantly, hip fractures. Collectively, these data show an inverse relationship between the risk of hip fracture and estrogen use and predict a 50% reduction in the rate of fractures with long-term estrogen use. In a prospective, randomized trial, Lindsay et al.[26] found that

Table IV. Effect of estrogens on rates of fracture

Study	*Patients*	*Study design*	*Length of study (yr)*	*Fracture site studied*	*Comments*
Gordan et al.[23] (1973)	120 Postmenopausal women	Prospective, uncontrolled, nonrandomized	25	Unspecified	Total of 1664 patient-years studied; all patients received estrogens within 2 years of entering study
Hammond et al.[24] (1979)	301 Cases 309 Control	Retrospective	5+	Unspecified	Thirty percent of treated used 0.625 mg, 33.4% used 1.25 mg of CEE; 15% of controls fractured, 8.6% of treated patients fractured
Hutchinson et al.[25] (1979)	80 Cases 30 Control	Retrospective, case-control	3	Hip and distal radius	Odds ratio 3.0; increases to 3.8 if estrogens begun within 5 years of menopause
Lindsay et al.[26] (1980)	58 Treated 42 Control	Prospective, nonrandomized	Mean of 9	Spine and radius	Significant reduction in wedge deformities of T9 and L2
Weiss et al.[27] (1980)	327 Cases 576 Control	Retrospective, case-control	1	Hip and distal radius	Relative risk 1.0 for no estrogen use, 0.43 for current estrogen use
Paganini-Hill et al.[28] (1981)	91 Cases 166 Control	Retrospective, case-control	5	Hip	Risk ratio for estrogen used >60 months was 0.42
Kreiger et al.[29] (1982)	98 Cases 884 Control	Retrospective, case-control	2	HIp	Odds ratio for fracture was 0.5 for estrogen use >6 months

CEE, Conjugated equine estrogen.

estrogens reduced vertebral deformities in oophorectomized women.

Effect of estrogens with progesterone in preventing bone loss

Progestational agents are often added to an estrogen regimen to prevent the complications of unopposed estrogen on the endometrium. Initial studies using either C-21 progestins (medroxyprogesterone) or 19-norprogestogens (norgestrel and norethindrone) combined with estrogens have shown that progesterone does not impair estrogen's bone-preserving function.[20, 21, 30] Used alone, 19-nor-17β-hydroxyprogesterone protects against bone loss.[31] In studying the use of norgestrel and norethindrone (also 19-norprogestins) alone, Hart et al.[32] determined that only norethindrone was effective over a 2-year period. Additional study is needed to confirm the osteotrophic role of progestogens when used as single-agent therapy.

Prevention versus treatment of osteoporosis with estrogens

Data support the practice of starting hormonal replacement therapy as soon after menopause begins as possible to achieve maximum beneficial effects. Because menopause-induced bone loss is greatest in the 5 to 10 years immediately after menopause and because estrogen will only prevent loss of bone mass, it is uncertain whether a woman should begin estrogen replacement therapy 10 to 15 years after menopause if osteoporosis is the only indication for its use.

The duration of therapy is less well defined. Existing data suggest that estrogen's protective effects persist only as long as it is being taken. Discontinuation of therapy causes rapid bone loss.[12] Epidemiologic data suggest that at least 6 years of therapy are need to reduce the risk of osteoporotic fractures significantly.[28] Postmenopausal women should receive estrogens for at least 15 years, and some investigators recommend longer term therapy. The risk of long-term estrogen use must be balanced against the long-term benefits.

Because the use of estrogen replacement therapy for preventing bone loss requires long-term dosing, patient education is essential. Only 30% of women comply with their prescribed estrogen regimen.[33] In one review, of those women receiving estrogens for the first time, 20% to 30% never had the prescription filled, 20% stopped therapy within the first 9 months, and another 10% used it on an intermittent basis (i.e., when they remembered to). Intermittent therapy may be sufficient to alleviate vasomotor symptoms, but is not adequate for preventing postmenopausal bone loss.

Conclusion

Estrogen replacement therapy that is begun at the time of menopause prevents bone loss and decreases fractures. The use of estrogen in established osteoporosis is less well accepted, because estrogens do not replace bone mass that has been lost. Postmenopausal women should take estrogens for at least 15 years, after which time continuing therapy may or may not be indicated.

REFERENCES

1. Riggs BL, Melton LJ. Involutional osteoporosis. N Engl J Med 1986;314:1676-86.
2. Lindsay R. Prevention of postmenopausal osteoporosis. Obstet Gynecol 1987;14:63-76.

3. Mazess RB. On aging bone loss. Clin Orthop 1982; 165:239-52.
4. Ettinger B. Prevention of osteoporosis: treatment of estradiol deficiency. Obstet Gynecol 1988;72:12S-7S.
5. Mashchak CA, Lobo RA, Donzono-Takano R, et al. Comparison of pharmacodynamic properties of various estrogen formulations. Am J Obstet Gynecol 1982;144: 511-8.
6. Lindsay R, Aitken JM, Anderson JB, et al. Long-term prevention of postmenopausal osteoporosis by estrogen. Lancet 1976;1:1038-40.
7. Ettinger B. Estrogen, progestogen and calcium in treatment of postmenopausal women. In: Genant HK, ed. Osteoporosis update. San Francisco: Radiology and Education Foundation.
8. Christiansen C, Christensen MS, McNair P, et al. Prevention of early postmenopausal bone loss: controlled 2 year study in 315 normal females. Eur J Clin Invest 1980; 10:273-9.
9. Albright F, Smith PH, Richardson AM. Postmenopausal osteoporisis: its clinical features. JAMA 1941;116:2465-74.
10. Aitken JM, Hart DM, Lindsay R. Oestrogen replacement therapy for prevention of osteoporosis after oophorectomy. Br Med J 1973;3:515-8.
11. Meema S, Bunker ML, Meema HE. Preventive effect of estrogen on postmenopausal bone loss. Arch Intern Med 1975;135:1436-40.
12. Lindsay R, MacLean A, Kraszewski A, et al. Bone response to termination of estrogen treatment. Lancet 1978;1: 1325-7.
13. Lindsay R, Hart DM, Forrest C, Baird C. Prevention of spinal osteoporosis in oophorectomized women. Lancet 1980;2:1151-4.
14. Genant HK, Cann CE, Ettinger B, et al. Quantitative computed tomography of vertebral spongiosa: a sensitive method for detecting early bone loss after oophorectomy. Ann Intern Med 1982;97:699-705.
15. Recker RR, Saville PD, Heaney RP. Effects of estrogens and calcium carbonate on bone loss in postmenopausal women. Ann Intern Med 1977;87:649-55.
16. Horsman A, Gallagher JC, Simpson M, Nordin BEC. Prospective trial of oestrogen and calcium in postmenopausal women. Br Med J 1977;2:789-92.
17. Nachtigall LE, Nachtigall RH, Nachtigall RD, et al. Estrogen replacement therapy I: a 10-year prospective study in the relationship to osteoporosis. Obstet Gynecol 1979; 53:277-81.
18. Christiansen C, Christensen MS, Transbol I. Bone mass in postmenopausal women after withdrawal of oestrogen/gestagen replacement therapy. Lancet 1981;1:459-61.
19. Lindsay R, Hart DM, Clark DM. The minimum effective dose of estrogen for prevention of postmenopausal bone loss. Obstet Gynecol 1984;63:759-63.
20. Ettinger B, Genant HK, Cann CE. Postmenopausal bone loss is prevented by treatment with low-dosage estrogen with calcium. Ann Intern Med 1987;106:40-5.
21. Riis B, Thomsen K, Christiansen C. Does calcium supplementation prevent postmenopausal bone loss? N Engl J Med 1987;316:173-7.
22. Genant HK, Baylink DL, Gallagher JC. Effect of estrone sulfate on postmenopasual bone loss. (submitted for publication)
23. Gordan GS, Picchi J, Roof BS. Antifracture efficacy of long-term estrogens for osteoporosis. Trans Assoc Am Phys 1973;86:326-32.
24. Hammond CB, Jelovsek FR, Lee KL, et al. Effects of long-term estrogen replacement therapy. I. Metabolic effects. Am J Obstet Gynecol 1979;133:525-36.
25. Hutchinson TA, Polansky SM, Feinstein AR. Postmenopausal oestrogens protect against fractures of hip and distal radius. Lancet 1979;2:705-9.
26. Lindsay R, Hart DM, Forrest C, et al. Prevention of spinal osteoporosis in oophorectomized women. Lancet 1980; 2:1151-3.
27. Weiss NS, Ure CL, Ballard JH, et al. Decreased risk of fractures of the hip and lower forearm with postmenopausal use of estrogens. N Engl J Med 1980;303:1195-8.
28. Paganini-Hill A, Ross RK, Gerkins VR, et al. Menopausal estrogen therapy and hip fractures. Ann Intern Med 1981;95:28-31.
29. Kreiger N, Kelsey JL, Holford TR, et al. An epidemiologic study of hip fracture in postmenopausal women. Am J Epidemiol 1982;116:141-8.
30. Gallagher JC, Goldgar D, Kable WT. Comparison of estrogen and progestin therapy on cortical and trabecular bone using SPA, DPA and QCT. Calcified Tissue International 1989;44:153.
31. Lindsay R, Hart DM, Purdie D, et al. Comparative effects of oestrogen and a progestogen on bone loss in postmenopausal women. Clin Sci Mol Med 1978;54:193-5.
32. Hart DM, Abdalla H, Clarke D, et al. Preservation of bone mass in postmenopausal women during therapy with estrogen and progestogens. In: Christiansen C, Arnaud CD, Nordin BEC, et al, eds. Osteoporosis: Proceedings of the Copenhagen International Symposium on Osteoporosis. Copenhagen: Aalborg Stiftsborgtrykkeri, 1984:697-9.
33. Ravnikar VA, Compliance with hormonal therapy. Am J Obstet Gynecol 1987;156:1332-4.

Estrogen replacement and cardiovascular disease: Serum lipids and blood pressure effects

William R. Hazzard, MD
Winston-Salem, North Carolina

Coronary heart disease, a major cause of morbidity and death, is the leading cause of death in older women, with an incidence that approaches that in men of comparable age. Estrogen favorably alters lipid metabolism and should therefore diminish the risk for coronary heart disease in estrogen users. Epidemiologic data from case-control and prospective cohort studies have suggested that estrogen use may confer protection from cardiovascular disease and decrease all-cause mortality rates in postmenopausal women. Because the age-adjusted mortality rate due to heart disease among American women is approximately four times the combined mortality rate due to endometrial and breast cancers, even modest changes in the risk of fatal heart disease after estrogen use would dramatically impact the overall risk-benefit equation. (Am J Obstet Gynecol 1989;161:1847-53.)

Key words: Estrogen, cardiovascular disease, lipids, blood pressure

Cardiovascular disease is a major cause of morbidity and death in modern society. Risk factors for cardiovascular disease include elevated blood pressure, impaired glucose tolerance, abnormal blood lipid levels, cigarette smoking, male sex, and advanced age. Elevated blood pressure and alterations in lipoprotein metabolism are major contributors to cardiovascular disease in general and to coronary heart disease in particular.[1-3]

Lipids, lipoproteins, and coronary heart disease risk

Prospective epidemiologic studies over the past three decades have documented a linear relationship between dietary fat consumption, serum total cholesterol, and the incidence of coronary heart disease.[4-15] These population studies were stimulated by the observation that patients with clinical manifestations of coronary heart disease had higher cholesterol levels than did control subjects. Longitudinal epidemiologic studies have documented an association between coronary heart disease risk in the general population and lipoprotein values, which are proportional to the serum total cholesterol.[6] Coronary heart disease risk increases as cholesterol values increase. Lipid components can be evaluated in terms of serum total cholesterol, triglycerides, and high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) cholesterol fractions. The relationship between increased triglyceride levels and coronary heart disease risk is not clear; other blood lipid measurements provide a better assessment of risk.

An inverse relationship exists between HDL cholesterol levels and coronary heart disease risk: the lower the HDL concentration, the greater the risk for coronary heart disease. This significant inverse relationship between HDL cholesterol levels and the incidence of myocardial infarction, angina pectoris, and cardiac death in people 50 years old or older holds true even when studies control for risk factors such as age, other cholesterol components, cigarette smoking, high blood pressure, weight gain, and diabetes mellitus.[3, 16]

Simplistically, elevations of any of the lipoprotein fractions, except HDL, are associated with an increased risk of coronary heart disease.[16-19] Elevated HDL levels, the fraction thought to be involved with clearing cholesterol from tissues and its subsequent metabolism and excretion, is associated with decreased coronary heart disease risk.[16, 17, 19]

Atherosclerosis and coronary heart disease share a common multifactorial origin. At any level of serum total cholesterol, risk will vary over a wide range depending on other associated risk factors. Reductions in any lipoprotein fraction other than HDL are assumed to prevent or reduce atheromatous plaque formation and prolong the lives of high-risk patients.[19]

Initial observations on estrogens and coronary heart disease risk

A definite rise in population total cholesterol and LDL cholesterol levels occurs across the menopause in women.[20] Postmenopausal status is accompanied by a gradual and progressive increase in the incidence of

From the Department of Internal Medicine, Bowman Gray School of Medicine.
Reprint requests: William Hazzard, MD, Professor and Chairman, Department of Internal Medicine, Bowman Gray School of Medicine, 300 S. Hawthorne Rd., Winston-Salem, NC 27103.
6/0/16684

coronary heart disease and its associated morbidity and death.[20] Coronary heart disease is the leading cause of death in older women, with an incidence that comes close to approximating that in men of comparable age. A common factor that may be responsible for the marked rise in this risk among postmenopausal women in general would appear to be the decline in estrogen levels and the resulting rise in LDL cholesterol levels.

Mortality rates due to coronary heart disease began to decline in the 1960s, and by 1976 had decreased by more than 30% in women and 20% in men.[21] This decline has been widely attributed to hygienic alterations in life-style. For instance, the decrease in mortality rates was accompanied by decreases in the proportion of male (by 26%) and female (by 8%) cigarette smokers between 1964 and 1976. During this same period, the percentage of men and women aged 55 to 64 years with serum cholesterol values greater than 260 mg/dl decreased by 14% and 29%, respectively.[21] Since the early 1960s, postmenopausal estrogen use has been widespread in the United States. Although not yet demonstrated in systematic population studies, some of the decline in coronary heart disease deaths in women since 1964 may be attributable to postmenopausal hormone replacement therapy. Other factors may include the decline in cigarette smoking, earlier detection and treatment of hypertension, decline in consumption of animal fats, other secular trends toward a more hygienic life-style shared in common with men, and the more effective treatment of this disease.

The possible protective role of exogenous estrogens has been controversial for decades. The early negative association between estrogens and atherosclerosis was based on the observation that men under 55 years of age had five to eight times the risk of coronary artery disease as did women of similar age. Researchers therefore suggested that estrogen might protect against progression of atherosclerosis. This association was further supported by the apparent increase in coronary artery disease among climacteric women. However, in the 1960s (the Coronary Drug Project), men with a previous history of myocardial infarction who received a high dose (5 mg/day) of conjugated estrogens had a risk of nonfatal myocardial infarction (6.2%) greater than that in the placebo group (3.2%).[22] In a subsequent evaluation, men given 2.5 mg/day of conjugated estrogens demonstrated a mortality rate of 19.9% compared with 18.8% for the placebo group.[23] As a result, both estrogen treatment arms were terminated early, and estrogen fell from favor as a possible form of secondary coronary disease prevention.

In contrast, other reports have suggested that estrogen use protects against the development of cardiovascular disease. Evidence from animal and autopsy studies suggests that exogenous estrogen may inhibit the development of atherosclerotic lesions.[24-26] Epidemiologic data from case-control and prospective cohort studies have suggested that postmenopausal estrogen use may confer a substantial degree of protection from cardiovascular disease.[24, 26-32]

Resolution of this paradox requires considering the pathophysiology of atherogenesis and the relative contributions of different phenomena at different stages in the natural history of atherogenesis. Women are physiologically protected by long-standing endogenous estrogen secretion; thus the process of atherosclerosis is retarded and superimposed thrombosis is unlikely. In such women, the net effect of estrogen is to prevent coronary disease.

In contrast, middle-aged or elderly men have had years of testosterone secretion with its adverse effects on lipoprotein cholesterol levels. Therefore, the effect of estrogen in men may be to enhance thrombosis, possibly resulting in coronary or cerebral artery occlusion. The effect of estrogen in increasing coagulation diathesis is well known: Estrogen not only increases platelet adhesiveness but also decreases the levels of factors that inhibit thrombosis, such as antithrombin III. Thus what is good for the goose may not be good for the gander!

Evaluating hormone replacement therapy relative to coronary heart disease risk, particularly in view of the small, but definite, risk of endometrial carcinoma associated with this form of therapy, is important. Of current concern is the effect of hormone replacement therapy on coronary heart disease risk factors such as blood pressure levels and lipoprotein profiles, and whether postmenopausal estrogens affect the risk for this disease.

Hormone replacement therapy and blood pressure

Possible evidence of an association between estrogen administration and hypertension was first published in 1967.[33, 34] Initially investigators assumed that hormone replacement therapy could either induce or aggravate hypertension. However, most reports published in the late 1970s and early 1980s failed to demonstrate a consistent, significant association between hormone replacement therapy and elevated blood pressure.[20, 24, 27, 33-35] Mashchak and Lobo[33] have reviewed epidemiologic case-control and prospective, double-blind clinical studies that clearly indicate that hormone replacement therapy does not increase the incidence of hypertension or aggravate preexisting hypertension:

Case-control studies

Davis et al.[36] (1961) reported the absence of an association between hormone replacement therapy and hypertension.

Stern et al.[37] (1976) demonstrated no relationship between current estrogen use and blood pressure alterations in a study that adjusted for age and weight.

Pallas et al.[38] (1977) found no significant increase in mean blood pressure (with or without controlling for age and weight) in women using conjugated estrogens alone. Plasma renin activity, plasma renin substrate, and renin reactivity, but not plasma renin concentration, were elevated.

Pfeffer[39] (1978) found a significant increase in the incidence of hypertension in nondiabetic women (58 to 79 years) taking hormone replacement therapy in a study controlled for age, diabetes mellitus, and stroke but not for weight. Mashchak and Lobo[33] (1979) found no change in blood pressure in either estrogen users or age-matched women who did not use estrogen followed for 5 months.

Barrett-Connor et al.[40] (1979), studying a group controlled for age and weight, found no significant difference between the systolic or diastolic blood pressures of estrogen users and control subjects. Estrogen users 60 to 64 years old had a significantly lower diastolic blood pressure than nonusers.

Persson et al.[41] (1983) showed a significantly higher prevalence of estrogen use in women with hypertension (17% versus 14%); however, 89% of the women were hypertensive before the start of hormone replacement therapy.

Prospective, randomized clinical trials

Lind et al.[42] (1979) observed a small, but statistically significant, fall in mean systolic and diastolic blood pressures in women of similar age (49 to 54 years) who were receiving conjugated estrogen therapy between 6 and 48 months after menopause. Both systolic and diastolic blood pressures returned to pretreatment levels by 3 months after the end of therapy.

Wren and Routledge[34] (1981), in a trial comparing conjugated equine estrogens and piperazine estrone sulfate, reported that 9% and 25% of subjects taking piperazine estrone sulfate and conjugated estrogens, respectively, had an increase in blood pressure, and 25% and 14%, respectively, had a decrease in blood pressure. A second study[35] (1983) demonstrated a slight, but statistically significant, fall in blood pressure when piperazine estrone sulfate was administered. This effect was not seen in women given conjugated estrogens for the same length of time. In fact, 15% of these patients had an increase in blood pressure of at least 15 mm Hg.

Lindsay and Herrington[43] (1983) followed postmenopausal women taking hormone replacement therapy for 3 years and suggested that hypertension in postmenopausal women was related more to obesity than to this therapy. Blood pressure tended to fall in women of normal weight, whereas diastolic and systolic blood pressures tended to rise in the placebo group. Among obese subjects, the blood pressure rise in the estrogen-treated group was not greater than that in the placebo group.

Luotola[44] (1983), in a crossover study, evaluated a normotensive and a hypertensive group's response to estradiol; blood pressure fell significantly in each 4-week treatment cycle for both study groups.

The rare occurrence of estrogen-induced hypertension cannot be disputed. However, these hypertensive episodes represent idiosyncratic reactions to hormone replacement therapy. The most carefully designed studies of blood pressure response to this therapy strongly suggest that blood pressure (both systolic and diastolic) is consistently, although minimally, lowered by estrogen administration.

Hormone replacement therapy, lipids, and lipoproteins

Both natural and surgical menopause are accompanied by adverse changes in serum lipids and lipoproteins that are associated with an increased risk of coronary heart disease.[45] As explained above, the adverse changes are related to cessation of ovarian estrogen production. We have long realized that hepatic protein synthesis and lipid metabolism are sensitive to the effects of exogenous estrogens and can be modified by the type of estrogen, dose, duration, and route of administration.[46-62] Estrogen-induced effects on liver protein synthesis vary depending on which protein is assessed; postmenopausal women's responses to hormone replacement therapy vary between and within organ systems.[46, 47, 63]

Overall, postmenopausal estrogens lower the serum concentration of LDL cholesterol* and increase the serum concentration of HDL cholesterol,† HDL_2,[26, 59, 62] apoprotein AI,[26, 59, 62] and apoprotein AII.[59] In most of the above-cited studies there was no change or a decrease in total cholesterol,[62] an increase in total triglycerides,[25, 57, 62] and no change in HDL_3[59, 62] after estrogen use. Hormone replacement therapy thus beneficially alters the LDL/HDL ratio in both the numerator and the denominator. The overall total cholesterol value may not change because decreases in LDL may be balanced, at least partially, by increases in HDL. An inverse relationship between the plasma concentration of HDL and apoprotein AI and the development of atherosclerotic cardiovascular disease and myocardial infarction has been reported.[59, 62] Therefore, the effect of postmenopausal hormone replacement therapy on lipid metabolism may be to diminish the risk of coronary heart disease in estrogen users.[63]

Hormone replacement therapy and coronary heart disease risk: Results of studies

In a 10-year prospective, double-blind study, Nachtigall et al.[64] compared the effects of high-dose (2.5 mg)

*References 25, 26, 52, 54, 56, 61, 62.
†References 25, 26, 53, 54, 59, 61, 62.

Table I. Case-control, cross-sectional studies of hormonal replacement therapy and coronary heart disease

Study	Population	Size	End point	Relative risk			
				Ever used		Current use	
				Crude	Adjusted	Crude	Adjusted
Rosenberg et al.[68] (1976)	Hospital	336 Cases 6730 Controls	Nonfatal MI	NR	NR	0.5	1.0
Pfeffer et al.[69] (1978)	Community	185 Cases 511 Controls	Fatal/nonfatal MI	0.9	0.9	0.6	0.7
Jick et al.[70] (1978)	Hospital	14 Cases 21 Controls	Nonfatal MI	NR	NR	4.2	NR
Rosenberg et al.[71] (1980)	Hospital	477 Cases 1832 Controls	Nonfatal MI	1.2 (Past use)	NR	1.0	NR
Ross et al.[72] (1981)	Community	133 Cases 133 Living controls	Fatal IHD	0.4	0.4	NR	NR
Bain et al.[73] (1981)	Community	64 Cases 1390 Controls	Nonfatal MI	0.9	0.8	0.7	0.7
Adam et al.[74] (1981)	Community	76 Cases 151 Controls	Fatal MI	0.6	NR	0.8	NR
Szklo et al.[75] (1984)	Hospital	39 Cases 39 Controls	Nonfatal MI	0.8	0.6	—	—

NR, Not reported; *MI,* myocardial infarction; *IHD,* ischemic heart disease.

conjugated equine estrogen and medroxyprogesterone acetate (10 mg) with placebo in 84 pairs of randomly chosen postmenopausal inpatients matched for age and diagnosis. Those in the treated group who began the study with elevated LDL/HDL ratios experienced a reduction in that ratio over the course of the study. In the control group, LDL/HDL ratios were either maintained or increased. The treatment group's response occurred within the first 6 months of the study, and lipoprotein levels changed little over the succeeding 0.5 years of follow-up. The treatment group's overall survival rate was higher, although not significantly so, than the placebo group's rate.

The Lipid Research Clinics Program Follow-up Study[25] is the most authoritative study of cardiovascular disease risk in postmenopausal women using estrogens. This study reported that the age-adjusted relative risk for death from cardiovascular disease among estrogen users compared with nonusers was 0.34 (95% confidence limits, range 0.12 to 0.81). Multivariate analysis for age, blood pressure, and cigarette smoking estimated the relative risk for atherosclerotic cardiovascular disease in estrogen users to be 0.37 (95% confidence limits, range 0.16 to 0.88), again a highly significant reduction. When investigators analyzed the role of alterations in HDL and LDL concentrations, they found that more than half of the beneficial effect on cardiovascular disease risk could be attributed to the statistically significant LDL and HDL alterations. In particular, alterations in the HDL concentrations were individually and significantly associated with reduced cardiovascular disease and all-cause mortality rates in this cohort. These data are comparable to those of other studies linking high plasma HDL levels to low coronary death rates[16-19] and suggest an important postmenopausal benefit for hormone replacement therapy.

These data appear to conflict with an earlier report from The Framingham Study[65] that indicated a 50% elevated risk of cardiovascular disease morbidity ($p < 0.01$) in postmenopausal estrogen users. This report also noted a twofold risk for cerebrovascular disease in estrogen users compared with nonusers. An increased incidence of myocardial infarction was observed, particularly among estrogen users who smoked cigarettes. Nonsmoking estrogen users experienced only an increased risk of stroke. However, the postmenopausal estrogen user group included all women who had ever used postmenopausal estrogens in the 8 years before the twelfth biennial examination (1970 to 1972), and the overall user rate was low (24.4%). The Framingham Study data further suggested that estrogen use at baseline was less than 15%.

A separate analysis of the data,[66] excluding angina pectoris as an end point, demonstrated a significant protective effect for estrogen use in women 50 to 59 years old. This analysis suggests that age at menopause should be considered a covariate and is inversely associated with cardiovascular disease risk at a marginally significant level ($p = 0.04$). In this age group there was no increase in all-cause mortality rates, and cardiovascular disease risk decreased in estrogen users. No association was found between estrogen use and cardiovascular disease risk in older nonsmoking women. The original analysis adjusted for HDL level and thus possibly underestimated the benefits of postmenopausal estrogen use.[67]

Lipid, lipoprotein, and blood pressure responses to hormone replacement therapy predict a reduction in

Table II. Prospective cohort studies of hormonal replacement therapy and coronary heart disease risk

Study	Population	Size	End point	Relative risk	
				Crude	Adjusted
Burch et al.[76] (1974)	Surgical Practice	737	Fatal CHD	0.4	—
Gordon et al.[45] (1978)	Community	18,334 Person-years	CHD	1.6	—
			Angina	2.3	—
Hammond et al.[77] (1979)	Hospital	301 Users 309 Nonusers	CVD	0.3	—
Petitti et al.[78] (1979)	Kaiser Permanente	16,759	Nonfatal/fatal MI	1.2	—
Nachtigall et al.[61] (1979)	Hospital	84 Matched pairs	Nonfatal/fatal MI	0.3	—
Henderson et al.[79] (1985)	Community	7,610	All-cause mortality	0.8	—
			Acute MI	0.5	—
Wilson et al.[65] (1985)	Community	1,234	CHD	1.9	—
Stampfer et al.[31] (1985)	Nurses	32,317	CHD	0.3	0.5
Petitti et al.[80] (1986)	Kaiser Permanente	16,638	Fatal CVD	0.5	—
Bush et al.[25] (1987)	LRC	2,270	Fatal CVD	0.3	—
Criqui et al.[32] (1988)	Community	1,868	CHD mortality	—	0.7 (all-cause)

CHD, Coronary heart disease; *CVD,* cardiovascular disease; *MI,* myocardial infarction; *LRC,* Lipid Research Clinics.

coronary heart disease risk for women receiving this therapy. To date, no randomized trials have been conducted to determine the risk for atherosclerotic cardiovascular disease in women taking estrogens. Therefore, support for the benefits of hormone replacement therapy has come from epidemiologic surveys of case-control, cross-sectional, and prospective cohort design. Most studies[24-32, 64] show that hormone replacement therapy reduces the risk of cardiovascular disease and all-cause mortality rates in postmenopausal women (Tables I and II). Postmenopausal estrogen users fare better for each case-specific end point and significantly better for all-cause mortality rates compared with nonusers.

If cardiovascular disease risk is influenced by postmenopausal estrogen use, as the epidemiologic data suggest, then the associated influence on death will be a primary factor in balancing the risks against the benefits of hormone replacement therapy. For example, even minor relative changes in the risk of fatal heart disease after estrogen use would have a major impact on the overall risk-benefit equation, because the age-adjusted mortality rate due to ischemic heart disease among women in the United States is approximately four times the combined mortality rate due to endometrial and breast cancers.[29,30] In one study that set the relative risk of cardiovascular disease at 1.0, when the risk was reduced by as little as 20% (relative risk = 0.8), there was a net fall in mortality rates of 10% for high-dose (1.25 mg conjugated equine estrogens) therapy and of nearly 18% for moderate-dose (0.625 mg conjugated equine estrogens) therapy.[79] If risk were reduced by 50% (relative risk = 0.5), the net decrease in mortality rate would be in excess of 33% and 41% with high- and moderate-dose therapy, respectively.[28] These estimations underline the importance for determining with certainty any dose-response relationship between estrogen use and coronary heart disease.

Clinicians must consider the possible effects of estrogen therapy on the entire risk-benefit equation. Furthermore, epidemiologists must continue to refine these calculations through additional research. Future research needs must address the following issues before any firm conclusions regarding the effect of hormone replacement therapy on cardiovascular disease are established:

- Further defining the effect of hormone replacement therapy on lipoprotein subfractions, apoproteins, and systolic and diastolic blood pressures
- Determining dose-response relationships between estrogen administration and serum lipids, lipoprotein levels, and blood pressure changes, with a better definition of the effects of different estrogen preparations, routes of administration, dose, and duration of action on these parameters
- Improving definition of the effects of adding progestational agents to hormone replacement therapy regimen relative to lipoprotein and blood pressure response
- Describing the effects of estrogen/progesterone in patients with hyperlipoproteinemia and hypertension
- Undertaking prospective studies of the effect of hormone replacement therapy on cardiovascular and total mortality rates
- Clarifying the interactions between hormone replacement therapy and known risk predictors of coronary heart disease

Summary

Studies have revealed no clear evidence for adverse estrogen-induced effects on blood pressure levels. Post-

menopausal women's blood pressure, both systolic and diastolic, is consistently lowered by estrogen administration, although the decrease is small. Hormone replacement therapy does not aggravate preexisting hypertension. Oral hormone replacement therapy reverses the negative postmenopausal trends in lipoprotein profiles by decreasing LDL cholesterol and increasing HDL cholesterol levels. The significant improvement in lipoprotein profiles, particularly increases in HDL concentrations, implies cardioprotection and should predict a reduced risk of atherosclerotic cardiovascular disease. Epidemiologic data demonstrate that deaths due to myocardial infarction are significantly reduced in postmenopausal estrogen users. Because of its effects on lipoprotein profiles and blood pressure, hormone replacement therapy has been associated with lower overall morbidity and mortality rates due to coronary and total mortality. The available data clearly support using hormone replacement therapy in postmenopausal women.

REFERENCES

1. Shaw J. Antihypertensive drugs—impact of different classes of coronary heart disease risk profiles. Clin Ther 1987;9(suppl D):31-6.
2. Gordon T, Kannel WB. Premature mortality from coronary heart disease. The Framingham Study. JAMA 1971; 215:1617-25.
3. Kannell WB. Hypertension: relationship with other risk factors. Drugs 1986;31(suppl 1):1-11.
4. Keys A. Coronary heart disease in seven countries. Circulation 1970;41:1-195.
5. Kannel WB, Dawber TR, Friedman GD, et al. Risk factors in coronary heart disease: an evaluation of several serum lipids as predictors of coronary heart disease. The Framingham Study. Ann Intern Med 1964;61:888-99.
6. Kannel WB, Castelli WP, Gordon T. Cholesterol in the prediction of atherosclerotic disease. Ann Intern Med 1979;90:85-91.
7. Kannel WB. Status of risk factors and their consideration in antihypertensive therapy. Am J Cardiol 1987;59:80A-90A.
8. Kannel WB. Hypertension and other risk factors in coronary heart disease. Am Heart J 1987;114:918-25.
9. McGee D, Gordon T. The Framingham Study: the results of the Framingham Study applied to four other US-based epidemiologic studies of cardiovascular disease. DHEW publ no (NIH) 76-1083. Washington, DC: US Government Printing Office, 1976.
10. Carlson LA, Bottiger LE. Ischemic heart disease in relation to fasting values of plasma triglycerides and cholesterol: Stockholm Prospective Study. Lancet 1972;22: 865-8.
11. Gordon T, Garcia-Palmieri MR, Kagan A, et al. Differences in coronary heart disease in Framingham, Honolulu and Puerto Rico. J Chron Dis 1974;27:329-44.
12. Wilhelmsen L, Wedel H, Tibblin G. Multivariate analysis of risk factors for coronary heart disease. Circulation 1973;48:950-8.
13. Westlund K, Nicolaysen R. Ten-year mortality and morbidity related to serum cholesterol: a follow-up of 3,751 men age 40-49. Scand J Clin Lab Invest 1972;30(suppl 127):1-24.
14. Bates HM. Prevalence of hyperlipidemia in a large sample population. J Cardiovasc Pharm 1982;4(suppl 2):196-200.
15. Weinberger MH. Antihypertensive therapy and lipids: evidence, mechanisms and implications. Arch Intern Med 1985;145:1102-5.
16. Yaari S, Even-Zohar S, Goldbourt U, et al. Associations of serum high density lipoprotein and total cholesterol with total, cardiovascular, and cancer mortality in a 7-year prospective study of 10,000 men. Lancet 1981;1:1011-5.
17. Gordon T, Castelli WP, Jhortland MC, et al. High density lipoprotein as a protective factor against coronary heart disease. Am J Med 1977;62:707-14.
18. The Pooling Project Research Group. Relationship of blood pressure, serum cholesterol, smoking habit, relative weight and ECG abnormalities to incidence of major coronary events: final report of the Pooling Project. J Chron Dis 1978;41:201-306.
19. Miller NE, Thelle DS, Forde OH, et al. The Tromso heart study—high density lipoprotein and coronary heart disease: a prospective case control study. Lancet 1977;1: 965-8.
20. Kaplan NM. Estrogen replacement therapy: effect on blood pressure and other cardiovascular risk factors. J Reprod Med 1985;39(suppl):802-3.
21. Healthy people—the Surgeon General's report on health promotion and disease prevention. DHEW publ no (PHS) 79-55071. Washington, DC: US Government Printing Office, 1979.
22. Coronary Drug Research Group. The coronary drug project, initial findings leading to modifications of its research protocol. JAMA 1970;214:1303-13.
23. Coronary Drug Research Group. The coronary drug project, findings leading to discontinuation of the 2.5 mg/day estrogen group. JAMA 1973;226:652-7.
24. Bush TL, Barrett-Connor E. Noncontraceptive estrogen use and cardiovascular disease. Epidemiol Rev 1985;7:80-104.
25. Bush TL, Barrett-Connor E, Cowan LD, et al. Cardiovascular mortality and noncontraceptive use of estrogen in women: results from the Lipid Research Clinics Program follow-up study. Circulation 1987;75:1102-9.
26. Knopp RH. Cardiovascular effects of endogenous and exogenous sex hormones over a woman's lifetime. Am J Obstet Gynecol 1988;158:1630-43.
27. Ross RK, Paganini-Hill A, Mack TM, et al. Estrogen use and cardiovascular disease. In: Mishell DR, Jr, ed. Menopause: physiology and pharmacology. Chicago: Year Book, 1987:209-23.
28. Henderson BE, Ross RK, Paganini-Hill A, et al. Estrogen use and cardiovascular disease. Am J Obstet Gynecol 1986;154:1181-6.
29. Ross RK, Henderson BE. Estrogen replacement in the prevention of cardiovascular disease. Hosp Pract 1986; (suppl 9):16-24.
30. Henderson BE, Paganini-Hill A, Ross RK. Estrogen replacement therapy and protection from acute myocardial infarction. Am J Obstet Gynecol 1988;159:312-7.
31. Stampfer MJ, Willett WC, Colditz GA, et al. A prospective study of postmenopausal estrogen therapy and coronary heart disease. N Engl J Med 1985;313:1044-9.
32. Criqui MH, Suarez L, Barrett-Connor E, et al. Postmenopausal estrogen use and mortality: results from a prospective study in a defined, homogeneous community. Am J Epidemiol 1988;128:606-14.
33. Mashchak CA, Lobo RA. Estrogen replacement therapy and hypertension. J Reprod Med 1985;30(suppl 10):805-10.
34. Wren BG, Routledge DA. Blood pressure changes: oestrogens in climacteric women. Med J Aust 1981;2:528-31.
35. Wren BG, Routledge DA. The effect of type and dose of oestrogen on the blood pressure of postmenopausal women. Maturitas 1983;5:135-42.
36. Davis ME, Jones RJ, Jarolim C. Long-term estrogen substitution and atherosclerosis. Am J Obstet Gynecol 1961;82:1003-18.
37. Stern MP, Brown BW Jr, Haskell WL, et al. Cardiovascular risk and use of estrogens or estrogen-progestogen combinations: Stanford three-community study. JAMA 1976; 235:811-5.
38. Pallas KG, Holzwarth GJ, Stern MP, et al. The effect of

conjugated estrogens on the renin-angiotensin system. J Clin Endocrinol Metab 1977;44:1061-8.
39. Pfeffer RI. Estrogen use, hypertension and stroke in postmenopausal women. J Chron Dis 1978;31:389-98.
40. Barrett-Connor E, Brown V, Turner J, et al. Heart disease risk factors and hormone use in postmenopausal women. JAMA 1979;241:2167-8.
41. Persson I, Adami HO, Lindberg BS, et al. Characteristics of estrogen-treated women. II. A descriptive epidemiological study of a Swedish population. Acta Obstet Gynecol Scand 1983;62:297-302.
42. Lind T, Cameron EC, Hunter WM, et al. A prospective, controlled trial of six forms of hormone replacement therapy given to postmenopausal women. Br J Obstet Gynaecol 1979;86(suppl 3):1-29.
43. Lindsay R, Herrington BS. Estrogens and osteoporosis. Semin Reprod Endocrinol 1983;1:55-61.
44. Luotola H. Blood pressure and hemodynamics in postmenopausal women during estradiol-17β substitution. Ann Clin Res 1983;15(suppl 38):1-121.
45. Gordon T, Kannel WB, Hjortland MC, et al. Menopause and coronary heart disease: the Framingham Study. Ann Intern Med 1978;89:157-61.
46. Geola FL, Frumar AM, Tataryn IV, et al. Biological effects of various doses of conjugated equine estrogens in postmenopausal women. J Clin Endocrinol Metab 1980; 51:620-5.
47. Elkik F, Gompel A, Mercier-Bodard C, et al. Effects of percutaneous estradiol and conjugated estrogens on the level of plasma proteins and triglycerides in postmenopausal women. Am J Obstet Gynecol 1982;143:888-92.
48. Place VA, Powers M, Darley PE, et al. A double-blind comparative study of Estraderm and Premarin in the amelioration of postmenopausal symptoms. Am J Obstet Gynecol 1985;152:1092-9.
49. Padwick ML, Siddle NC, Lane G, et al. Oestriol with oestradiol vs oestradiol alone: a comparison of endometrial, symptomatic and psychologic effects. Br J Obstet Gynaecol 1986;93:606-12.
50. Riis BJ, Thomsen K, Strom V, et al. The effect of percutaneous estradiol and natural progesterone on postmenopausal bone loss. Am J Obstet Gynecol 1987; 156:61-5.
51. Kauppila A, Kivinen S, Stenback F, et al. Tamoxifen and natural progesterone as supplements to low-dose postmenopausal estrogen therapy. Gynecol Obstet Invest 1988;25:58-65.
52. Riis BJ, Johansen J, Christiansen C. Continuous oestrogen-progestogen treatment and bone metabolism in postmenopausal women. Maturitas 1988;10:51-8.
53. Sporrong T, Hellgren M, Samsioe G, et al. Comparison of four continuously administered progestogen plus oestradiol combinations for climacteric complaints. Br J Obstet Gynaecol 1988;95:1042-48.
54. Colvin PL, Auerbach BJ, Applebaum-Bowden D, et al. Effect of estrone vs 17-β-estradiol on lipoproteins in postmenopausal women. Clin Res 1988;36:269A.
55. Selby PL, Peacock M. Dose dependent response of symptoms, pituitary, and bone to transdermal oestrogen in postmenopausal women. Br Med J 1986;293:1337-9.
56. Jensen J, Riis BJ, Strom V, et al. Long-term effects of percutaneous estrogens and oral progesterone on serum lipoproteins in postmenopausal women. Am J Obstet Gynecol 1987;156:66-71.
57. Paterson MEL, Sturdee DW, Moore B, et al. The effect of various regimens of hormone therapy on serum cholesterol and triglyceride concentrations in postmenopausal women. Br J Obstet Gynaecol 1930;87:552-60.
58. Mattsson LA, Cullberg G, Samsioe G. A continuous estrogen-progestogen regimen for climacteric complaints. Acta Obstet Gynecol Scand 1984;63:673-7.
59. Ottosson UB. Oral progesterone and estrogen/progestogen therapy: effects of natural and synthetic hormones on subfractions of HDL cholesterol and liver proteins. Acta Obstet Gynecol Scand 1984;(suppl 127):5-37.
60. Lagrelius A, Fredricsson B, Hirt M, et al. Clinical experience with a low-dose combination of estradiol valerate and levonorgestrel. A double-blind comparative study between SH D 386 F and Cyclabil. Effects of symptoms, lipids and endometrial condition. Acta Obstet Gynecol Scand 1986;134(suppl):97-101.
61. Bush TL, Miller VT. Effects of pharmacologic agents used during menopause: impact on lipids and lipoproteins. In: Mishell DR, Jr, ed. Menopause: physiology and pharmacology. Chicago: Year Book, 1987:187-208.
62. LaRosa JC. Effect of estrogen replacement therapy on lipids: implications for cardiovascular risk. J Reprod Med 1985;30(suppl):811-3.
63. Mishell DR Jr. Estrogen replacement therapy: measuring benefit v cardiovascular risk. J Reprod Med 1985;30 (suppl 10):795-6.
64. Nachtigall LE, Nachtigall RH, Nachtigall RD, et al. Estrogen replacement therapy II. A prospective study in the relationship to carcinoma and cardiovascular and metabolic problems. Obstet Gynecol 1979;54:74-9.
65. Wilson PWF, Garrison RJ, Castelli WP. Postmenopausal estrogen use, cigarette smoking and cardiovascular morbidity in women over 50. The Framingham Study. N Engl J Med 1985;313:1038-43.
66. Eaker ED, Garrison RJ, Castelli WP. Risk factors for coronary heart disease among women: 30 years of follow-up from the Framingham Study. Presented at the American Heart Association Twenty-fifth Annual Conference on Cardiovascular Disease Epidemiology, Tucson, Ariz, March 7-9, 1985.
67. Stubblefield PG. Postmenopausal estrogen and cardiovascular disease. N Engl J Med 1987;316:342-3.
68. Rosenberg L, Armstrong B, Jick H. Myocardial infarction and estrogen therapy in postmenopausal women. N Engl J Med 1976;294:1256-9.
69. Pfeffer RI, Whipple GH, Kurosuki TT, et al. Coronary risk and estrogen use in postmenopausal women. Am J Epidemiol 1978;107:479-97.
70. Jick H, Dinan B, Rothman KJ. Noncontraceptive estrogens and nonfatal myocardial infarction. JAMA 1978; 239:1407-8.
71. Rosenberg L, Slone D, Shapiro S, et al. Noncontraceptive estrogens and myocardial infarction in young women. JAMA 1980;244:339-42.
72. Ross RK, Mack TM, Paganini-Hill A, et al. Menopausal oestrogen therapy and protection from death from ischemic heart disease. Lancet 1981;1:858-60.
73. Bain C, Willett W, Hennekens CH, et al. Use of postmenopausal hormones and risk of myocardial infarction. Circulation 1981;64:42-6.
74. Adam S, Williams V, Vessey MP. Cardiovascular disease and hormone replacement treatment: a pilot case-control study. Br Med J 1981;282:1277-8.
75. Szklo M, Tonascia J, Gordis L, et al. Estrogen use and myocardial infarction risk: a case control study. Prev Med 1984;13:510-6.
76. Burch JC, Byrd BF, Vaughn WK. The effects of long-term estrogen on hysterectomized women. Am J Obstet Gynecol 1974;118:778-82.
77. Hammond CB, Jelovsek FR, Lee KL, et al. Effects of long-term estrogen replacement therapy. I. Metabolic effects. Am J Obstet Gynecol 1979;133:525-36.
78. Petitti DB, Wingerd J, Pellegrin F, et al. Risk of vascular disease in women. Smoking, oral contraceptives, noncontraceptive estrogens and other factors. JAMA 1979; 242:1150-6.
79. Henderson BE, Ross RK, Paganini-Hill A. Estrogen use and cardiovascular disease. J Reprod Med 1985;30:814-20.
80. Petitti DB, Perlman JA, Sidney S. Postmenopausal estrogen use and heart disease. N Engl J Med 1986;315: 131-2.

Compliance considerations with estrogen replacement: Withdrawal bleeding and other factors

Ricardo G. Hahn, MD
Memphis, Tennessee

Withdrawal bleeding and other side effects such as edema, bloating, premenstrual irritability, lower abdominal cramps, dysmenorrhea, and breast tenderness limit compliance with hormonal replacement therapy. Although many of these troublesome side effects can be managed by adjusting the dose or changing the source of the estrogen or progestin components, postmenopausal women view withdrawal bleeding as the most negative factor influencing their decision to use hormonal replacement therapy. Additionally, the potential link between postmenopausal estrogen use and subsequent endometrial hyperplasia and cancer concerns potential users. Cyclic progestins protect the endometrium from hyperplastic changes but may not prevent withdrawal bleeding. Both patient and physician education, including the nature of menopause and the protective role of estrogens in osteoporosis and cardiovascular disease, are critical to improving compliance with hormonal replacement therapy. (Am J Obstet Gynecol 1989;161:1854-8.)

Key words: Estrogen, withdrawal bleeding, compliance

American women currently have life spans of some 80 years. With menopause occurring at a mean age of 51 years, women can expect to live one third of their lives in the postmenopausal state.[1, 2]

A significant number of undesirable physiologic changes in the postmenopausal years result from decreased levels of circulating estrogen. The use of hormonal replacement therapy for relieving vasomotor symptoms and slowing vaginal atrophy is well established. Estrogen also has a role in protecting against osteoporosis.[3-8] Additionally, recent studies have reported cardiovascular benefits from hormonal replacement therapy.[9, 10]

Despite the positive benefit/risk ratio of hormonal replacement therapy, compliance is a problem because of side effects (especially withdrawal bleeding) and other factors, including fear of cancer. This article will discuss these factors and provide suggestions for improving compliance with hormonal replacement.

How side effects can limit compliance

Many investigators have proposed that withdrawal bleeding and other side effects from hormonal replacement therapy limit compliance.[1, 11-13] For example, the rate of compliance with this therapy was only 30% in one study of 301 postmenopausal women.[11] In another investigation, 15 of 18 women receiving combination therapy dropped out of the study.[12]

In addition to withdrawal bleeding, side effects include edema, bloating, premenstrual irritability, lower abdominal cramps, dysmenorrhea, and breast tenderness. Although troublesome, these effects, which occur in 5% to 10% of patients taking combination therapy, may be managed by adjusting the dosage or changing the source of estrogen or progestin components.[14, 15] Edema, bloating, and irritability can be managed by reducing the estrogen dose, although adding a diuretic is occasionally necessary.[14] Changing progestins may alleviate the symptoms of lower abdominal cramps and dysmenorrhea, but a prostaglandin-inhibiting analgesic may be needed.[14, 15]

Withdrawal bleeding may be the most significant factor in the patient's decision to begin, continue, or discontinue hormonal replacement therapy.[1, 12] As stated above, in a comparison of two groups of postmenopausal women,[12] 15 of 18 women with intact uteri who were receiving combination therapy dropped out of the study because of withdrawal bleeding. These women, who were receiving the combination of 0.625 mg conjugated equine estrogens for 21 days with 2.5 mg norethindrone added from day 12 through day 21, had been informed that bleeding was to be expected during days 21 to 30 of the treatment cycle. None of 22 women hysterectomized for nonmalignant pathologic conditions who were receiving only 0.625 mg of CEE for 21 days of each month discontinued therapy. In a survey by Ferguson et al.,[1] women using hormonal replace-

From the Department of Family Medicine, University of Tennessee at Memphis.
Reprint requests: Ricardo G. Hahn, MD, Department of Family Medicine, University of Tennessee, 1121 Union Ave., Memphis, TN 38104.
6/0/16770

Table I. Factors that influenced (or would influence) the decision to use hormonal replacement therapy

Factor	*Postmenopausal*		*Premenopausal* (n = 65)
	Currently taking hormonal replacement therapy (n = 37)	*Have never taken hormonal replacement therapy* (n = 125)	
Physician recommended it	1.3	2.7	2.3
Would stop hot flashes	1.4	2.5	2.1
Would not cause cancer	1.5	2.7	1.9
Would require checkups	2.0	2.2	2.6
Test could determine risk of osteoporosis	2.1	2.3	2.0
Would have to be taken for rest of life	2.6	3.8	3.7
Could be given through a skin patch	2.8	3.1	2.7
Friend was taking it without problems	2.0	3.0	2.4
Friend was taking it with problems	3.5	3.4	3.6
Would cause to have periods	3.6	3.4	3.8

Reprinted with permission from Ferguson KJ, Hoegh C, Johnson S. Arch Intern Med 1989;149:133-6, copyright 1989, American Medical Association.

Rating scale: 1 = Very positive to 5 = very negative.

Table II. Patterns of vaginal bleeding with normal and hyperplastic endometrium during unopposed cyclic estrogen therapy

Type of therapy	*Patients with withdrawal bleeding*		*Patients with breakthrough bleeding*		*Patient with no vaginal bleeding*	
	No.	*%*	*No.*	*%*	*No.*	*%*
Cyclic high dose						
Normal endometrium	14	64	11	69	22	71
Endometrial hyperplasia	8	36	5	31	9	29
Cyclic low dose						
Normal endometrium	3		5	56	23	96
Endometrial hyperplasia	1		4	44	1	4

Reprinted with permission from Whitehead MI. Maturitas 1978;1:87-98.

Cyclic high dose = conjugated equine estrogens 1.25 mg/day, piperazine estrone sulfate 3.0 mg/day, estradiol valerate 2.0 mg/day; cyclic low dose = half the high dose, daily.

ment therapy rated withdrawal bleeding as the most negative of a list of factors influencing their decision to use this therapy (Table I).

The atrophic postmenopausal endometrium responds to exogenous hormones by developing proliferative, secretory, and bleeding phases almost identical to those that occur during the normal ovulatory cycle. The so-called withdrawal bleeding can be heavy and painful in some patients.[12, 15]

Unopposed estrogen in some premenopausal and postmenopausal women can result in endometrium hyperplasia, an abnormal increase in the amount of proliferative endometrium. Possible causes include disordered estrogen metabolism, decreased levels of binding protein that allow greater estrogen access into cells, and increased sensitivity of the endometrium to estrogenic stimulation.[16] Two distinct forms of hyperplasia, cystic and adenomatous, exhibit different cytologic findings and premalignant potentials. Cystic hyperplasia converts to adenocarcinoma only at a 1% rate; 12% of adenomatous hyperplasias become carcinomas after 18 months and 30% undergo malignant changes within 10 years.[16]

American and European data have established a link between unopposed estrogen use and endometrial cancer. However, data do not necessarily demonstrate a direct link between endometrial status and vaginal bleeding patterns.[16-18] Withdrawal bleeding and breakthrough bleeding are not accurate predictors of endometrial hyperplasia (Table II).[17]

Maximum antiestrogenic effects appear to be achieved by adding progestins for 10 to 13 days of the monthly cycle.[14] In one series in which the incidence of hyperplasia was 18% to 32% with unopposed cyclic estrogen, the incidence was lowered to 3% to 4% when progestins were added for 7 days, and to zero when progestins were added for 12 to 13 days of the cycle.[19, 20] In another study, 5 to 10 days of progestin therapy reduced the frequency of hyperplasia to 2%; with 13 days of progestin therapy no hyperplasia was found.[21] Other studies confirm this effect.[17, 22, 23]

Although cyclic progestins may protect the endo-

Table III. Attitudes toward menopause

Attitude	Postmenopausal		Premenopausal
	Currently taking hormonal replacement therapy (n = 37)	*Have never taken hormonal replacement therapy* (n = 125)	
Menopause is a medical condition	1.9	3.7	3.1
Women with distressing symptoms should be taking hormonal replacement therapy	2.1	3.7	3.5
Women cannot control menopausal changes	3.3	3.7	4.0
Sexual interest and comfort increase after menopause	3.4	2.9	3.6
Psychologic problems are due to life changes, not hormonal changes	3.0	3.7	4.3
Women who have trouble are those who expect it	4.5	3.7	4.3
Risks of estrogen outweight benefits	4.6	3.8	4.4
Natural approaches are better than hormonal replacement therapy	4.6	2.6	3.0
Male partners of menopausal women see them as less sexually desirable	5.4	6.0	5.3
A woman feels like less of a woman after menopause	5.6	6.3	5.1

Reprinted with permission from Ferguson KJ, Hoegh C, Johnson S. Arch Intern Med 1989;149:133-6, copyright 1989, American Medical Association.

Scale: 1 = strongly agree to 7 = strongly disagree.

metrium from hyperplastic changes, withdrawal bleeding can nonetheless occur. Epidemiologic and biochemical arguments tend to support a differential effect among different estrogens when used cyclically. Wren et al.[24] suggested a more marked response of conjugated estrogens compared with estrone sulfate on uterine tissue (including endometrial hyperplasia), an increase in blood pressure, and decreased effectiveness in controlling vasomotor instability (hot flashes).

Many researchers have tested continuous combination therapy as a way to produce amenorrhea, alleviate symptoms, reduce the incidence of side effects, and avoid hyperplasia.[13, 15, 25-28] In a study comparing cyclic with continuous therapy, Prough et al.[13] followed two groups of postmenopausal women for 9 months. Group I was given 0.625 mg conjugated equine estrogen and 2.5 mg medroxyprogesterone acetate daily. Group II received 0.625 mg conjugated equine estrogen for days 1 to 25 and 10 mg medroxyprogesterone acetate during days 16 to 25. Vaginal bleeding was eliminated in all but 2 of the 16 women in group I; both had one episode of spotting. In group II, 8 of 10 patients continued to have withdrawal bleeding. After 9 months of therapy, all patients in the first group had an inactive endometrium. In the second group, nine patients had an inactive endometrium; in one patient the endometrium converted from inactive to proliferative.

In a study comparing different continuous regimens in 75 women, Magos et al.[15] used four combinations of daily conjugated equine estrogen (0.625 or 1.25 mg) and norethindrone (0.35 or 1.05 mg). At 3-month intervals, patients who experienced any bleeding were assigned to receive another 0.35 mg dose of norethindrone. At the start of the study, 65.2% of women taking 0.625 mg conjugated equine estrogen combinations and 46.9% of women taking 1.25 mg conjugated equine estrogen combinations became amenorrheic immediately. After dosage adjustments at the 1-year mark, 95.1% of all patients were amenorrheic. The incidence of unscheduled bleeding decreased with increased duration of treatment. Vasomotor symptoms were controlled, and no abnormal histologic findings developed during therapy.

Other factors that limit compliance

In addition to side effects, other factors contribute to noncompliance with hormonal replacement therapy. These include factors attributable to both patients and physicians.

In an ongoing field study of 2500 postmenopausal women, Ravnikar[29] found that women used hormonal therapy sporadically. The major reported reason for stopping therapy was the patient's fear of cancer. Among patients receiving first-time hormonal prescriptions, 20% stopped therapy within 9 months, 10% took the medication sporadically, and 20% to 30% never had their prescriptions filled.

Ferguson et al.[1] surveyed 289 women by question-

naire. Respondents were divided into three groups: postmenopausal women who currently use hormonal replacement therapy, postmenopausal women who have never used hormonal replacement therapy, and premenopausal women. (Women who had discontinued hormonal replacement therapy were excluded because the subgroups of those who stopped on their own or stopped due to physician suggestion were too small for valid analysis.) Table I summarizes the factors that influenced or would influence the decision to use hormonal replacement therapy. The most negative factor for all three groups of women was the fact that therapy would cause them to have periods. The most positive factors included physician recommendation, "would stop hot flashes," and "would not cause cancer."

Ferguson et al.[1] also examined these women's attitudes toward menopause (Table III). Women using hormonal replacement therapy were more likely to agree that menopause is a medical condition and that symptoms can be treated with this therapy. Most women disagreed or were neutral regarding the relative risks and benefits of estrogen therapy. Although 89% of women currently using hormonal replacement therapy knew that lack of estrogen was a significant factor in the development of osteoporosis, only 27% of postmenopausal women not using hormonal replacement therapy knew this. There was an apparent lack of knowledge of the risk factors for osteoporosis, with women using hormonal replacement therapy being the most knowledgeable.

The role of physicians in patient compliance is important. In the study by Ferguson et al.,[1] the major factor influencing hormonal replacement therapy use was physician recommendation. Another important finding was that of 125 postmenopausal women who had never used hormonal replacement therapy, 64% had never discussed this option with their physicians. Of this group, 75% agreed that a physician recommendation would have a positive effect regarding therapy use. All significant positive predictors of hormonal replacement therapy use ("natural approaches to menopause are less preferable," "women experiencing hot flashes should use hormonal replacement therapy," "menopause is a medical condition," "estrogen can reduce the risk of osteoporosis") can be influenced by physician teaching.

In a survey of 50 primary care providers, Holzman et al.[30] found that most clinicians were more concerned about the risks of endometrial cancer than about possible future fractures and thus did not readily prescribe estrogens.

Weinstein and Schiff[31] have developed a model to show that when all the epidemiologic data of risks and benefits are compared, the effectiveness of treatment in terms of longevity and quality of life is apparent.

Thus both patient and physician education are necessary if compliance with hormonal replacement therapy is to be increased. Research suggests that giving patients more information about hormonal therapy could significantly alter utilization patterns. Important factors to discuss include the nature of menopause, emphasizing that it is a medical condition with symptoms that can be alleviated with hormonal therapy. The protective role of estrogens in osteoporosis and cardiovascular disease must be balanced with the risks of cancer.

The physician's role in self-education is as important as his or her role in educating patients. A knowledge of available alternative regimens is essential because symptoms, side effects, and withdrawal bleeding may be managed by adjusting dosages or changing the estrogen and progestin formulations. The physician should also be aware that perimenopausal women with dwindling or intermittent periods may accept withdrawal bleeding more easily than older women. Additionally, recent data on the use of a Pipelle (instead of a Vabra aspirator) may offer patients who need serial endometrial biopsies a less painful alternative.[32, 33]

Physicians are typically familiar with the absolute contraindications (e.g., estrogen-dependent tumors or acute liver disease) and relative contraindications (e.g., a past history of endometriosis) to estrogen therapy. Equally important is an understanding of the large population of women for whom estrogens are indicated. Improving both patient and physician education will help increase compliance with hormonal therapy.

Areas for future research that may influence compliance include (1) improved combination therapy, perhaps prepackaged in dose packs, and (2) drugs that more specifically reduce the incidence of side effects, osteoporosis, and cardiovascular disease without producing end organ stimulation.

REFERENCES

1. Ferguson KJ, Hoegh C, Johnson S. Estrogen replacement therapy: a survey of women's knowledge and attitudes. Arch Intern Med 1989;149:133-6.
2. Richards-Kuston CJ, Kase NG. Diagnosis and management of perimenopausal bleeding. Obst Gynecol Clin North Am 1987;14:169-89.
3. Davis ME, Lanzl L, Cox AB. Detection, prevention, and retardation of menopausal osteoporosis. Obstet Gynecol 1970;36:187-98.
4. Lindsay R, Hart DM, Aitken JM, et al. Long-term prevention of postmenopausal osteoporosis by oestrogen. Lancet 1976;1:1038-41.
5. Nachtigall LE, Nachtigall RH, Nachtigall RD, et al. Estrogen replacement therapy I: a ten-year prospective study in the relationship to osteoporosis. Obstet Gynecol 1979;53:277-81.
6. Weiss NS, Ure CL, Ballard JH, et al. Decreased risk of fractures of the hip and lower forearm with postmenopausal use of estrogen. N Engl J Med 1980;303:1195-8.
7. Paganini-Hill A, Ross RK, Gerkins UR, et al. Menopausal

estrogen therapy and hip fractures. Ann Intern Med 1981;95:28-31.
8. Kiel DP, Felson DT, Anderson JJ, et al. Hip fractures and the use of estrogens in postmenopausal women: the Framingham study. N Engl J Med 1987;317:1169-74.
9. Bain C, Willet W, Hennekena CH, et al. Use of postmenopausal hormones and risk of myocardial infarction. Circulation 1981;64:42-6.
10. Ross RK, Paganini-Hill A, Mack TM. Menopausal estrogen therapy and protection from death from ischemic heart disease. Lancet 1981;1:858-60.
11. Hammond CB, Jelovsek FR, Lee KL, et al. Effects of long-term estrogen replacement therapy II. Neoplasia. Am J Obstet Gynecol 1979;133:537-47.
12. Hahn RG, Nachtigall RD, Davies TC. Compliance difficulties with progestin supplemented replacement therapy. J Fam Pract 1984;18:411-4.
13. Prough SG, Aksel S, Wiebe RH, et al. Continuous estrogen/progestin therapy in menopause. Am J Obstet Gynecol 1987;157:1449-53.
14. Gambrell RD. Clinical use of progestins in the menopausal patient. J Reprod Med 1982;27:531-8.
15. Magos AL, Brincat M, Studd WW, et al. Amenorrhea and endometrial atrophy with continuous oral estrogen and progestogen therapy in postmenopausal women. Obstet Gynecol 1985;65:496-9.
16. Whitehead MI. Prevention of endometrial abnormalities. Acta Obstet Gynecol Scand Suppl 1986;134:81-91.
17. Whitehead MI. The effects of oestrogens and progestogens on the postmenopausal endometrium. Maturitas 1978;1:87-98.
18. Schiff I, Sela NK, Cramer D, et al. Endometrial hyperplasia in women on cyclic or continuous estrogen replacements. Fertil Steril 1982;37:79-82.
19. Studd JWW,Thom MH, Paterson MEL, et al. The prevention and treatment of endometrial pathology in postmenopausal women receiving exogenous estrogen. In: Pasetto N, Pavletti R, Ambrus JL, eds. The menopause and postmenopause. Lancaster, England: MTP Press, 1980: 127-39.
20. Whitehead MI, Townsend PT, Pryse-Davies J, et al. Effects of various types and dosages of progestogens on the postmenopausal endometrium. J Reprod Med 1982; 27:539-48.
21. Thom MH, White PJ, Williams RM, et al. Prevention and treatment of endometrial disease in climateric women receiving oestrogen therapy. Lancet 1978;2:455-7.
22. Sturdee DW, Wade-Evans T, Paterson MEL, et al. Relationships between bleeding pattern, endometrial histology and oestrogen treatment in menopausal women. Br Med J 1978;1:1575-7.
23. Wentz WB. Progestin therapy in endometrial hyperplasia. Gynecol Oncol 1974;2:362-7.
24. Wren BG, Brown LB, Routledge DA. Differential clinical response to oestrogens after menopause. Med J Aust 1982;2:329-32.
25. Staland B. Continuous treatment with natural oestrogens and progestogens. A method to avoid endometrial stimulation. Maturitas 1981;3:145-56.
26. Mattson LA, Cullberg G, Samsive G. Evaluation of a continuous oestrogen-progestogen regimen for climateric complaints. Maturitas 1982;4:95-102.
27. Weinstein L. Efficacy of a continuous estrogen-progestin regimen in the menopausal patient. Obstet Gynecol 1987;69:929-32.
28. Luciano AA, Turksoy RN, Caleo J, et al. Clinical and metabolic responses of menopausal women to sequential vs continuous estrogen and progestin replacement therapy. Obstet Gynecol 1988;71:39-43.
29. Ravnikar VA. Compliance with hormone therapy. Am J Obstet Gynecol 1987;156:1332-4.
30. Holzman GB, Ravitch MM, Metheny W, et al. Physician's judgments about estrogen replacement therapy for menopausal women. Obstet Gynecol 1984;63:303-11.
31. Weinstein CW, Schiff I. Cost-effectiveness of hormone replacement therapy in the menopause. Obstet Gynecol Surv 1983;38:445-55.
32. Cornier E. The pipelle: a disposable device for endometrial biopsy. Am J Obstet Gynecol 1984;148:109-10.
33. Kaunitz AM, Masciello A, Ostrowski M, et al. Comparison of endometrial biopsy with the endometrial pipelle and vabra aspirator. J Reprod Med 1988;33:427-30.

The cancer question: An overview of recent epidemiologic and retrospective data

Brian E. Henderson, MD
Los Angeles, California

Unopposed estrogen stimulates mitotic activity in endometrial and breast tissue. Numerous case-control studies have evaluated the relationship between estrogen use and the risk of endometrial and breast cancers. In general, exogenous, unopposed estrogen use increases the risk of endometrial cancer by tenfold and of breast cancer by twofold after long-term use of high doses. Estrogen's positive effects on osteoporosis and coronary heart disease must be considered when evaluating the potential risks associated with its use in postmenopausal women. (Am J Obstet Gynecol 1989;161:1859-64.)

Key words: Estrogen, breast cancer, endometrial cancer

Experimental, clinical, and epidemiologic evidence indicates that hormones play a major role in causing several human cancers. Based on his studies of estrogens and mammary cancer in mice, in 1947 Bittner[1] first proposed the concept that hormones can increase the incidence of neoplasia. This concept has been refined into epidemiologic hypotheses related to cancers of the breast, endometrium, prostate, ovary, and testis.[2, 3] These hypotheses are based on the association between neoplasia and excessive hormonal stimulation of the specific target organ, the normal growth and function of which is controlled by one or more steroid or polypeptide hormones.

This review will focus on our current knowledge of hormonal replacement therapy and its carcinogenic potential on the endometrium and breast.

Endometrial cancer

Unopposed estrogen stimulates endometrial cell biosynthesis, potentially leading to endometrial hyperplasia due to incomplete shedding of the endometrium (Fig. 1).[4] A proliferative endometrium can progress to cystic or adenomatous hyperplasia, which can then progress to atypical adenomatous hyperplasia, reportedly a premalignant stage.[5-7] The likelihood of developing hyperplasia appears to be directly related to the dose of estrogen prescribed.[8]

Case reports of endometrial cancer occurring in women after the use of estrogens have appeared in the medical literature for more than 30 years. Efforts to evaluate this relationship in a controlled fashion began in 1975, with two case-control studies[9, 10] that found estrogen use increased the risk of endometrial cancer. Since then, numerous case-control studies, some of which are summarized in Table I,[9-26] have reported similar conclusions. In general, exogenous, unopposed estrogen therapy (primarily Premarin) was found to increase the risk of developing endometrial cancer 2- to 15-fold compared with the risk in nonusers. In absolute terms, the overall incidence of adenocarcinoma of the endometrium in women aged 50 to 74 years who have never used estrogen is relatively low (estimated at 1/1000 women per year).[18] Therefore, a 10-fold increased relative risk, a representative value for "ever-users," indicates an increase in absolute risk from 1:1000 to 1:100 women per year.

Evidence suggests that adenocarcinoma of the endometrium after estrogen use is generally low stage and low grade and is associated with fewer cases of myometrial invasion.[11, 13, 17, 19, 25, 27] For example, Chu et al.[27] studied 320 women with endometrial cancer; 79% (285 of 363) had used estrogens for more than 1 year. Estrogen users tended to have well-differentiated tumors and no myometrial invasion. In addition, the women who had used estrogen had primarily stage 0 or I cancers, with a very low incidence of stage II or III tumors (2%) compared with those who had never used estrogen (12%; $p = 0.005$).

Not surprisingly, several studies suggest that estrogen users who develop endometrial cancer survive longer than nonusers who develop the same cancer. Elwood and Boyes[28] found that estrogen users had survival rates at 5 years of 89.1%, compared with 73.5% for nonusers. After adjusting the rates for expected deaths of age-matched women, they calculated relative survival rates for users and nonusers of 94.2% and 81.3%, respectively. At another Canadian study site, Collins et al.[29] similarly found that the estrogen users' 5-year survival rates (92% ± 2%) exceeded those of nonusers (68% ± 2%) (Fig. 2). Furthermore, for the women with en-

From the Department of Preventive Medicine, Kenneth Norris Jr. Comprehensive Cancer Center, University of Southern California School of Medicine.
Reprint requests: Brian Henderson, MD, Department of Preventive Medicine, Kenneth Norris Jr. Comprehensive Cancer Center, University of Southern California School of Medicine, Los Angeles, CA 90033.
6/0/16771

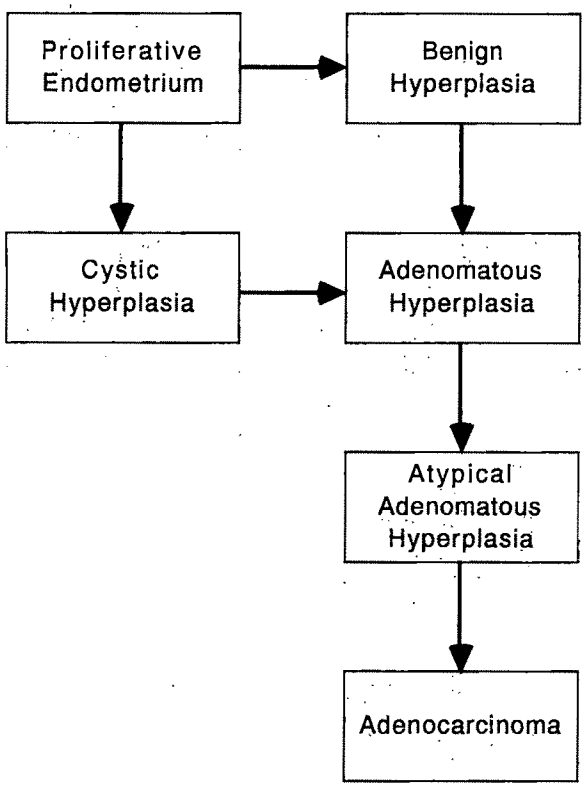


Fig. 1. Role of unopposed estrogen in development of endometrial hyperplasia and neoplasia. (Reprinted with permission from Gambrell RD Jr. J Reprod Med 1982;27:531-38.)

dometrial cancer, the risk of any death for nonusers was 2.7 times greater than that for estrogen users.

Disease risk is related to both dose and duration of estrogen use.[9, 10, 11, 17] High-dose users (>0.625 mg of conjugated estrogen per day) had a risk of developing endometrial cancer similar to that of low-dose users (<0.625 mg/day) and controls after short courses of therapy (<3.5 years).[19] In contrast, those who took high doses over long periods (≥3.5 years) had a greater risk of developing cancer than did low-dose users.

The findings from the above case-control studies are supported by data from several cohort studies.[6, 30-32] Never-users of estrogen had a risk of endometrial cancer 10 times less than that of long-term users.[30, 32] Estrogen-progesterone users had a relative risk of endometrial cancer of 0.9, a value lower than that observed among those who used estrogen alone (1.4).[33]

The benefit of adding a progestogen to estrogen replacement therapy to reduce estrogen-induced endometrial mitotic activity has been clearly established in clinical practice.[4, 34, 35] However, the possible adverse effects of progestogen on the risks of heart disease and breast cancer indicate that the dose of progestogen should be minimized and that simultaneous oral progestogen dosing is not suitable for all women, particularly those with a prior hysterectomy.[36] Whitehead et al.[37] showed that duration of progestogen therapy is more important than the dose of progestogen given; however, the optimal type, dose, and duration of progestogen therapy have not been established.

Table I. Endometrial cancer risk relative to use of estrogen replacement therapy: Case-control studies

Study	Relative risk*	
	Ever users	*Long-term users*
Smith et al.[9]	4.5	—
Ziel and Finkle[10]	7.6	13.9
Mack et al.[11]	5.6	8.8
Gray et al.[12]	3.1	11.6
McDonald et al.[13]	2.0	7.9
Wigle et al.[14]	2.2	5.2
Horwitz and Finkelstein[15]	12.0	—
Hoogerland et al.[16]	2.2	6.7
Antunes et al.[17]	6.0	15.0
Weiss et al.[18]	7.5	8.2
Hulka et al.[19]	—	4.2
Shapiro et al.[20]	3.9	6.0
Jelovsek et al.[21]	2.4	4.8
Spengler et al.[22]	3.2	8.6
Stavraky et al.[23]	4.2	14.4
Kelsey et al.[24]	—	8.2
LaVecchia et al.[25]	2.7	—
Henderson et al.[26]	1.4	3.1

From Peterson HB, Lee NC, Rubin GL. In: Mishell, DR, Jr, ed. Menopause: physiology and pharmacology. Chicago: Year Book Medical Publishers, 1987:279.

*Risk relative to never-users.

Breast cancer

Breast cancer risk is determined primarily by the total cumulative exposure of breast tissue to bioavailable estrogens and the associated cumulative mitotic activity. In contrast, progesterone does not have a growth-mediating effect on breast tissue comparable to its effect on the endometrium.

Most early studies that assessed the possible effects of estrogen on breast cancer risk were uncontrolled follow-up studies. In the most credible of these studies, Hoover et al.[38] reported 25% more cases of breast cancer in their cohort of menopausal estrogen users than what would have been expected based on general population rates (49 observed versus 39 expected). A more substantial increase occurred among women using high estrogen doses over a long period.

Early case-control studies to evaluate the relationship between menopausal estrogens and breast cancer were often limited by small numbers of subjects, insufficient data on dose and duration of use, and the definite possibility of bias. A series of carefully conducted case-control studies using healthy population controls have recently been published (Table II).[39-46] Overall, long-

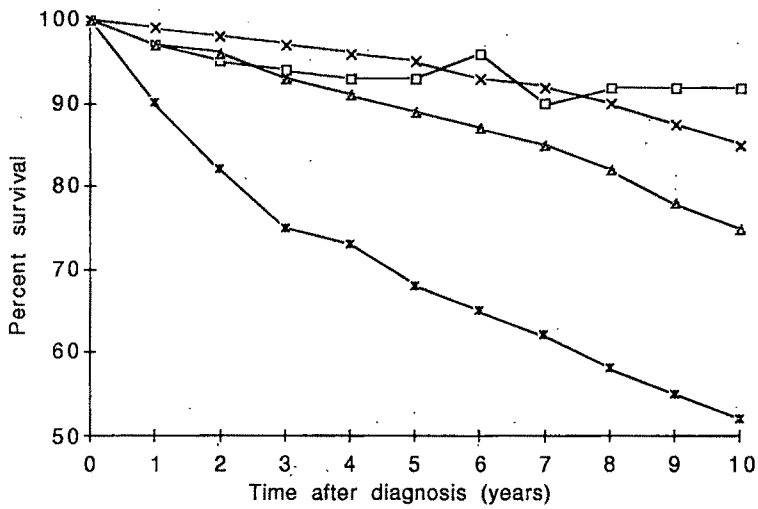


Fig. 2. Survival of women with endometrial cancer and a history of noncontraceptive estrogen use in Ontario, Canada. X, Estrogen user controls (age-adjusted mortality); □, estrogen users with endometrial cancer; ∗, women with endometrial cancer who are not estrogen users; △, non-estrogen user controls (age-adjusted mortality). (From Collins J, Allen LH, Donner A. Lancet 1980;2:961-63.)

Table II. Relative risk of breast cancer after estrogen replacement therapy using population controls: Recent case-control studies

Study	*No. cases/controls (% controls exposed)*	*Relative risk**		*Comments*
		Ever user	*Long-term users*	
Brinton et al.[39] (1986)	1127/1308 (41%)	1.1	15+ yr, 1.7	Results for women with natural menopause
	485/517 (59%)	1.0	15+ yr, 1.3	Results for women with hysterectomy, no BSO
	320/393 (77%)	1.1	15+ yr, 1.4	Results for BSO
				Overall, high in BBD with 10+ years of estrogen use
Hiatt et al.[40] (1984)	119/119 (90%)	0.7	3+ yr, 1.8	All subjects had BSO
Hoover et al.[41] (1981)	345/611 (29%)	1.4	5+ yr, 1.7	High in family history
Hulka et al.[42] (1982)	152/620 (18%)	1.8	10+ yr, 1.7	Results for women with natural menopause
	21/117 (50%)	1.3	Not provided	Results for women with hysterectomy
				High in women with natural menopause with family history, users of injectable estrogens
McDonald et al.[43] (1986)	96/307 (40%)	1.3†	6+ yr, 1.2	Results for BSO
	43/120 (67%)	0.5†	6+ yr, 0.5	Results for women with hysterectomy, no BSO
	35/89 (79%)	0.8†	6+ yr, 0.7	Results for women with natural menopause
Nomura et al.[44] (1986)	181/181 (50%)	1.1	6+ yr, 1.9	Both results include 21 premenopausal women
	160/159 (62%)	0.9	6+ yr, 1.3	high in family history, BBD
Ross et al.[45] (1980)	101/187 (45%)	1.4	TMD, 2.5	Results for women with natural menopause
	26/66 (56%)	0.8	TMD, 0.7	Results for BSO
				High in women with natural menopause with BBD
Wingo et al.[46] (1987)	295/401 (67%)	1.3	15+ yr, 1.7	Results for BSO
	564/652 (25%)	1.1	15+ yr, 2.0	Results for women with hysterectomy, no BSO
	510/592 (16%)	0.8	5+ yr, 0.7	Results for women with natural menopause
				High in BSO with family history

BSO, Bilateral salpingo-oophorectomy; *BBD,* benign breast disease; *TMD,* total cumulative dose greater than 1500 mg.

*Risk relative to never-users.

†Ever users are women who have used estrogens for at least 1 year; ever users and long-term users are compared with women with either less than 1 year of use or no estrogen use.

term estrogen use was associated with small to moderate increases in the risk of breast cancer, although some variation existed across studies based on ovarian status (intact ovaries versus ovaries removed) or hysterectomy status (surgical versus natural menopause).

In women with intact ovaries, Ross et al.[45] found that those who had received a total cumulative conjugated estrogen dose in excess of 1500 mg had a 2.5-fold increased risk relative to nonusers. No such increase was observed for oophorectomized women. In the Cancer and Steroid Hormone (CASH) study,[46] risk with long-term use was elevated only for women who re-

Table III. Relative risk of breast cancer after estrogen replacement therapy using hospital controls: Recent case-control studies

Study	No. cases/controls (% controls exposed)	Relative risk*		Comments
		Ever users	Long-term users	
Hulka et al.[42] (1982)	152/309 (18%)	1.7	10+ yr, 0.7	Results for women with natural menopause
	21/63 (52%)	1.2	Not provided	Results for women with hysterectomy
				High in women with natural menopause with family history, users of injectable estrogens
Nomura et al.[44] (1986)	181/183 (53%)	1.0	6+ yr, 1.2	Both results include premenopausal women
	160/161 (66%)	0.7	6+ yr, 0.8	
Kaufman et al.[47] (1984)	1610/1606 (30%)	1.0		Overall
	NA	0.9†	10+ yr, 1.3	Results for women with natural menopause
	NA	0.9†	10+ yr, 0.3	Results for women with hysterectomy
	NA	0.5†	10+ yr, 0.5	Results for BSO
Kelsey et al.[48] (1981)	41/219 (44%)	0.9	50+ mgM, 1.0	Results for BSO
	286/1089 (76%)	0.9	50+ mgM, 0.6	Results for at least one ovary
				Analyses include premenopausal women
Sherman et al.[49] (1983)	113/113 (45%)	0.6	Not provided	High in overweight women
Jick et al.[50] (1980)	60/7 (44%)	1.1	Not provided	Results for women with hysterectomy
	37/61 (70%)	3.4	Not provided	Results for women with natural menopause
LaVecchia et al.[51] (1986)	1108/1281 (4%)	1.8	2+ yr, 2.0	Combines women with natural menopause and women with hysterectomy, but risk with 2+ yr use is greater only in women with natural menopause

BSO, Bilateral salpingo-oophorectomy; *mgM,* milligram months.
*Risk relative to users.
†Equal to or more than.

ported surgical menopause. Hiatt et al.[40] reported an increased risk with long-term use by women who had undergone bilateral oophorectomy. Finally, Hoover et al.[41] and Brinton et al.[39] reported an elevated risk of breast cancer for women with and without intact ovaries after replacement estrogen therapy. Estrogen users with 15 or more years of exposure had a 50% elevation in risk, amounting to a 2% cumulative absolute excess risk in women aged 65 to 79 years.

Studies using hospital controls as the comparison group generally found no evidence to suggest that breast cancer risk was increased either overall or with long duration of use[42, 44, 47-49] (Table III). Two other studies reported significantly elevated risks associated with estrogen use by women who had natural menopause.[50, 51] Hulka et al.[42] reported comparably low risks with either hospital-based or community-based controls. A possible explanation exists for this lack of effect: controls in hospital-based studies have more contact with the health care system and are therefore more likely to use elective drugs than the population as a whole.

Buring et al.[52] evaluated the risk of breast cancer associated with postmenopausal hormone use among a cohort of nurses who primarily used conjugated estrogens unopposed by progestogens. These women were at no increased risk due to ever having used postmenopausal hormones before the initial survey in 1976; however, long-term use conferred a moderate risk (5 to 9 years of use, relative risk = 1.5; 10+ years of use, relative risk = 0.9).

Based on case-control studies using population controls, it seems sensible to conclude the following: long-term use of estrogen replacement therapy leads to about a 75% increase in breast cancer risk after 15 years. Certain subgroups of women may be relatively more susceptible to the carcinogenic effects of estrogens than others, including postmenopausal women with a positive family history of breast cancer[41, 42, 44, 46] and women with surgically proved benign breast disease,[37, 39, 45] especially if the estrogen use followed the diagnosis of the benign disease.[53]

Estrogen users who develop breast cancer may survive longer than nonusers who develop the same cancer. Bergkvist et al.[54] found that the relative survival rate was significantly higher ($p = 0.02$, by about 10% at 5 and 8 years) in estrogen users 50 years old or older who had discontinued estrogen therapy less than 1 year before the cancer diagnosis.

The value of adding a progestogen to estrogen replacement therapy to minimize breast cancer risk has not been suitably evaluated in substantial, well-conceived studies. However, we have reason to believe that the addition of progestogen will actually increase the risk of breast cancer beyond that associated with estrogen use alone.[55] Results from studies of premenopausal women receiving depot-medroxyprogesterone acetate provide some information on the possible in-

dependent effect of this progesterone on breast cancer risk. In two of three studies, the relative risk was 1.0[56, 57]; the association between this drug and breast cancer was significant in the third study[58] (which was potentially biased). In a recent cohort study, women who took progestogen and estrogen in combination had a higher risk of breast cancer than women who used estrogen alone.[59]

Other considerations

As reviewed by Genant et al., estrogens prevent osteoporosis and its most serious medical consequence, hip fracture. Estrogens also lower the risk of coronary heart disease. Because death rates from coronary heart disease among American women are four times those from endometrial and breast cancers combined, estrogen's benefit in this respect could far outweigh any carcinogenic potential. In fact, we recently calculated that for nonhysterectomized women aged 65 to 74 years, a daily dose of estrogen (equivalent to 0.625 mg conjugated estrogen) for 10 years would result in an annual saving of 302 lives per 100,000 women. For hysterectomized women the estimated saving was even more dramatic (328 lives per 100,000 women).[60]

REFERENCES

1. Bittner JJ. The causes and control of mammary cancer in mice. Harvey Lect 1947;42:221-46.
2. Henderson BE, Ross RK, Pike MC, et al. Endogenous hormones as a major factor in human cancer. Cancer Res 1982;43:3232-39.
3. Henderson BE, Ross RK, Bernstein L. Estrogens as a cause of human cancer: the Richard and Hinda Rosenthal Foundation Award Lecture. Cancer Res 1988;48:246-53.
4. Gambrell RD Jr. Clinical use of progestins in the menopausal patient. J Reprod Med 1982;27:531-38.
5. Campbell PE, Barter GA. The significance of atypical hyperplasia. J Obstet Gynaecol Br Commonw 1961;68:668-72.
6. Gambrell RD Jr, Massey FW, Castaneda TA. Use of the progestogen challenge test to reduce the risk of endometrial cancer. Obstet Gynecol 1980;55:732-38.
7. Gusberg SB. The individual at high risk for endometrial cancer. AM J OBSTET GYNECOL 1976;126:535-42.
8. Campbell S, McQueen J, Minardi J, et al. The modifying effect of progestogen on the response of the postmenopausal endometrium to exogenous estrogens. Postgrad Med J 1978;2(suppl):59-64.
9. Smith DC, Prentice R, Thompson DJ, et al. Association of exogenous estrogens and endometrial cancer. N Engl J Med 1975;293:1164-67.
10. Ziel HK, Finkle WD. Increased risk of endometrial carcinoma among users of conjugated estrogens. N Engl J Med 1975;293:1167-70.
11. Mack TM, Pike MC, Henderson BE, et al. Estrogens and endometrial cancer in a retirement community. N Engl J Med 1976;294:1262-67.
12. Gray LA Jr, Christopherson WM, Hoover R. Estrogens and endometrial cancer. Obstet Gynecol 1977;49:385-89.
13. McDonald TW, Annegers JF, O'Fallon WM, et al. Exogenous estrogens and endometrial carcinoma: case-control and incidence study. AM J OBSTET GYNECOL 1977; 127:572-80.
14. Wigle DT, Grace M, Smith ESO. Estrogen use and cancer of the uterine corpus in Alberta. Can Med Assoc J 1978;118:1276-78.
15. Horwitz R, Feinstein AR. Alternative analytic methods for case-control studies of estrogens and endometrial cancer. N Engl J Med 1978;299:1089-94.
16. Hoogerland DL, Buchler DA, Crowley JJ, et al. Estrogen use—risk of endometrial carcinoma. Gynecol Oncol 1978;6:451-58.
17. Antunes CMF, Stolley PD, Rosenshein MB, et al. Endometrial cancer and estrogen use. Report of a large case-control study. N Engl J Med 1979;300:9-13.
18. Weiss NS, Szekely DR, English DR, et al. Endometrial cancer in relation to patterns of menopausal estrogen use. JAMA 1979;242:261-64.
19. Hulka BS, Kaufman DG, Fowler WC Jr. Predominance of early endometrial cancers after long-term estrogen use. JAMA 1980;244:2419-22.
20. Shapiro S, Kaufman DW, Slone D, et al. Recent and past use of conjugated estrogens in relation to adenocarcinoma of the endometrium. N Engl J Med 1980;303:485-89.
21. Jelovsek FR, Hammond CB, Woodard BH, et al. Risk of exogenous estrogen therapy and endometrial cancer. AM J OBSTET GYNECOL 1980;137:85-91.
22. Spengler RF, Clarke EA, Woolever CA, et al. Exogenous estrogens and endometrial cancer: a case-control study and assessment of potential biases. Am J Epidemiol 1981;114:497-506.
23. Stavraky KM, Collins JA, Donner A, et al. A comparison of estrogen use by women with endometrial cancer, gynecologic disorders, and other illnesses. AM J OBSTET GYNECOL 1981;141:547-55.
24. Kelsey JL, LiVolsi VA, Holford TR, et al. A case-control study of cancer of the endometrium. Am J Epidemiol 1982;116:333-42.
25. LaVecchia C, Franceschi S, Gallus G, et al. Oestrogens and obesity as risk factors for endometrial cancer in Italy. Int J Epidemiol 1982;11:120-26.
26. Henderson BE, Casagrande JT, Pike MC, et al. The epidemiology of endometrial cancer in young women. Br J Cancer 1983;47:749-56.
27. Chu J, Schweid AI, Weiss NS. Survival among women with endometrial cancer: a comparison of estrogen users and nonusers. AM J OBSTET GYNECOL 1982;143:569-73.
28. Elwood JM, Boyes DA. Clinical and pathological features and survival of endometrial cancer patients in relation to prior use of estrogens. Gynecol Oncol 1980;10:173-86.
29. Collins J, Allen LH, Donner A. Oestrogen use and survival in endometrial cancer. Lancet 1980;2:961-63.
30. Jick H, Watkins RN, Hunter JR, et al. Replacement estrogens and endometrial cancer. N Engl J Med 1979; 300:218-22.
31. Hammond CB, Jelovsek FR, Lee KL, et al. Effects of long-term estrogen replacement therapy II. Neoplasia. AM J OBSTET GYNECOL 1979;133:537-47.
32. Paganini-Hill A, Ross RK, Henderson BE. Endometrial cancer and patterns of use of oestrogen replacement therapy: a cohort study. Br J Cancer 1989;59:445-47.
33. Persson I, Adami H-O, Bergkvist L, et al. Risk of endometrial cancer after treatment with oestrogens alone or in conjunction with progestogens: results of a prospective study. Br Med J 1989;298:147-51.
34. Gal D, Edman CD, Vellios F, et al. Long-term effect of megestrol acetate in the treatment of endometrial hyperplasia. AM J OBSTET GYNECOL 1983;146:316-22.
35. Studd JWW, Thom MH, Paterson MEL. The prevention and treatment of endometrial pathology in postmenopausal women receiving exogenous oestrogens. In: Pasetto W, Pavletti R, Lambrus JL, eds. The menopause and postmenopause. Lancaster, England: MTP Press, 1980: 127-39.
36. Henderson BE, Ross RK, Lobo RA, et al. Re-evaluating the role of progestogen therapy after the menopause. Fertil Steril 1988;49(suppl):9S-15S.

37. Whitehead MI, Townsend PT, Pryse-Davies J, et al. Effects of various types and dosages of progestogens on the postmenopausal endometrium. J Reprod Med 1982;27:539-47.
38. Hoover R, Gray LA, Cole P, et al. Menopausal estrogens and breast cancer. N Engl J Med 1976;295:401-5.
39. Brinton LA, Hoover R, Fraumeni JF. Menopausal oestrogens and breast cancer risk: an expanded case-control study. Br J Cancer 1986;54:825-32.
40. Hiatt RA, Bawal R, Friedman GW, et al. Exogenous estrogens and breast cancer after oophorectomy. Cancer 1984;54:139-44.
41. Hoover R, Glass A, Finkle WD, et al. Conjugated estrogens and breast cancer risk in women. JNCI 1981;67:815-20.
42. Hulka BS, Chambless LE, Deubner DC, et al. Breast cancer and estrogen replacement therapy. AM J OBSTET GYNECOL 1982;143:638-44.
43. McDonald JA, Weiss NS, Daling JR, et al. Menopausal estrogen use and the risk of breast cancer. Breast Cancer Res Treat 1986;7:193-99.
44. Nomura AMY, Kolonel LN, Hirohata T, et al. The association of replacement estrogens with breast cancer. Int J Cancer 1986;37:49-53.
45. Ross RK, Paganini-Hill A, Gerkins VR, et al. A case-control study of menopausal estrogen therapy and breast cancer. JAMA 1980;243:1635-39.
46. Wingo PA, Layde PM, Lee NC, et al. The risk of breast cancer in postmenopausal women who have used estrogen replacement therapy. JAMA 1987;257:209-15.
47. Kaufman DW, Miller DR, Rosenberg L, et al. Noncontraceptive estrogen use and risk of breast cancer. JAMA 1984;252:63-67.
48. Kelsey JL, Fischer DB, Holford JR, et al. Exogenous estrogens and other factors in the epidemiology of breast cancer. JNCI 1981;67:327-33.
49. Sherman B, Wallace R, Bean J. Estrogen use and breast cancer: interaction with body mass. Cancer 1983;51:1527-31.
50. Jick H, Walker AM, Watkins RN, et al. Replacement estrogens and breast cancer. Am J Epidemiol 1980;112:586-94.
51. LaVecchia C, Decarli A, Parazzini F, et al. Noncontraceptive oestrogens and the risk of breast cancer in women. Int J Cancer 1986;38:853-58.
52. Buring JE, Hennekens CH, Lipnick RJ, et al. A prospective cohort study of postmenopausal hormone use and risk of breast cancer in U.S. women. Am J Epidemiol 1987;125:939-47.
53. Thomas DB, Persing JP, Hutchinson WG. Exogenous estrogens and other risk factors for breast cancer in women with benign breast diseases. JNCI 1982;69:1017-25.
54. Bergkvist L, Adami H-O, Persson I, et al. Prognosis after breast cancer diagnosis in women exposed to estrogen and estrogen-progestogen replacement therapy. Am J Epidemiol 1989;130:221-28.
55. Key TJA, Pike MC. The role of oestrogens and progestagens in the epidemiology and prevention of breast cancer. Eur J Cancer Clin Oncol 1988;24:29-43.
56. Depot-medroxyprogesterone acetate (DMPA) and cancer: memorandum from a WHO meeting. Bull WHO 1986;64:375-82.
57. Liang AP, Levenson AG, Layde PM, et al. Risk of breast, uterine corpus and ovarian cancer in women receiving medroxyprogesterone injections. JAMA 1983;249:2909-12.
58. Lee NC, Rosero-Bixby L, Oberle MW, et al. A case-control study of breast cancer and hormonal contraception in Costa Rica. JNCI 1987;79:1247-54.
59. Bergkvist L, Adami H-O, Persson I, et al. The risk of breast cancer after estrogen and estrogen-progestin replacement. N Engl J Med 1989;321:293-97.
60. Ross RK, Pike MC, Henderson BE, et al. Stroke prevention and oestrogen replacement therapy. Lancet 1989;1:505.

Estrogen replacement therapy: What the future holds

Charles B. Hammond, MD
Durham, North Carolina

Although hormonal replacement therapy has proved beneficial for many postmenopausal women, several issues remain to be resolved through future research. Improved combination therapy, better delivery systems, and optimal dosing may enhance compliance, which is essential for maximum benefit from therapy. Epidemiologic studies are necessary to clarify the role of estrogen in osteoporosis and cardiovascular disease. Additionally, the contribution of hormonal replacement therapy to the development of breast and endometrial cancers needs to be clarified. (AM J OBSTET GYNECOL 1989;161:1864-8.)

Key words: Hormone replacement therapy, menopause, research

From the Department of Obstetrics and Gynecology, Duke University Medical Center.
Reprint requests: Charles Hammond, MD, Department of Obstetrics and Gynecology, Duke Medical Center, Box 3853, Durham, NC 27710.
6/0/16683

Women in the United States presently have a life expectancy of 81 years, 30 years beyond the usual age of menopause. When ovarian function ceases, a state of relative estrogen deficiency develops. A woman will live one third of her life without the beneficial effects

of her natural estrogens. Common hypoestrogenic-associated conditions such as vasomotor instability, emotional lability, sleep disturbances, genitourinary atrophy, and osteoporosis develop in 75% to 85% of postmenopausal women. Hormonal replacement therapy is effective in treating these conditions; in most cases it is superior to alternative modes of therapy.[1] In addition to its other benefits, hormonal replacement therapy may reduce the increasing risk of atherosclerosis in postmenopausal women.[2] This therapy is associated with few relative risks and side effects. Hormonal replacement therapy should be considered a preventive measure; for effective prophylaxis, early administration and long-term use are required. Concerns over possible risks of breast and endometrial cancer, coronary artery disease, thrombosis, hypertension, and effects on glucose tolerance have been obstacles to using this therapy. This article will discuss the increasing role of hormonal replacement therapy in both postmenopausal women and in women with induced estrogen deprivation. Future uses and research directions will be suggested.

Formulation and route of administration

Free estrogen is the biologically active form of the hormone. More than 90% of circulating estrogen is protein bound, 30% to sex hormone binding globulin and 60% to albumin, leaving, 2% to 3% unbound. The concentration of free hormone is a function of total estrogen concentration, sex hormone binding globulin concentration, and metabolic clearance rate, which is inversely proportional to the extent of protein binding.[3] Free estrogens exert their metabolic effects by binding to high-affinity, specific low-capacity estrogen receptors in the nucleus of the target cell. The potency of a specific estrogen is determined by the length of time it occupies the steroid receptor.[4] There is no uniform estrogen bioassay: multiple factors determine the efficacy of a particular estrogen compound, including the efficiency of absorption and metabolic clearance, dose and route of administration, and the particular system or effect under evaluation.[4] Dose, route, and frequency of administration vary among different estrogen preparations. Currently, potency is generally assessed by changes in the maturation index of the vaginal mucosa, cervical mucous ferning, and endometrial proliferation. Similar biologic effects are achieved with diethylstilbestrol 5 mg, estradiol 5 mg, conjugated equine estrogens 3.75 mg, estrone sulfate 3.75 mg, ethinyl estradiol 0.05 mg, and mestranol 0.08 mg.

Conjugated equine estrogens, obtained from the urine of pregnant mares, are the most commonly prescribed estrogens of hormonal replacement therapy in the United States. Most circulating estrogen is equilin, which is both potent and long-lasting due to its lipophilic properties that allow storage and slow release from adipose tissues.[5] Conjugated estrogens are effectively absorbed after oral or intravaginal administration. A daily dose of 0.625 mg oral conjugated estrogen or 1.25 mg intravaginal conjugated estrogen raises the total circulating estrogen level to approximately 93 pg/ml (the mean serum level of estrogen after menopause is 35 ng/l).[4]

Other available agents for hormonal replacement therapy include estradiol, estrone, or estriol. Because these estrogens are insoluble in water and are rapidly metabolized in the gastrointestinal tract, they must be esterified or sulfated for oral use. Adding an ethinyl group at the 17 position produces 17β-estradiol; this compound is almost completely absorbed after oral administration. Micronization, by which the particles are made small enough to permit gastrointestinal absorption (20×10^6 m or less), is another technique that makes oral therapy possible.[3, 4] Micronized estrogen preparations may be administered orally, intravaginally, or sublingually. Intravaginal administration results in rapid and effective systemic absorption. In a study of postmenopausal women, subjects experienced a decrease in vasomotor and vaginal symptoms after 15 days of treatment; moderate to marked improvement in symptoms related to atrophic vaginitis was noted in 53 of 54 patients.[6]

17β-Estradiol is also available in the United States as a transdermal patch in dosages of 0.05 and 0.1 mg. The transdermal patch raises estrogen levels from 40 to 80 pg/ml, but estrogen levels fluctuate around the physiologic level and achieving a steady state is not easy.[3] The patch is convenient to use and needs to be replaced only every 3 days. A once-a-week oral estrogen agent, quinestrol (the 3-cyclopentylether of ethinyl estradiol), is also available. This preparation is absorbed through the lymphatic system, stored in adipose tissue, and released gradually.[4]

Thus a variety of estrogen preparations and routes of administration are available. The transdermal forms have only recently been shown to have similar beneficial effects on lipids and bone that the oral preparations confer. If relative dose is considered, no one preparation is clinically more likely to cause major side effects.

Progesterone cotherapy

In the 1970s, evidence indicated that using unopposed estrogens was associated with a significant increase in endometrial cancer.[7] Unopposed estrogens may also cause problems such as abnormal vaginal bleeding for which diagnostic evaluations and surgical treatments may be needed. In a retrospective study spanning 17 years, more than one fourth of women using unopposed estrogen required a hysterectomy to treat nonmalignant complications.[8] The ad-

dition of a progestogen to hormonal replacement therapy can usually avoid both the malignant and nonmalignant gynecologic problems associated with unopposed estrogen. Because natural progesterone is poorly absorbed through the gastric mucosa, synthetic forms are used. Available progestogens include 17-acetyloxyprogestin (medroxyprogesterone acetate) and 19-norprogestins (norgestrel, norethindrone, and norethynodrel). Gastrointestinal absorption varies among patients. Like estrogens, these progestogens may be administered orally, vaginally, and parenterally. A micronized form of progesterone that has been used in Europe produces serum levels greater than 10 ng/ml and a secretory endometrium.[3]

Both 17- and 19-progestins reduce calcium creatinine ratios. Some studies have suggested that regimens of estrogen plus a progestin actually have a beneficial effect on bone metabolism, increasing bone mineral mass rather than just stabilizing it.[9, 10] Progestins' effect on lipid metabolism is the reverse of that of estrogens: they elevate low-density lipoprotein cholesterol while lowering high-density lipoprotein cholesterol. The amount of androgenic activity of a specific progestogen correlates with its lipid effects. To date, oral micronized progesterone has not been shown to affect lipid metabolism adversely.[3]

Despite their adverse effects on lipids, progestins seem necessary to protect the endometrium from hyperplasia. Future areas of research might focus on the development of a transdermal progestin preparation and the development of an estrogen-progesterone minipill containing the proper balance of both agents so that adverse effects are minimized and beneficial effects maximized.

Patient selection, dosage, and compliance

When selecting patients for hormonal replacement therapy, one must balance the potential benefits against the risks. Women with symptoms related to estrogen deficiency should be considered candidates for hormonal replacement therapy as should women at risk of developing osteoporosis or those with a strong family history of cardiovascular disease. Considerable data now suggest that estrogen-progestin therapy can significantly reduce death among postmenopausal women.[11] Hormone replacement therapy should be started as soon as ovarian failure occurs (with the cessation of menses) or in the perimenopausal period if significant symptoms of estrogen deficiency begin. Absolute contraindications include a known or suspected estrogen-dependent neoplasia, undiagnosed genital bleeding, active thrombophlebitis or thromboembolic disorder, and active liver disease. Relative contraindications include a past history of estrogen-dependent thrombophlebitis, uterine leiomyomata, unstable hypertension, and poorly controlled glucose intolerance.[3]

The dose of estrogen used should be the minimum required to control symptoms and to provide protection against osteoporosis. No one estrogen or progestin has been found to be superior when equivalent doses are considered. Conjugated equine estrogen and estrone sulfate at doses of 0.625 mg have prevented osteoporosis in most women.[12, 13] Cyclic administration of estrogen and progestins is no longer recommended. Rather, estrogen should be given continuously, 365 days a year, and a progestogen should be given concomitantly for the first 10 to 14 days of the month. This method of administration provides protection against the development of endometrial carcinoma and produces a predictable bleeding pattern that reflects the status of the endometrium.[3] If menstrual bleeding occurs before the tenth day of the month, a proliferative endometrium is likely present. In this case, the dose of progestin may need to be increased and endometrial sampling is necessary. If bleeding begins after day 10 of the month but before day 18, there is full secretory transformation.[14]

Hormone replacement therapy should be continued until the patient is in her eighties or beyond because rapid bone loss occurs after its discontinuation. The appropriate dose of progestin that will protect the endometrium and produce the least effect on lipids is still unknown. However, a dose of 0.35 mg norethindrone or 5 mg medroxyprogesterone acetate administered for 14 days a month appears to provide adequate protection to the endometrium.[3]

Compliance may be a problem due to several effects of this therapy, including initial mastalgia, vaginal discharge, and resumption of menses. Withdrawal bleeding is a primary compliance problem. When hormonal replacement therapy is administered with 10 to 14 days of a progesterone, as described above, 80% to 90% of women will have regular withdrawal bleeding. If both estrogen and progestogen are given every day, it now seems that less numbers of women experience bleeding.[8]

Future research is needed in three primary areas. The optimal dose of progestins needed to optimize protective effects on the endometrium while minimizing detrimental effects on lipid metabolism must be determined through prospective, randomized studies. In women who have undergone hysterectomies, we must yet determine whether adding a progestin is necessary. This controversy exists due to a few studies showing that progestins may protect against the higher risk that exists for breast cancer with high doses of estrogens. The current approach is to individualize therapy based on the patient's risk of developing breast cancer. The frequency and need for endometrial sampling has still not been established. Women receiving estrogen plus progestin probably do not need periodic endometrial sampling. However, some physicians still recommend

that endometrial sampling be done every 1 to 3 years. Finally, withdrawal bleeding remains a compliance problem; different forms of estrogens and progestogens should be developed to address this problem.

Osteoporosis

Osteoporosis becomes a significant problem in the postmenopausal woman. After menopause, bone matrix formation by osteoblast is reduced, leading to reduced bone density, increased rate of bone loss, and increased susceptibility to atraumatic fractures. One in three postmenopausal women in the United States develops some complication of osteoporosis. Postmenopausal women lose 1% or more of their bone mass per year. Therefore, if left untreated, an 80-year-old white woman may have lost 30% to 50% or more of her skeletal mass.[3] Slowing the development of osteoporosis is the most efficient approach to this problem. Reported possible preventive methods include hormonal replacement therapy, dietary calcium supplementation, modification of life-style risk factors such as smoking and alcohol consumption, regular weight-bearing exercise programs, vitamin D and fluoride supplementation, and calcitonin use.

Unequivocal evidence shows that estrogen is of critical importance in maintaining bone density in women.[3, 15] The mechanism by which estrogen acts to prevent bone loss is not known; however, several large studies have demonstrated a causal relationship between estrogen deprivation and accelerated bone loss. In a 10-year, prospective, double-blind, placebo-controlled study, women who received estrogen-progestin therapy within 3 years of menopause demonstrated a dramatic difference in bone mineral density compared with women who received placebo. All patients receiving hormonal replacement therapy showed an improvement or no increase in osteoporosis and experienced significantly fewer fractures.[16] When hormonal replacement therapy is stopped there is a rapid and progressive loss of bone mineral content, at a rate of approximately 2.5% per year. Within 4 years the bone mineral content of women who discontinued therapy is equivalent to that of women who never began it.[3] The dose of estrogen necessary to protect postmenopausal women against osteoporosis is established at 0.625 mg conjugated estrogen or estrone sulfate per day.[3] For the greatest protection, hormonal replacement therapy should start as close as possible to menopause and be maintained for the balance of life.[17]

Areas in which research is needed include estrogen deficiency–related disorders, estrogens' role and the dosage of estrogen necessary in treating established osteoporosis, and the role of calcitonin in postmenopausal osteoporosis, whether estrogen is a primary or secondary treatment modality. Estrogens clearly play an important role in treating and preventing osteoporosis. However, the optimal regimen of hormonal replacement therapy combined with calcium, diet, life-style modifications, and other measures needs to be established.

Cardiovascular disease

Coronary artery disease is a multifactorial problem associated with at least four identifiable risk factors: hypertension, hyperlipidemia, diabetes, and cigarette smoking. Coronary artery disease is responsible for approximately 500,000 deaths among women in the United States each year.[3] Many epidemiologic case-control studies have shown that postmenopausal hormonal replacement therapy lowers the relative risk for coronary artery disease; however, a few studies indicate little or no protection.[17] Until further data from controlled epidemiologic studies are available, this therapy should be considered a possible mode of preventing coronary artery disease. Data show that after menopause the incidence of coronary artery disease rapidly increases. The importance of estrogen is supported by studies showing that women with premature surgical menopause have a 2.2-fold increase in their relative risk of developing coronary artery disease. This increased risk can be reduced to normal by hormonal replacement therapy.[3] One possible mechanism for this protective effect may be estrogen's beneficial effects on the lipid profile. As stated earlier, estrogen lowers levels of total and low-density lipoprotein cholesterol and increases levels of high-density lipoprotein cholesterol. However, the exact mechanism by which estrogen protects against coronary artery disease remains to be established. Studies on estrogen's effect on platelet adhesiveness and its direct effects on the vessels are needed.

Adverse effects of estrogens

Estrogens' role in the development of endometrial cancer has raised significant controversy and concern over the years. Recently, concerns regarding a similar effect on the incidence of breast cancer have been raised. These data are much less convincing of such a relationship. Prolonged stimulation of the endometrium by estrogen in the absence of progesterone is known to cause hyperplasia in both premenopausal and postmenopausal women.[3] Adenocarcinoma more often seems to develop in women who have endometrial hyperplasia. The primary reason for endometrial hyperplasia, and subsequent carcinoma, in women taking unopposed estrogens appears to be the absence of progesterone. Progestational agents have a protective effect on the endometrium because they inhibit the replenishment of estrogen receptors and increase activity of 17β-estradiol dehydrogenase, which converts estradiol to the less potent estrone. Thus a pseudodecidual endometrium is created and endometrial shedding occurs.[18] The addition of cyclic progesterone for

14 days each month reduces the incidence of endometrial hyperplasia to nearly zero. In our current approach to postmenopausal hormonal replacement therapy, which uses a combination of estrogen and progesterone, the risk of endometrial cancer is no greater than in a patient not receiving therapy and actually may be less.[11]

Breast cancer is a multifactorial disease, and the role of estrogen in its generation is not clear. The potential risk is based primarily on data on estrogen's ability to induce mammary tumors in mice. In one case-control study of 1610 women taking estrogen, the relative risk for an increase in the likelihood of developing carcinoma of the breast was zero.[19] Most clinical studies do not show an increased risk, but several studies have generated some concern. The role of hormonal replacement therapy with added progestins remains controversial in this area. Equally controversial is the decision to add (or not add) progestin to estrogen therapy in women whose uterus has been removed. Another important area of investigation is the safety of hormonal replacement therapy in women with a past history of endometrial or breast carcinoma. Clearly, many more epidemiolgic studies are needed to answer these questions.

Summary

Hormone replacement therapy plays an important role in the preventive medical care of postmenopausal women. There are relatively few contraindications to its use. Programs are needed to educate physicians and women on the role of estrogen in overall postmenopausal well-being. Improved compliance is essential if women are to attain maximum benefit from therapy. Improved combination therapy, better delivery systems, and optimal dosing may enhance compliance. Multicenter epidemiologic studies must be performed to clarify the role of estrogen in bone and cardiovascular disease. Studies to determine the role of estrogens in the development of breast and endometrial carcinoma need to be performed. Despite the need for future investigation, it is increasingly clear that in 1989, for maximum benefit, most postmenopausal women except those with absolute contraindications should receive hormonal replacement therapy.

REFERENCES

1. Utian WH. The true clinical features of postmenopause and oophorectomy and their response to estrogen therapy. S Afr Med J 1972;46:732-7.
2. Johansson BW, Juij L, Kullander S, et al. Some late effects of bilateral oophorectomy. Acta Obstet Gynecol Scand 1975;54:449-61.
3. Hammond CB, Ony SJ. Endocrine aspects of the menopause. In: Shearman RP, ed. Clinical reproductive endocrinology. London: Churchill-Livingstone, 1985: 185.
4. Hammond CB, Maxson WS. Estrogen replacement therapy. Clin Obstet Gynecol 1986;29:407-30.
5. Adams WP, Hasegawa J, Johnson RN, Haring R. Conjugated estrogens bioequivalence: comparison of four products in postmenopausal women. J Pharmacol Sci 1979;68:986-91.
6. Martin PL, Yen SSC, Burnier AM, Herman H. Systemic absorption and sustained effects of vaginal estrogen creams. JAMA 1979;242:2699-700.
7. Weiss NS, Szekely R, Austin DF. Increasing incidence of endometrial cancer in the United States. N Engl J Med 1976;294:1259-62.
8. Ettinger B. Optimal use of postmenopausal hormone replacement. Obstet Gynecol 1988;72(suppl):31S-6S.
9. Mandel FP, Davidson BJ, Erlik Y, et al. Effects of progestins on bone metabolism in postmenopausal women. J Reprod Med 1982;27:511-4.
10. Christianson C, Riis BT, Nilas L, et al. Uncoupling of bone formation and resorption by combined estrogen and progestogen therapy in postmenopausal osteoporosis. Lancet 1985;2:800-1.
11. Henderson BE, Ross RK, Lobo RA, et al. Re-evaluting the role of progestin therapy after the menopause. Fert Steril 1988;49(suppl):9S-15S.
12. Lindsay R, Hart DM, Clark DM. The minimum effective dose of estrogen for prevention of postmenopausal bone loss. Obstet Gynecol 1984;63:759-63.
13. Genant HK, Baylink DL, Gallagher JC. Effect of estrone sulfate on postmenopausal bone loss (submitted for publication).
14. Padwick ML, Pryse-Davies J, Whitehead MI. A simple method for determining the optimal dosage of progestin in postmenopausal women receiving estrogens. N Engl J Med 1986;315:930-4.
15. Speroff L. Estrogen replacement today: a preventive health care issue. Hosp Pract 1986;3(suppl):3-8.
16. Nachtigall LE, Nachtigall RH, Nachtigall RD, et al. Estrogen replacement therapy: a 10-year prospective study in the relationship of osteoporosis. Obstet Gynecol 1979; 53:277-81.
17. Metzger DA, Hammond CB. Are estrogens indicated for the treatment of postmenopausal women? Drug Intell Clin Pharm 1988;22:493-6.
18. Gambrell RD. Estrogens, progestogens and endometrial cancer. J Reprod Med 1977;18:301-6.
19. Kaufman DW, Miller DR, Rosenberg L, et al. Noncontraceptive estrogen use and the risk of breast cancer. JAMA 1984;252:63-7.